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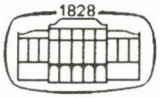

AKADÉMIAI KIADÓ
BUDAPEST

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MEASURED AND CALCULATED PRESSURES AND PRESSURE DIFFERENCES OF TINPLATE CANS UNDER STERILIZATION CONDITIONS

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The authors measured the inside and outside pressures of tinplate cans (V_0 = 3230 cm^3) in three experimental runs, simulating sterilization processes with water filled cans. A method for the calculation of inside pressure was also created, using finite differences and stepping with 10^{-4} bar pressure difference (inside minus outside pressure) increment. The calculation method includes the volume change vs. pressure difference relation based on previous laboratory measurements (KÖRMENDY et al., 1994).

The mean difference between measured and calculated inside pressures varied between 0.048 to 0.073 bar. The mean relative difference as related to the measured inside pressure varied between 2.37 to 8.02%. Calculated inside pressures fairly followed measured values. However, the mean relative difference as related to the difference of inside and outside pressures reached higher values.

Keywords: deformation of tinplate cans, sterilization of food

The aim of this work was to develop appropriate methods, which enable an expert to calculate the variation of pressure inside a tinplate can during the sterilization process and after it under storage conditions and to compare measured and calculated results.

The difference between inside and outside pressure (Δp) should not transgress critical pressure differences, which either cause the permanent outbulging of the end plates or the collapse at the mantle.

Another aspect at creating the calculating method was the versatility to quickly alter those parameters and time dependent variations, which influence the evolution of the pressure difference. Such parameters typically belong to the closing conditions of the can and time dependent variations may include the outside pressure's and temperature's and the due inside temperatures' change in time.

Since RIGHI (1955) the calculation of pressure inside partially filled cans became an elementary or advanced textbook material (CHARM, 1971; HEISS, 1980; HEISS & EICHNER, 1984). Inside container pressure and relating head space volume

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can be calculated explicitly by solving a quadratic equation, provided linear relation exists between volume change (ΔV) and pressure difference (Δp) in a narrow domain around the inside pressure and head space volume in request. Other solving procedures are also possible as iteration (trial and error method) or the method of finite differences. Some authors applied quadratic solution (RIGHI, 1955; KÖRMENDY, 1989), some used iteration or equivalent graphic solution (CHARM, 1971). PALMIERI and co-workers (1992) used a numerical solution.

The authors tried here to use a finite difference method (see para. 2.3.), though solution is available with other means too, as quoted before.

The whole work is based on the previous (experimental) results of FERENCZY (1986), KÖRMENDY and FERENCZY (1989), KÖRMENDY and co-workers (1994). The calculation methods here are apt only for food materials in which isotropic pressures are created.

The effect of dissolved gases, the amount of water vapour in the head space and water transport between head space and liquid water were also neglected. The authors regarded in the volume change vs. pressure difference relation ($\Delta V = f(\Delta p)$) the phenomena of hysteresis created by dome formation of end plates and subsequent propping effect as it had been evolved in a previous publication (KÖRMENDY et al., 1994).

1. Materials and measurement methods

1.1. Type of the can and its properties

Tinplate cans of Type 7/2–153 (Anglo-American equivalent about # 603 × 700) were used for experiments. Detailed description of geometry and properties (including the methods used for measuring the properties) is available in the publication of KÖRMENDY and co-workers (1994).

1.2. Apparatus and instruments for experiments

A horizontal retort (Stock Pilot Rotor) has been used for sterilization.

Two pressures and two temperatures have been measured by a Testostore 9510 (Testoterm GmbH & Co.) instrument with data storage capability. Accuracy: ±30 mbar up to 2 bar and ±1.5% of the measured value above 2 bar for pressure and ±0.5 °C up to 100 °C and ±0.5% above 100 °C for temperature. Two pressure transducers measured the retort pressure and the inside pressure of the can. Two temperature sensors measured the retort temperature and the water (fluid) temperature in the can.

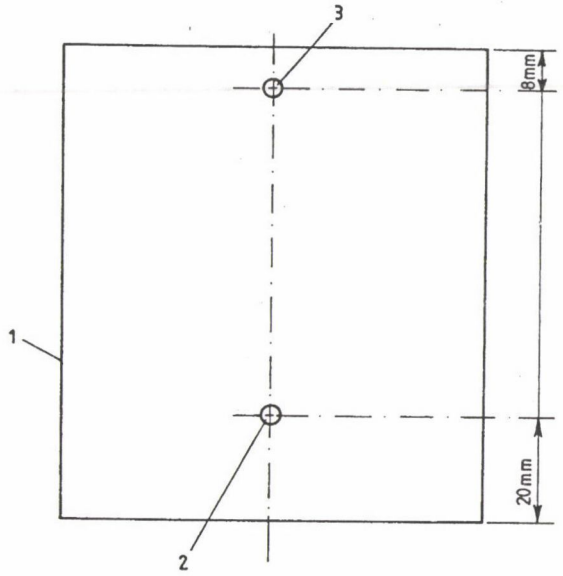

Fig. 1. Locations of sensors inside the can. 1: contour lines of the can; 2: sensor for measuring fluid (water) temperature and inside pressure; 3: sensor for measuring the head space temperature

Another instrument (Ellab Company, CTD–FDQ thermometer) served for measuring the temperature in the head space of the can (accuracy: ±0.1 °C).

Locations of pressure transducer and temperature sensors in the can are demonstrated in Fig. 1. Sealed openings served for their introduction into the can.

1.3. Experimental procedure

A closed tinplate can equipped with transducer and sensors has been filled up with m_e = 2.9892 kg hot water of 75 °C. The volume of water amounted to about 93% of the total volume of the can. The opening for filling has been closed by introducing into the same opening the head space temperature sensor. Small stuffing boxes served for the sealing of the pressure transducer's and temperature sensors' ducts. Instant setting adhesive was also applied.

The filled up can and accessories were closed into the retort and sterilization process started. Water and head space temperatures, pressure in the can, retort temperature and pressure were measured simultaneously, generally in 1 min intervals. An experimental sterilization lasted for about 2–2.5 h.

Altogether 3 experimental runs were completed within a week, always using a new can.

Retort temperature was kept constant by applying an automatic control apparatus in the constant temperature section. Retort temperature in the heating and

cooling periods and retort pressure during the whole process was manually regulated. Pressurized air was used for controlling the retort pressure. Cooling water had been flowed through the retort in the cooling period (as usual practice), while excess retort pressure was regulated by gradually decreasing the air pressure.

The cans in Run 1 and 2 collapsed at the end period of the process (see Figs 3 and 5) at $\Delta p = -1.01$ bar pressure difference. No evaluation has been made on a can after its collapsing.

2. Calculation methods

2.1. General principles

The authors created methods on already known principles to calculate the inside pressure and the relating pressure difference of tinplate cans under sterilization conditions. Preliminary knowledge is needed for the calculations regarding certain parameters at the moment of closing, like inside temperatures, volume and mass of the food, total volume of the can, environmental pressure and temperature. Further necessary constants and relations are as follows:

- Average water activity of the food and the mean volumetric thermal expansion coefficient of the can wall, both in the temperature range of the heat treatment. The volumetric thermal expansion coefficient of the can wall (Table 1) has been taken from the literature (HEISS & EICHNER, 1984).
- The density vs. temperature relation of food.
- Volume change vs. pressure difference relations ($\Delta V = f(\Delta p)$) as measured and calculated in accordance with the publication of KÖRMENDY and co-workers (1994), including such constants as the mean temperature and initial (unloaded) volume (T_0, V_0, $\Delta p = 0$, $\Delta V = 0$). (See the end of the introductory part.)
- Food and headspace temperature, retort temperature and pressure vs. time relations.

The equations include some simplifications: Dissolved gas content of the food material has been neglected, neither gas formation, nor its solution has been accounted for. Head space gas was regarded as air. Water vapour tension in the head space was acconted for as due to saturated condition. The mass of head space vapour and its transport towards liquid water has also been neglected, as calculation showed that vapour mass per liquid water mass varied between 10^{-5} to 10^{-4}.

2.2. Basic equations

One of the basic equations expresses the gas (head space) volume in the can as created by simultaneous mechanical and thermal can wall deformation and the

thermal expansion of food. Another equation gives the inside pressure in the can as the sum of the vapour pressure of food and the partial pressure of air.

$$V_G = (V_0+\Delta V)[1+\alpha_{vw}(T_w-T_0)]-m_e/\rho \quad (1)$$

$$p = a_w p_w+(RT_G/V_G)(m_G/M_G) \quad (2)$$

Additional equations relate volume change to pressure difference (see para. 2.1.) at constant temperature T_0:

$$\Delta V = f(\Delta p) \quad (3)$$

$$\Delta p = p-p_R \quad (4)$$

2.3. Finite difference method

The authors' finite difference method is based partly on the differentiation of Eqns (1) and (2) by time and partly on the use of a sufficiently small constant pressure difference step (increment) $|\delta(\Delta p)|$ for increasing or reducing the actual pressure difference. Differentiation and rearrangement resulted in two explicit formulas, which are presented here in a symbolic way:

$$d(\Delta p)/dt = F_1(dT_w/dt, dT_G/dt, dT_m/dt, dp_w/dT_G, dp_R/dt, df(\Delta p)/d(\Delta p), d\rho/dT_m, T_w, T_G, p_G, V_G, f(\Delta p), \rho) \quad (5)$$

$$dV_G/dt = F_2(Z, d(\Delta p)/dt) \quad (6)$$

Z in Eqn (6) symbolizes all the variables present in the right hand side of Eqn (5). Constants in the equations are m_e, T_0, V_0, α_{vw}, the constants of ρ vs. T_m, p_w vs. T_G and ΔV vs. Δp relations.

Constants of major importance for starting finite difference calculations are tabulated in Table 1.

More detailed form of Eqn (5) and Eqn (6) can obtained by contacting the authors.

Sterilization time has been devided into subsequent sections, so that all the variations of T_w, T_G, T_m and p_R could be well approximated by linear changes inside a section. Consequently time derivatives dT_w/dt,... etc. could be taken as constants in a section. Figures 8 to Fig. 13 illustrate such (reasonably) simplified sections.

Table 1

Constants of major importance belonging to the experimental sterilization runs. Pressures (p) are in bar, temperatures (T) in °C, volumes (V) in cm³

Serial No. of run	Common values	Data at closing conditions							
		T_{wz} (b)	T_{Gz}	T_{mz}	p_{zc} (c)	p_{zm}	m_G, g	V_{ez}	V_{Gz}
1		59.63	69.2	72.4	1.02	1.02	0.1248	3061	171
	$m_e = 2.9892$ kg $T_0 = 18$ °C (a) $V_0 = 3230$ cm³								
2		58.73	70.5	70.5	1.00	0.99	0.1201	3058	174
	$\alpha_{vw} = 3.65.10^{-5}$ K^{-1} $\lvert\delta(\Delta p)\rvert = 10^{-4}$ bar								
3		61.35	71.29	74.6	1.02	1.01	0.1166	3065	167

(a) Temperature of measuring ΔV vs. Δp relations

(b) Calculated according to para. 2.6.

(c) p_{zc}: barometric pressure, p_{zm}: measured inside the can

Calculation starts always at $\Delta p = 0$, $\Delta V = 0$, other values being valid at the moment of closing (initial conditions). Then stepping forward or backward with $\delta(\Delta p)$ (increasing or reducing Δp) begins. The $d(\Delta p)/dt$ is calculated for each step using Eqn (5), but step direction is accepted only, if the sign of $d(\Delta p)/dt$ is equal to the sign of $\delta(\Delta p)$, as time t can only increase. Step turns in the reverse direction if the two signs differ.

The due time increment is calculated as

$$\delta t = \delta(\Delta p)/[d(\Delta p)/dt]$$

and stepping proceeds up to the end of a time section, where results are available. All variables in Eqn (1) to Eqn (6) are repeatedly evaluated at the end of each valid (accepted) step.

The inclusion of time in the calculations makes seemingly unnecessary complications. However, the authors' interest was if the problem could be handled in a time dependent way, regarding further improvements by including time derivatives of measured values.

The authors checked the time dependent finite difference method by a traditional trial and error method. Differences in calculated pressures were negligible (see para. 3.2.), justifying the use of the time dependent finite difference method.

2.4. Calculation of density and vapour tension of water vs. temperature

Water density as a function of temperature has been calculated with a polynomial of second degree. The constants have been obtained by fitting, using the tabulated values of RAZNJEVIC (1964) between zero and 150 °C (temperature interval 1 °C). The result of fitting is:

$$\rho = 1001.6916 - 0.1399\,T - 0.02877\,T^2$$

where $[\rho] = \mathrm{kg\,m^{-3}}$, [T] = °C.

Temperature dependent vapour tension of water has been calculated by interpolating with the Clapeyron equation between zero and 150 °C (temperature interval 10 °C). Thus the appropriate constants in the Clapeyron equation are systematically replaced in each subsequent 10 °C interval. Accurate tabulated results (RAZNJEVIC, 1964) are obtained at the interval bounds.

2.5. Mean difference ($\overline{d_p}$) between measured and calculated inside pressures. Mean relative differences ($\overline{rdp}$, $\overline{rd\Delta p}$)

Mean difference ($\overline{d_p}$) has been calculated by Eqn (7) as follows:

$$\overline{d}_p = \left[\frac{1}{t_u} \int_0^{t_u} (p_m - p_c)^2 dt \right]^{0.5} \tag{7}$$

The equation is based on the calculating of the mean square of deviations as presented inside the square brackets.

$\overline{drp}$ symbolizes the mean relative difference of measured and calculated inside pressures as related to measured pressure. The theoretical relation is:

$$\overline{drp} = \frac{1}{t_u} \int_0^{t_u} \frac{|p_m - p_c|}{p_m} dt \tag{8}$$

Equation (8) was approximated by calculating the time section means and forming their section time weighted average.

$\overline{dr\Delta p}$ is the mean relative difference of measured and calculated pressure differences as related to measured pressure difference. The relation is the same as before, but $|p_m - p_c| = |\Delta p_m - \Delta p_c|$ is related to $|\Delta p_m| = |p_m - p_R|$. $dr\Delta p$ symbolizes a local value (at some selected process time).

2.6. Calculation of wall temperature and missing head space temperature

Wall temperature of the can was calculated as the arithmetic mean of the retort and fluid temperatures, i.e. $T_w = 0.5(T_R + T_m)$. However, in atmospheric air (at and after closing the can) the formula $T_w = 0.25\,T_R + 0.75\,T_m$ was used because of the much lower outside heat transfer coefficient.

Head space temperature for a missing measured value was calculated as the arithmetic mean of the wall and fluid temperatures, regarding a can under heat treatment in the retort.

Missing head space temperature at closing the can (and in the subsequent period in atmospheric air) has been determined by a trial and check method. Diverse head space temperatures were selected between $T_{Gz} = 0.5(T_{wz} + T_{mz})$ and T_{mz} for the calculation of the inside pressure. The mean difference ($\overline{d_p}$) according to Eqn (7) has been determined for each head space temperature. That value of T_{Gz} has been substitued for the missing value, which belonged to the minimum of $\overline{d_p}$.

3. Results

3.1. Experimental results

Three experimental sterilization runs have been carried out in accordance with para. 1.3. Constants of major importance are tabulated in Table 1.

Figure 2, Fig. 4 and Fig. 6 illustrate the retort temperature, head-space and water temperature in the can, as measured in the three runs. Figure 3, Fig. 5 and Fig. 7 illustrate the measured pressure inside the can and the retort pressure in each run.

In Run 1 and Run 2 the cans collapsed from transgressing the critical pressure difference for outside overpressure at the end period of sterilization.

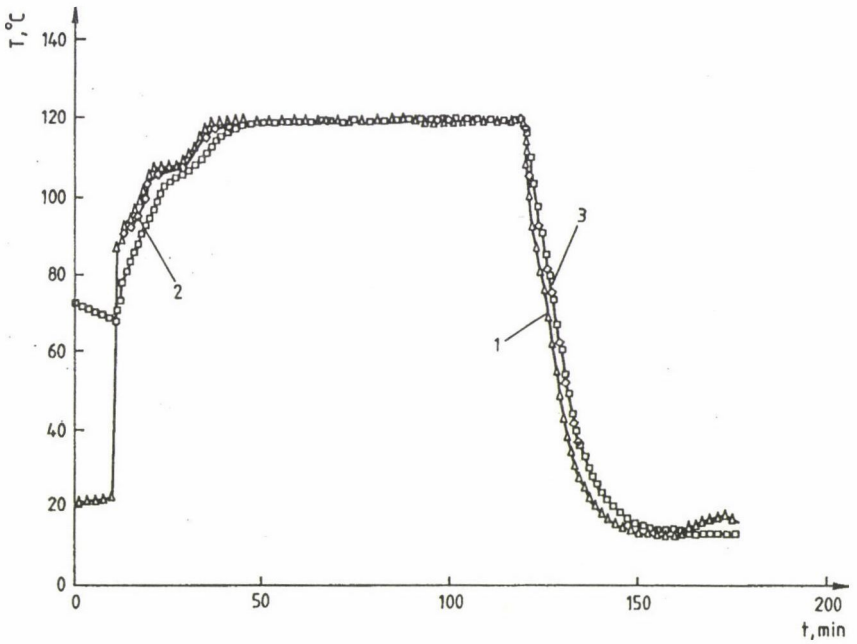

Fig. 2. Measured temperatures vs. time, Run 1. 1: outside (retort) temperature; 2: head space temperature; 3: fluid (water) temperature

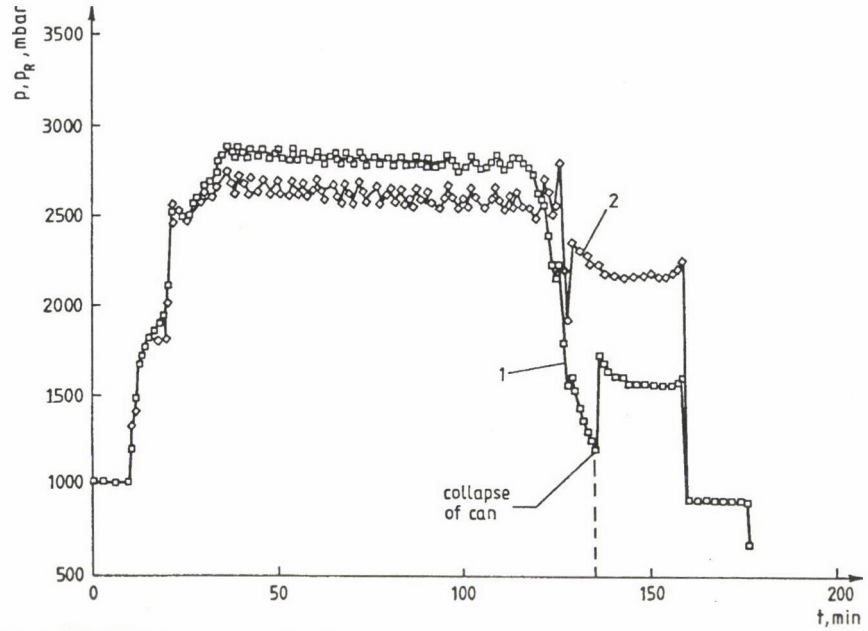

Fig. 3. Measured pressures vs. time, Run 1. 1: inside pressure; 2: outside (retort) pressure (p_R)

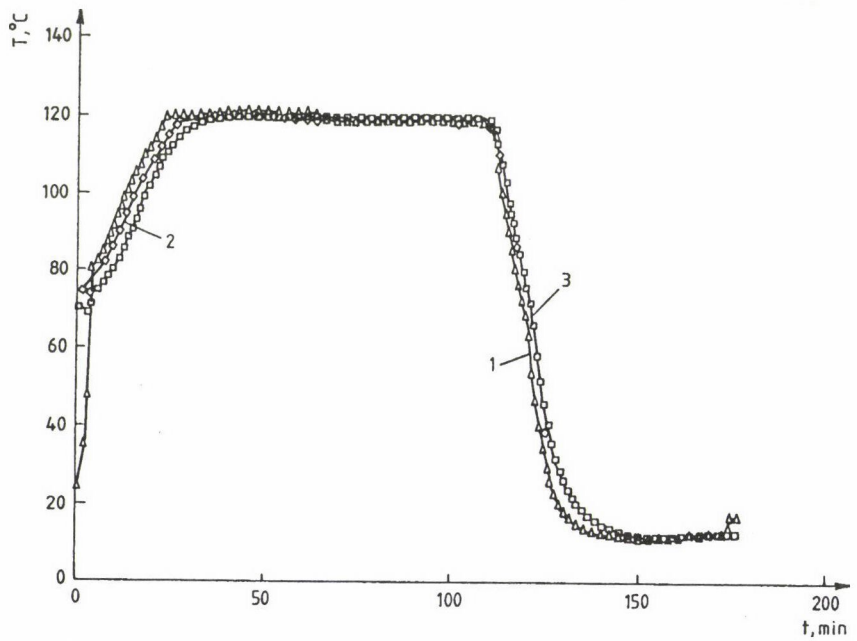

Fig. 4. Measured temperatures vs. time, Run 2. Markings and symbols are the same as for Fig. 2

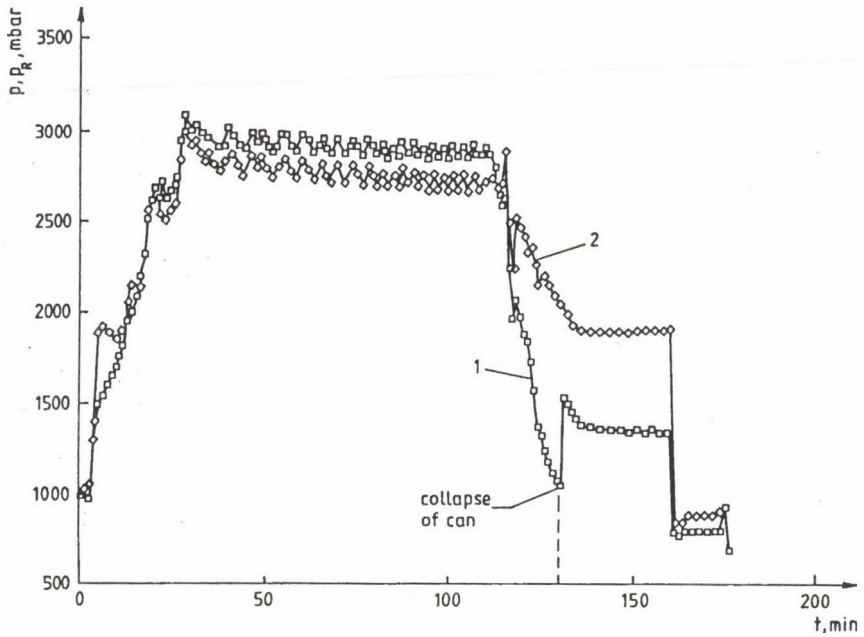

Fig. 5. Measured pressures vs. time, Run 2. Markings and symbols are the same as for Fig. 3

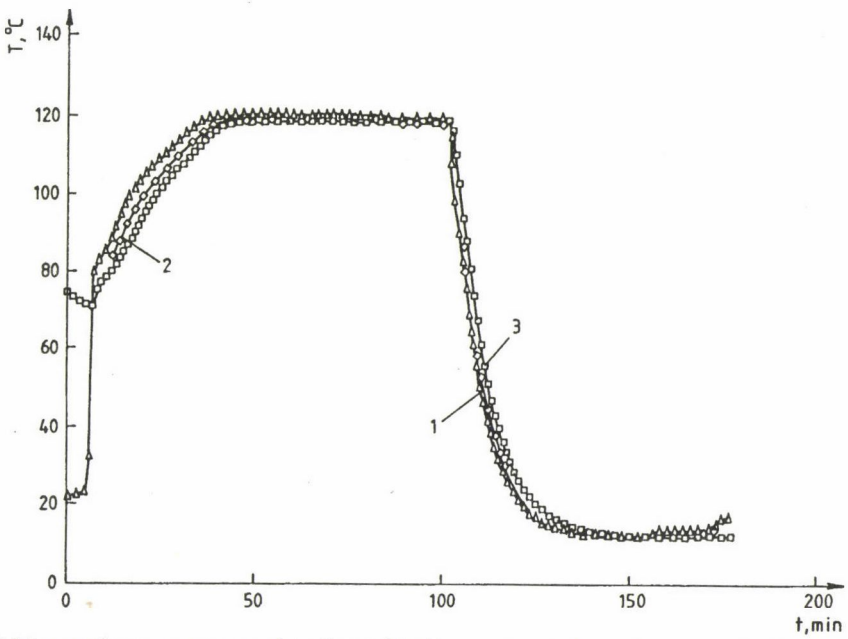

Fig. 6. Measured temperatures vs. time, Run 3. Markings and symbols are the same as for Figs 2 and 4

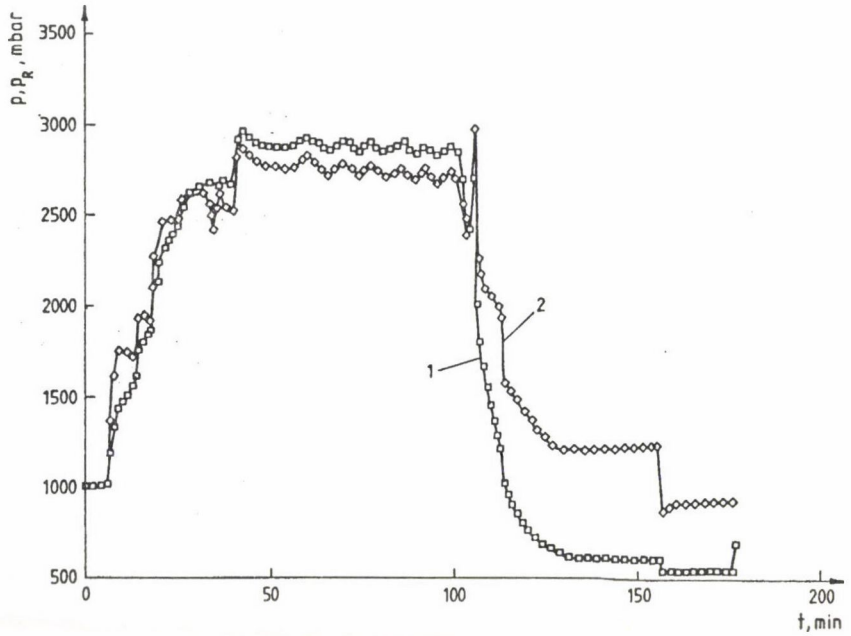

Fig. 7. Measured pressures vs. time, Run 3. Markings and symbols are the same as for Figs 3 and 5

3.2. Calculated results

Figure 8, Fig. 10 and Fig. 12 illustrate the calculated can wall temperatures (see para. 2.6.) and the simplified (linearized) time dependent variation of temperatures inside a can for the three experimental runs.

Figure 9, Fig. 11 and Fig. 13 illustrate the simplified time dependent variation of the measured inside pressure and the relating calculated one, moreover, the relating measured and calculated pressure differences for each run.

Results regarding mean differences of measured and calculated pressures (d_p, see para. 2.5.) have been presented in Table 2, together with the mean relative differences ($\overline{rdp}$, $\overline{rd\Delta p}$). The local values of $dr\Delta p$ at the maximum absolute values of measured pressure differences were also evaluated for each run (see para. 2.5. and Section 4).

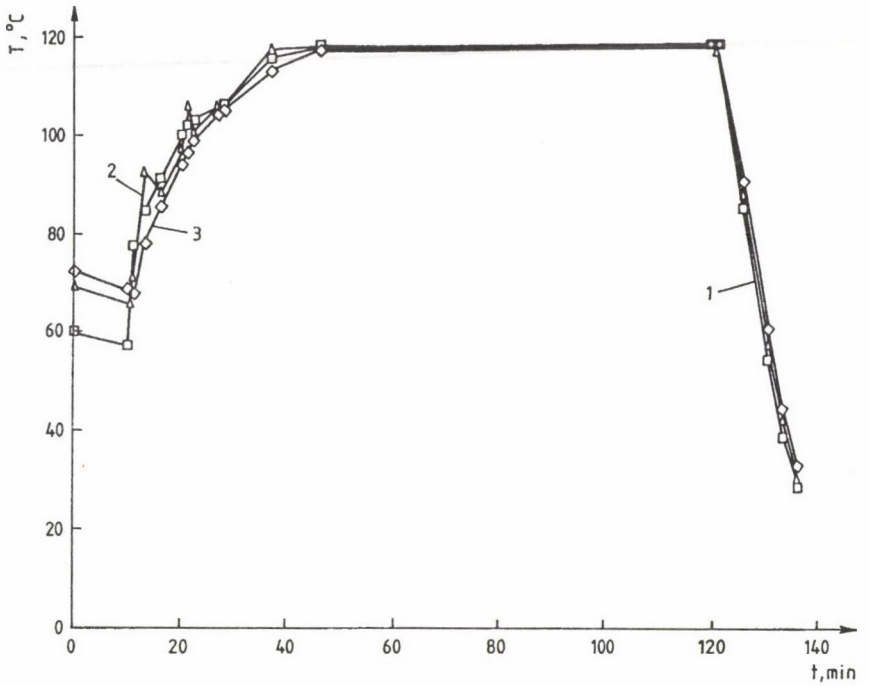

Fig. 8. Measured temperatures (T_G, T_m) and calculated wall temperatures (T_w) at the bounds of sections, Run 1. True variation is approximated by linear change inside a section. 1: wall temperature (T_w); 2: head space temperature (T_G); 3: fluid (water) temperature (T_m)

Checking of the time dependent finite difference method by a traditional trial and error method resulted in 1.10^{-4} to $1.5\,10^{-3}$ bar mean differences between calculated pressures. Dividing the previous values by the mean differences between measured and calculated pressures the answers are 0.29 to 2.5 per cent.

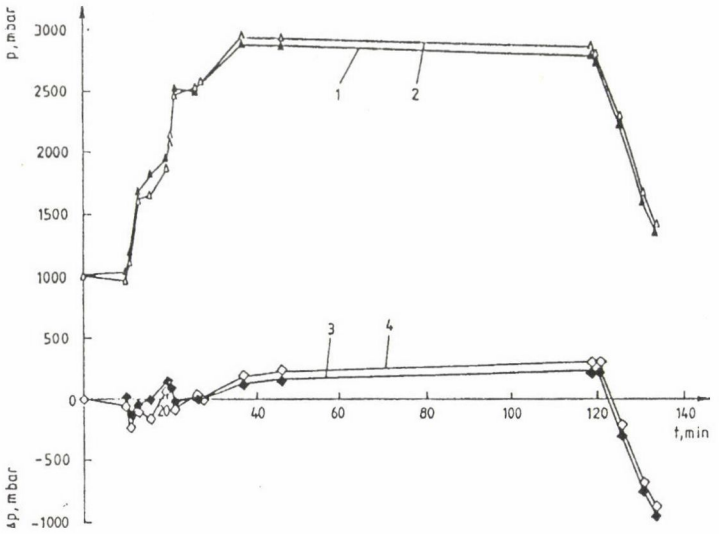

Fig. 9. Measured and calculated pressures and pressure differences at the bounds of sections, Run 1. True variation is approximated by linear change inside a section. 1: measured inside pressure (p_m); 2: calculated inside pressure (p_c); 3: measured pressure difference (Δp_m); 4: calculated pressure difference (Δp_c)

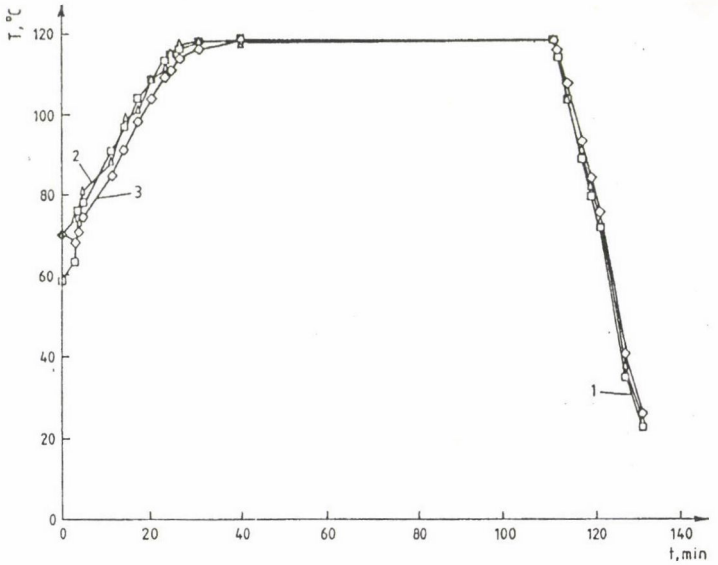

Fig. 10. Measured temperatures (T_G, T_m) and calculated wall temperatures (T_w) at the bounds of linear sections, Run 2. Markings and symbols are the same for Fig. 8

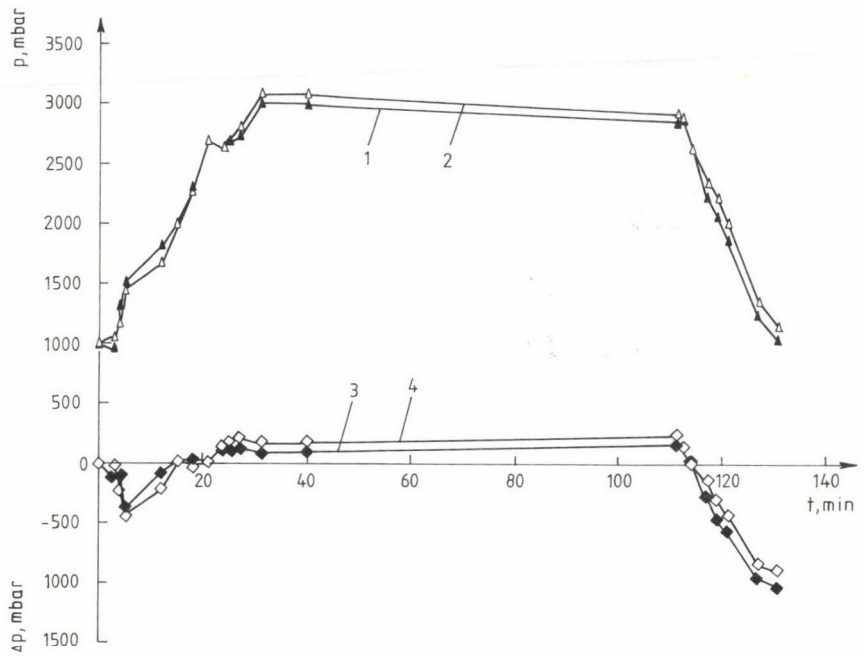

Fig. 11. Measured and calculated pressures and pressure differences at the bounds of sections, Run 2. Markings and symbols are the same as for Fig. 9

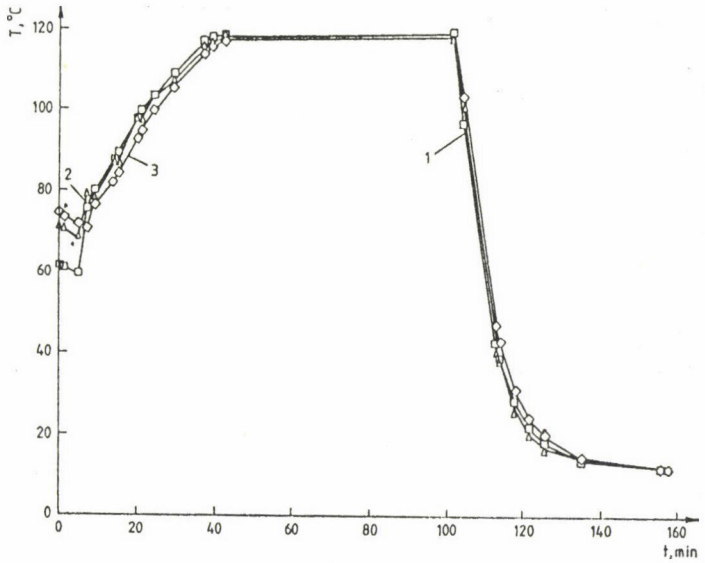

Fig. 12. Measured temperatures (T_G, T_m) and calculated wall temperatures (T_w) at the bounds of linear sections, Run 3. Markings and symbols are the same as for Figs 8 and 10

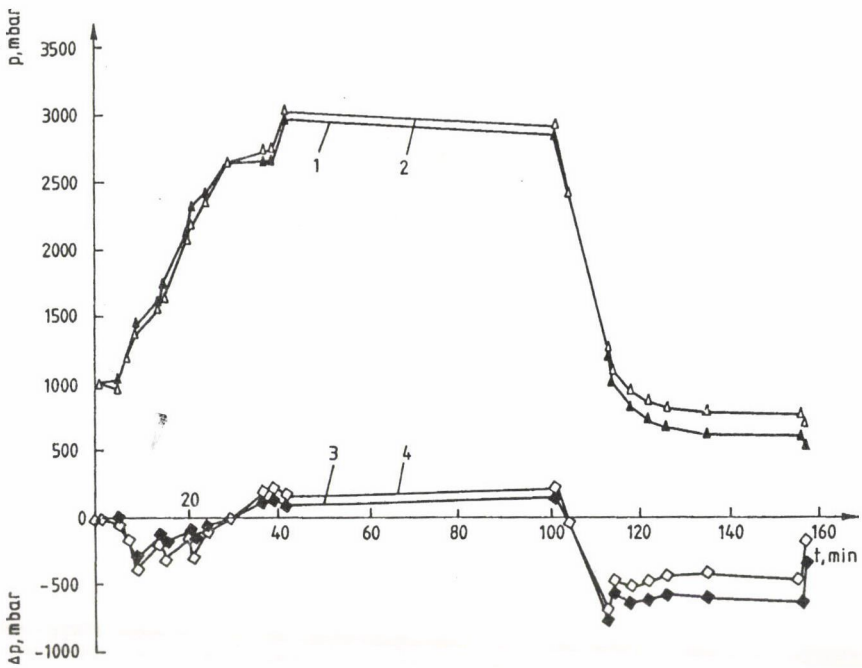

Fig. 13. Measured and calculated pressures and pressure differences at the bounds of sections, Run 3. Markings and symbols are the same as for Figs 9 and 11

4. Conclusions

Three experimental runs were made on water filled tinplate cans of V_0 = 3230 cm^3 volume in a horizontal retort. The runs imitated common sterilization process. Inside and outside pressures of cans and the appropriate temperatures were measured during the process. A calculation method was also elaborated using finite differences and stepping forward and backward with a pressure difference increment of $|\delta(\Delta p)| = 10^{-4}$ bar. The calculation method implies the previously published deformation vs. load relations (at constant temperature) together with the due interpolation formulas (KÖRMENDY et al., 1994). Volumetric thermal expansion of the tinplate can and its content, water vapour pressure and the partial pressure of air also contribute to the calculation method. Temperature dependent water vapour pressure was calculated by applying the Clapeyron equation.

Initial head space temperature was found by a trial and check method in each run. Its value was around 70 °C (see Table 1).

Measured and calculated pressures and pressure differences are presented in Figs. 9, 11 and 13.

Table 2 shows the main statistical results obtained from comparing measured and calculated data. The mean difference ($\overline{d_p}$) varied between 0.034 and 0.073 bar and was always less than 0.1 bar. The mean relative difference related to the measured inside pressure ($\overline{drp}$) varied between 2.37 and 8.02% and it can be considered quite favourable. The maximum of the absolute value of the difference of measured and calculated inside pressures varied between 0.0743 and 0.0753 bar for inside overpressure ($p > p_R$) and between 0.1546 and 0.1703 bar for outside overpressure ($p < p_R$). Positive sign in Table 2 indicates that the measured value was higher than the calculated one, while negative sign indicates the contrary.

Some considerations are needed regarding the values of $\overline{dr\Delta p}$. The rather high values (46.68, 51.95, 48.28%) can be contributed to the fact that measured differences $|p_m - p_R|$ were low during the greatest part of heat treatment time (see Figs 9, 11 and 13) and the predicted volume change (ΔV) is the most uncertain just in the zone around $\Delta p = 0$ (see $\Delta V = f(\Delta p)$ relations in the publications of KÖRMENDY and FERENCZY (1989); KÖRMENDY and co-workers (1994)). At the maximum absolute values of measured pressure differences ($p_m - p_R = -0.93$, -0.94 and -0.64 bar) the values of $dr\Delta p$ are 5.88, 11.70 and 17.3% for Runs 1, 2 and 3, respectively.

Results suggest that the interpolation equations in the publication of KÖRMENDY and co-workers (1994) might need some reconsideration. Moreover, the experimental method regarding the measurement of the parameters at closing the can needs some improvement in the future. The fact, that the initial head space temperature was determined by fitting, undoubtedly lessens the predictive power of the authors method. Sensitivity investigation, relating the variance of calculated pressure to input data variances with their partial weights, is expected only in the future. The inclusion of time dependency in the finite difference method did not effect results as demonstrated at the end of para 3.2.

Further questions remained for the future:

Should the water vapour tension be related only to the head space temperature or to some weighted average of the head space and liquid water temperatures? Has the thermal expansion of the can wall significant influence on the calculated results? Previous investigation on glass containers (KÖRMENDY et al., 1992) revealed, that the influence of cap displacement was much higher on calculational reliability than the influence of thermal expansion of glass (the volumetric thermal expansion coefficient of glass is about 55 to 70 percent of that of the tinplate).

Table 2

Comparison of measured and calculated inside pressures and pressure differences
(see para. 2.5.)

Serial No. of run	t_u (min)	$\overline{d_p}$ (bar)	$\overline{rdp}$ (%)	Maximum of $\lvert p_m+p_c \rvert$ in bar (a) Inside overpressure	Outside overpressure	$\overline{rd\Delta p}$ (%)
1	133	0.048	2.37	(+) 0.0753	(–) 0.1572	46.68
2	127	0.073	2.72	(+) 0.0751	(–) 0.1546	51.95
3	157	0.034	8.02	(+) 0.0743	(–) 0.1703	48.28

(a) Positive sign indicates $p_m > p_c$, negative signs indicate $p_m < p_c$

List of symbols

$f( ), F_1( ), F_2( )$	Functional relations (Eqn (3), Eqn (5), Eqn (6))
a_w	Water activity
$\overline{d_p}$	Mean difference between measured and calculated pressure (see para. 2.5., bar)
$\overline{drp}$	Mean relative difference of measured and calculated inside pressures as related to the measured inside pressure (see para. 2.5., bar)
$\overline{dr\Delta p}$	Mean relative difference of measured and calculated inside pressures as related to the pressure difference $p_m - p_R$ (see para. 2.5., bar)
$dr\Delta p$	The same as $\overline{dr\Delta p}$, but its local value
m_e	Mass of fluid content (food, water) in a can (kg)
m_G	Mass of gas (air) in a can (g)
M_G	Molar weight of gas (air, g mol^{-1})
p, p_c, p_m	Inside pressure in a can, its calculated or measured value respectively (bar, mbar, Pa)
p_G	Partial pressure of gas (air) in a can (bar, Pa)
p_R	Outside pressure (retort, atmospheric) of a can (bar, mbar, Pa)
p_w	Water vapour tension (bar, Pa)
R	Universal gas constant ($J.mol^{-1}.K^{-1}$)
t	Time (min, s)
t_u	Last sterilization time (min, s)
T	Temperature (°C, K)
T_G	Gas (air) temperature in a can (°C, K)
T_m	Mean temperature of fluid (food, water) in a can (°C, K)
T_0	Mean temperature of ΔV vs. Δp relation measurement (°C, K)
T_R	Retort (outside) temperature around a can (°C, K)
T_w	Mean temperature of can wall (°C, K)
V	Total volume of a can at T_0 when loading with Δp (m^3, cm^3)
V_e	Fluid (food, water) volume in a can (m^3, cm^3)
V_G	Gas (air) volume in can (m^3, cm^3)
V_0	Total volume of a can at T_0 in unloaded condition (m^3, cm^3)
Z	Symbol for the collective representation of all variants on the left-hand side of Eqn (5)
α_{vw}	Volumetric thermal expansion coefficient of the can wall (K^{-1})
$\delta(\Delta p)$	Elementary step (forward or backward) used for changing pressure difference, its absolute value being called as "increment" (bar, Pa)

δt	Elementary time interval due to $\delta(\Delta p)$ (min, s)
Δp	Pressure difference (see Eqn (4), bar, mbar, Pa)
$\Delta V = V - V_0$	Volume change caused by Δp reduced to temperature T_0 (cm^3, m^3)
ρ	Density of the fluid (food, water) in a can (kg m^{-3}, g dm^{-3})

Indices:

Subindex z refers to the conditions at the moment of closing the can. Subindices c and m refer to calculated and measured values for p, p_z and Δp

*

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Literature

CHARM, S. E. (1971): *Fundamentals of Food Engineering*. The AVI Publishing Company, Inc., Westport (Connecticut). pp. 558-563.

FERENCZY, I. (1986): Konzervdoboz deformációjának mérése és a hazai dobozgyártás fejlesztése. (Measurement of the deformation of cans and the development of can manufacture in Hungary.) Doctor's thesis. Kertészeti és Élelmiszeripari Egyetem (University of Horticulture and Food Industry), Budapest.

HEISS, R. (1980): Über den Druckverlauf beim Sterilisieren von Lebensmitteln. *Z. Lebensmittelunters. u. -Forsch.*, *31*(3), 117-120.

HEISS, R. & EICHNER, K. (1984): *Haltbarmachen von Lebensmitteln*. Springer-Verlag, Berlin, pp. 209-212.

KÖRMENDY, I. (1989): Konzervdobozok deformációja. Mérési és számítási módszerek. (Deformation of tinplate cans. Methods of measurement and calculation.) -in: SZENES, M. (Ed.) *Fém csomagolószerek*. (Metal containers for packaging purposes.) Műszaki Könyvkiadó, Budapest, pp. 146-156.

KÖRMENDY, I. & FERENCZY, I. (1989): Results of measurements of deformation versus load relations in tin cans. *Acta Alimentaria*, *18*, 333-351.

KÖRMENDY, I., ERDÉLYI, M., KONCZ, L., MÉSZÁROS, L., MONSPART-SÉNYI, J., PÁTKAI, GY. (1992): Az élelmiszertartósító hőkezelés területén a KÉE Konzervtechnológia Tanszékén elért eredmények, Part II. (Results in the field of preservation by heat treatment of food at the Department of Canning Technology of the University of Horticulture and Food Industry.) *Élelmezési Ipar*, *46*, 213-219.

KÖRMENDY, I., KONCZ, L. & SÁRKÖZI, I. (1994): Deformation versus load relations of tinplate cans. Interpolation between extreme loading cycles. *Acta Alimentaria*, *23*, 265-283.

PALMIERI, A., DIPOLLINA, G. & SQUITIERI, G. (1992): Studio dei fattori che influenzano le deformazioni dei contenitori metallici durate il trattamento termico. *Industria Conserve*, *67*, 434-440.

RAZNJEVIC, K. (1964): *Hőtechnikai táblázatok*. (Thermophysical charts.) Műszaki Könyvkiadó, Budapest.

RIGHI, H. (1955): Bestimmung der Wirkdrucke beim Sterilisieren von Dosenkonserven. -in: HEISS, R. & RIGHI, H. *Fortschritte in der Technologie des Konservierens von Gemüse und Obst*. Verlag Dr. Serger und Hempel, Braunschweig, pp. 167-214.

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INVESTIGATION OF GLIADIN CONTENT OF WHEAT FLOUR BY ELISA METHOD

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In our work the gliadin content of 9 Hungarian wheat varieties (Martonvásári) was investigated by sandwich ELISA method. The immunochemical method was satisfactory to measure the gliadin content since the wheat flour samples were extracted with different solvents and components that could disturb the reproducibility of ELISA test were exhaustively removed. The gliadin content of wheat varieties was determined by adopted ELISA test, the estimated quantity of gliadin was higher than that measured by gravimetry.

Keywords: gliadin, ELISA, wheat varieties, SDS PAGE

Distinction of gliadin content of wheat varieties can be important because the gliadin is part of the storage proteins and these proteins influence the baking quality.

The total level of gluten as well as its quality in various flours affect their suitability for use in different baked products (WRINGLEY & MCMASTER, 1989). Determination of gluten based on immundiffusion in gels is relatively of low sensitivity (KACZKOWSKI et al., 1985). Several authors have described sandwich ELISA for the detection of gluten in foods (WIDEMAN et al., 1982; TRONCONE et al., 1986; AYOB et al., 1988). These authors all have used polyclonal antibody with specificity for α-gliadin as one of the binding partners in the sandwich ELISA. Antibody specificity characteristic, together with alteration of the majority of antigenic determinants in gluten following heating (MEIER et al., 1984) resulted in the failure of these assays to yield quantitative results after foods have been baked or processed. The antibody is inappropriate when gives crossreaction and unsuitable for analysis of food potentially containing prolamins or tend to yield false positives with maize (TRONCONE et al., 1986). The only way of the quantitative estimation is to determine its content without destroying the protein structure in solvent (MILLS et al., 1989).

Our aim was to estimate the gliadin content of wheat varieties grown in Martonvásár. The storage protein of wheat was tested by ELISA method based on SKERRIT and HILL's work (1990).

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1. Materials and methods

The gliadin content of wheat flour was subtracted from 9 wheat varieties (Mv–4, Mv–9, Mv–10, Mv–15, Mv–16, Mv–14–85, Mv–8, Mv–107, Mv–21), given by the Faculty of Physical Science of Eötvös Loránd University, Budapest. Wheat grains of 15% moisture content were ground and sifted with a 0.25 mm sieve.

In our test 1 g wheat flour was mixed in 50% v/v propanol, or 70% v/v ethanol by stirring in water-bath at 37 °C, for 2 h. The stirring was continued at room temperature for 2 h. The solvent was removed by centrifugation and the pellet was discarded. The supernatant was mixed three times with 1.5% v/v NaCl, then centrifuged. The salt soluble proteins were removed. The precipitated protein was dissolved in 50% v/v propanol or 70% v/v ethanol dialysed exhaustively against 0.01% v/v acetic acid. Fractions containing total gliadin were purified from wheat flour (SHEWRY et al., 1983). The gliadin bands were separated by SDS PAGE (LAEMMLI, 1970). The polyclonal antibody to total gliadin was raised in rabbits. The animals were purchased from the Kisállattenyésztő Intézet in Gödöllő. The test animals were immunised both subcutaneously and intramusculary. The injections were given with total gliadin emulsified in Freund complete adjuvant (first injection) then with Freund incomplete adjuvant, all subsequent injections mixed with 0.01% v/v acetic acid. A final booster injection was given four days prior to sacrifice the animal. The ELISA microplates were purchased from the Sülysápi Műszeripari és Műanyagfeldolgozó GMK. All chemicals were ordered from the REANAL Company, Budapest.

The antibody was absorbed on to the microplates with the coating buffer consisting of 2.688 g $NaHCO_3$ and 1.908 g Na_2CO_3 diluted in 1000 cm^3 distillated water and adjusted to pH 9.6. For washing the plates, for diluting the samples and standards PBS puffer (8.0 g NaCl, 0.2 g KCl, 2.86 g $Na_2HPO_4 \cdot 2H_2O$, 0.27 g KH_2PO_4 in 1000 cm^3 distilled water, pH 7.4) was used. The washing buffer contained 1% v/v Tween 20 detergent. The anti rabbit – goat IgG–HRP conjugate was produced by NAKANE and KAWASI, 1974.

The antibody titration curves were obtained by measuring absorbance on the microtitration plates, which were coated with total gliadin diluted from a stock solution 1 mg cm^{-3} (in 70% ethanol) to 12 ng (1000 μg, 200 μg, 40 μg, 8 μg, 1600 ng, 64 ng, 12 ng). After incubation plates were washed three times with PBS containing Tween 20. The immune serum was diluted in PBS 80, 160, 320, 640, 1280, 2560, 5020 and 10040 times. The negative and a weak positive sera were diluted and measured the same way. After incubating and washing the plate wells the anti rabbit – goat IgG–HRP conjugate diluted 1:1500 v/v in PBS was added to each well, and incubated for 2 h, 37 °C. After a further 3-cycle washing 0.1 cm^3 3,3'5,5'-tetramethyl benzidine substrate (TMB) was added to each well and the plates were incubated for

15 min. The reaction was then stopped with 70 μl/well of 4 mol l^{-1} sulfuric acid. The absorbance of the wells was determined at 450 nm by Titertek Multiscan plate reader (Labsystem). The chequerboard ELISA was useful to optimize the conditions of antigen coating. Determining the titer of the serum we could check all the array controls.

Samples of wheat flours and other cereals for cross reactions (barley, maize, rice) were produced in the same way as the total gliadin. Estimation of gliadin content of wheat flours was carried out by the sandwich ELISA method. The microtitration plate wells were filled with 0.1 cm^3 of serum 1:200 v/v which was diluted from a stock solution in 0.05 mol l^{-1} Na-carbonate/bicarbonate buffer (pH 9.6) and incubated overnight at 4 °C. The plates were washed three times with PBS-Tween 20 using Titertek Microplate Washer (Labsystem). The gliadin standard was obtained, by addition of the gliadin diluted in PBS at 3000 ng, 1000 ng, 300 ng, 100 ng, 50 ng, 25 ng, 12.5 ng, 6.25 ng concentrations to the microplate. Samples of wheat varieties and other cereals were added to the wells in 50, 100, 300 times dilutions. The plates were dried, then HRP-enzyme was measured into the wells. After incubation for 2 h, at 37 °C, the plates were washed and TMB substrate was added according to the indirect ELISA. The gliadin content of samples was estimated on the basis of the calibration curve.

2. Results and discussion

In Table 1 the concentration values of gliadin in ng cm^{-3} are given according to the measured absorbance. The standard curve was established by computer (Fig. 1). The absorbance values were measured on different plates (Table 2) the sd values are low enough, the reproducibility is appropriate. In the regression analysis the independent variables were the gliadin concentrations. Using simple linear regression to develop linear model the calibration range were splited into two parts. The results of this model are not roboust because of the few measurement's data in these two ranges. Validation of the exponential model gave the best result for the prediction of data. The calibration equation form was $\ln Y = a+bx$; $a = 2.63$; $b = 5.09$; Fig. 1 shows the plot of fitted line of regression model. The fitting of the exponential model was verified by F-test as Table 3 explains.

Table 1

Standard deviation of gliadin standard curve values measured by sandwich ELISA method

Gliadin ($ng\ cm^{-3}$)	Absorbance on the plates (λ = 450 nm)				$\bar{x}$ n = 4	± sd
3000	1.086	1.082	1.094	1.119	1.095	0.016
1000	0.719	0.811	0.753	0.820	0.775	0.048
300	0.519	0.584	0.550	0.632	0.571	0.048
100	0.338	0.461	0.431	0.534	0.441	0.081
50	0.158	0.306	0.207	0.347	0.254	0.087
25	0.041	0.122	0.108	0.194	0.116	0.062

Table 2

Inter-assay of gliadin standards measured by sandwich ELISA method

Concentration of the gliadin ($\mu g\ cm^{-3}$)	Absorbance between the microplates				$\bar{x}$ n = 4	± sd
10	1.409	1.615	1.409	1.115	1.387	0.205
5	1.066	1.267	1.260	1.153	1.186	0.095
2.5	0.869	0.893	0.889	0.912	0.890	0.018
1.25	0.797	0.714	0.758	0.695	0.741	0.046
0.61	0.439	0.410	0.404	0.443	0.424	0.020
0.30	0.262	0.263	0.239	0.271	0.259	0.014
0.15	0.231	0.202	0.176	0.202	0.022	

Table 3

The result of regression analysis of exponential model

	Analysis of variance				
Source	Sum of squares	DF	Mean square	F ratio	Prob. level
Model	111.82	1	111.82	569.07	0.00
Error	5.50	28	0.196		
Total cor.	117.32	29			
Correl. coeff.	0.976				
Stnd error of est.	0.443				

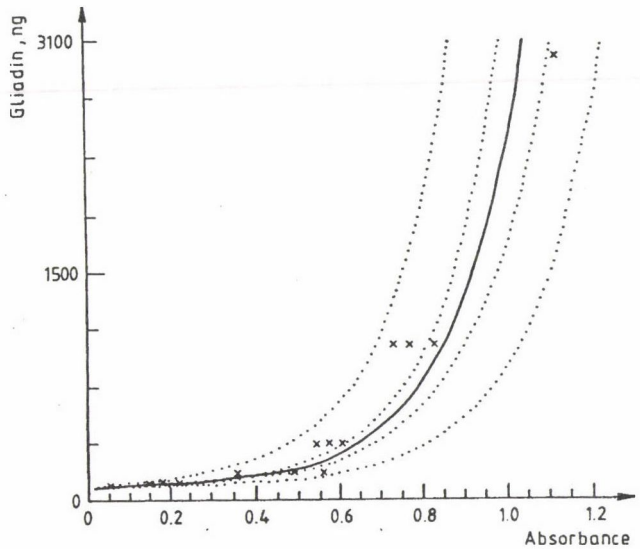

Fig. 1. The model of standard curves of sandwich ELISA method plotted by computer based on the average optical density values

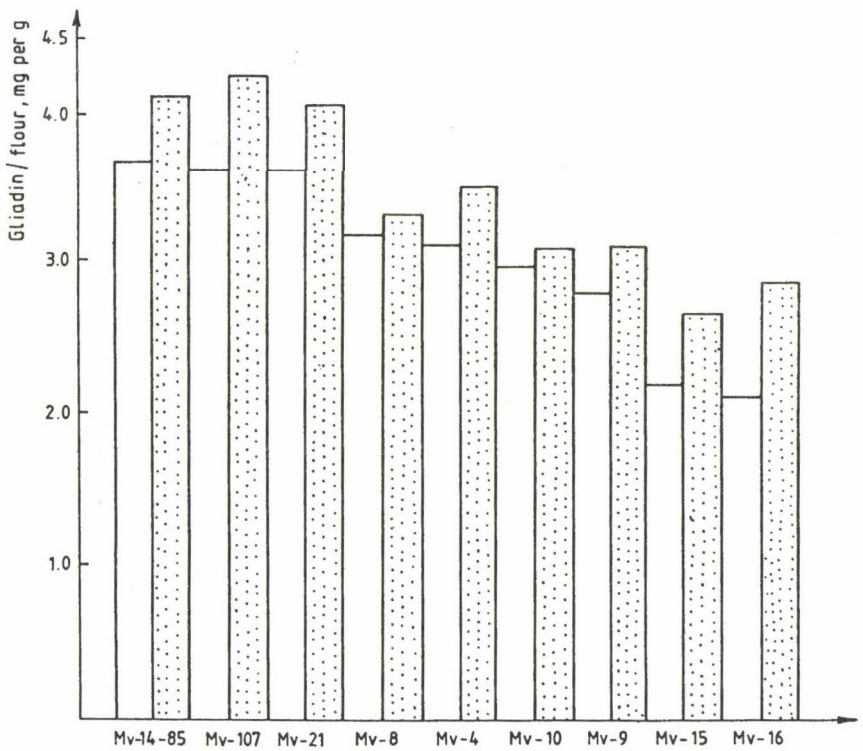

Fig. 2. The estimated quantity of 9 wheat varieties. ⊞: ELISA, □: gravimetry

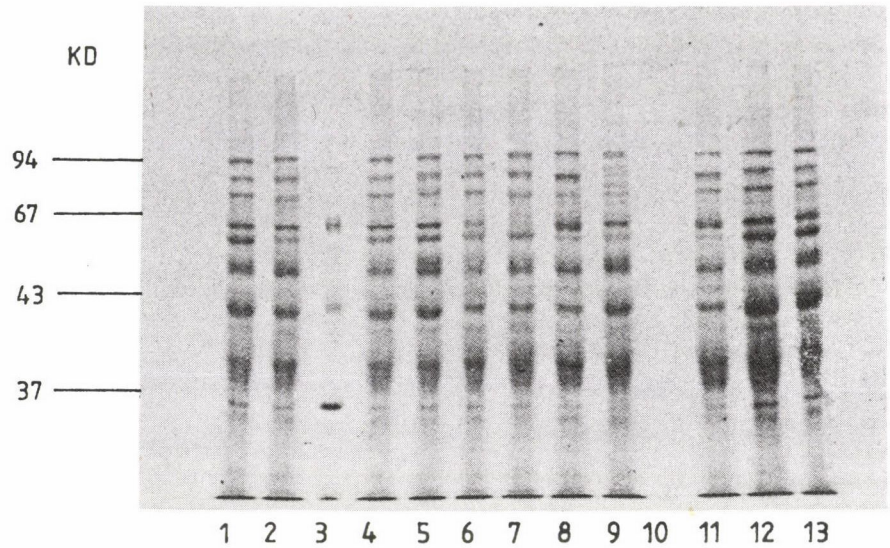

Fig. 3. Gliadin patterns of wheat separated by SDS PAGE varieties (from left) Mv-4, Mv-8, LMW standard Mv-9, Mv-10, Mv-15, Mv-16, Mv-14–85, Mv-21–85, Mv-107–86

The specificity of polyclonal immune-serum was characterized with common determinants of barley, maize and rice fluor. Our serum gave positive reaction with barley and maize. When the unheated wheat flours were tested the cross-reactions did not disturb the investigation. The estimated quantity of gliadin is summarized in Fig. 2. The quantity of gliadin measured by ELISA approximated the gravimetric measurements. The ELISA test was made in the 25–3000 ng range, it is too wide but that is not suprising since the polyclonal immune serum is not so sensitive as the monoclonal one. The affinity between the antigen and antibody may be stronger. The reliability is suitable which is based on the low sd values. Evaluating Fig. 3, it can be seen that the protein bands of gliadin might contain some glutenin contamination of high molecular weight fractions. The wheat flour samples were prepared by different solvents and the other components were exhaustively removed, so that they could not disturb the test. Inspite of efficient cleaning of the gliadin fractions of wheat flours there might be some contamination with other components (starch, lipid or other protein fraction glutenin) as some authors mentioned that before. These protein bands appeared in gel slab (SDS PAGE) in the high molecular weight fraction of gliadin which might be mixed with LMW (low molecular weight) fraction of glutenin. The ELISA test could not indicate that because of the homology of gluten and the exterior surface of gliadin and glutenin may be similar on the same place and gives the same immunochemical reaction.

3. Conclusion

The adopted ELISA method was suitable only for quantitative determination of prepared wheat flour samples (gliadin). When other components could not be removed, the gliadin sample was the simple ethanol solution of wheat flours, and the results were ambigous.

The well-prepared samples gave sufficient results in all case. In this adopted ELISA test the estimated gliadin content of wheat varieties was higher than that measured by gravimetry.

Literature

AYOB, M. K., RITTENBURG, J., ALLEN, J. C. & SMITH, C. J. (1988): Development of a rapid enzyme linked immunosorbent assay (ELISA) for gliadin determination in food. *Fd Hydrocolloids*, *2*, 39–49.

KACZKOWSKI, J., KUROESKA, E. & MOSKAL, M. (1985): Immunological method of wheat gluten detection in food products designed for celiac disease patients. *Acta Alimentaria Polon.*, *11*, 177–183.

LAEMMLI, U. K. (1970): Cleavage of structural proteins during the assembly of head of bacteriophage T_4. *Nature*, *227*, 680.

MEIER, P., WINDEMAN, H. & BAUMGARTNER, E. (1984): Determination of the α-gliadin content of heated food containing gluten or being "gluten free". *Z. Lebensmittelunters. u. -Forsch.*, *178*, 361–365.

MILLS, E. N. C., SPINKS, A. & MORGAN, M. R. A. (1989): A two-site Enzyme-linked immunosorbent assay for wheat gliadins. *Fd agric. Immunology*, *1*, 19–27.

NAKANE, P. K. & KAWASI, A. (1974): Peroxidase labeled antibody a new method of conjugation. *J. Histochem. Cytochem.*, *22*, 1084.

SHEWRY, P. R., MIFEIN, B. J., LEW, E. J. L. & KASARDA, D. D. (1983): The preparation and characterization of an aggregated gliadin fraction from wheat. *J. exp. Botany*, *34*, 1403–1413.

SKERRIT, J. H. & HILL, A. S. (1990): Monoclonal antibody sandwich enzyme immunoassays for determination of gluten of foods. *J. agric. Fd. Chem.*, *38*, 1771–1778.

TRONCONE, R., VITALE, M., DONATELLO, A., FARRIS, E., ROSSI, G. & AURICCHIO, S. (1986): A sandwich enzyme-immunoassay for wheat gliadin. *J. immunol. Methods*, *92*, 21–35.

WIDEMAN, H., FRITSHY, F. & BAUMGARTNER, E. (1982): Enzyme-linked immunosorbent assay for wheat for α-gliadin and whole gliadin. *Biochim. Biophys. Acta*, *709*, 110–121.

WRINGLEY, C. W. & MCMASTER, G. J. (1989): Wheat right for bread but much more too. – in: POMERANZ, Y. (Ed.) *Wheat is uniquex*. AACC, St. Paul, pp. 21–39.

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CORROSION OF IRON, TIN AND ALUMINIUM IN FRUIT JUICES

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The corrosion behaviour of iron, tin and aluminium, in different fruit juices (apple, cherry, lemon, orange, grapefruit, pineapple and maracuja) was investigated.

Potentiostatic polarization and Tafel extrapolation methods were used to determine corrosion parameters. Weight loss method was used in order to determine the corrosion rate. Tin, iron and aluminium contents in fruit juices after exposure of samples for juice action were determined by atomic absorption spectrophotometry. For comparison with literature data, the same metals well also analyzed in canned fruit juices after 12 and 18 months of storage.

The results obtained gave an insight into the aggressiveness of certain fruit juices, which depends not only on the composition but also on the storage conditions.

Keywords: aluminium, iron, tin, corrosion, electrochemical methods, fruit juices

In canned fruit juices, due to the aggressiveness of the product, corrosion of metal cans and contamination of juices with metal ions occur during transportation and storage (ARVANITOYANNIS, 1990; SADLER et al., 1992). Metal dissolution causes disagreeable flavour (so-called "metallic flavour") as well as other organoleptic changes and reduction of the nutritive value. The presence of metal ions mentioned above is permitted in a determined level but within two years of storage it might increase to a higher concentration dangerous to public health (SOUCI et al., 1981, EWAIDAH, 1992).

To get an insight into corrosion taking place in tinplate and aluminium cans, electrochemical behaviour of iron, tin and aluminium in different fruit juices has been investigated (UHLIG, 1977; NAGY & NICKDEL, 1986; WEBER, 1987).

Electrochemical measurements were carried out using tin, iron and aluminium electrodes. As test solutions various fruit juices, bought from the local shop, were used. Furthermore, the influence on metal content of imported fruit juices filled into cans and combined packaging materials, were studied during two years of storage.

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1. Materials and methods

The following fruit juices, packaged into "Tetra-brick" package were used as test media: apple juice (produced by "Vindija"), cherry juice (produced by "Takovo"), canned lemon, orange, grapefruit and pineapple juices (imported from Greece) and maracuja (in foil laminated packs imported from Austria).

Electrochemical measurements were carried out in a conventional electrolytic cell (UHLIG, 1977).

The following working electrodes were prepared:

- Tin electrode (99.995% purity); exposed area of 0.639 cm^2.
- Iron electrode (99.792% purity); exposed area of 0.729 cm^2.
- Aluminium electrode (99.750% purity); exposed area of 1.00 cm^2.

The active electrode area was ensured with an epoxy resin coating.

Smooth platinum foil (1.28 cm^2) was used as counter electrode, while saturated calomel electrode (SCE) was used as reference electrode.

Potentiostatic polarization data were obtained using the Wenking LB 75M potentiostat and Wenking SMP 72 potentiometer. The potential of the working electrodes were also checked on digital voltmeter (Digimer 30, "Iskra", Kranj).

From the polarization curves, using Tafel's extrapolation method, corrosion current density and corrosion potential (corrected for the standard hydrogen electrode potential – SHE) were determinated. The scan rate used 40 mV min^{-1}.

pH values of the test media were determined on pH-meter (Radiometer, M22, Copenhagen), and the total acidity was determined by potentiometric titration (POMERANTZ & MELOAN, 1971).

Weight loss method was performed in order to estimate the corrosion rate.

The quantities of Fe, Sn and Al in juices were estimated by AAS method after 28 days of samples exposure to fruit juices at 277 K (4 °C). The juices were ashed at 550 °C (823 K) and stabilized till constant mass and than wet digested. The solutions were diluted with hydrochloric acid to the final volume (25 cm^3).

Atomic absorption spectrophotometry (Varian Techtron Model 1200) at wavelengths of 235.4 nm, 248.3 nm and 309.3 nm was used to determine the amounts of dissolved tin, iron and aluminium, respectively.

Series of dilutes standards of metals were used for the calibration curves.

2. Results and discussion

The results obtained representing the average of six repeated measurements from three series are presented in Tables 1–4 and Fig. 1.

As it can be seen from the results (Table 1) the pH values of the investigated juices were between 2.10 ± 0.15 and 3.70 ± 0.20. A good accordance between pH and

total acidity was observed, i.e. the higher the pH value the lower the total acidity. Thus maracuja, apple and pineapple juices showed lower total acidity and higher pH values, while in case of lemon and orange juice the higher total acidity content resulted in lower pH values. The exception was noticed for grapefruit having pH of 3.70 ± 0.20 and total acidity of $(0.78 \pm 0.10)\%$. For cherry juice the values of 2.90 ± 0.10 and $(0.99 \pm 0.1)\%$ were obtained for pH and total acidity, respectively. These values were close to an average of the results obtained (i.e. pH 3 and 1% of total acidity), what corresponds to moderately corrosive media.

Table 1

pH and total acidity values of fruit juices

Fruit juices	Prevailing organic acid	pH	Total acidity (%)
Apple	Malic	3.15 ± 0.10	0.71 ± 0.12
Cherry	Malic	2.90 ± 0.10	0.99 ± 0.14
Lemon	Citric	2.10 ± 0.15	1.16 ± 0.11
Orange	Citric	2.24 ± 0.10	1.05 ± 0.05
Grapefruit	Malic	3.70 ± 0.20	0.78 ± 0.10
Pineapple	Malic	3.70 ± 0.15	0.78 ± 0.11
Maracuja	Malic	3.12 ± 0.05	0.70 ± 0.12

Average values of three series of six samples and standard deviation ($\pm$)

Tin polarization curves, obtained for apple, orange, pineapple and cherry juices as well as iron and aluminium curves in apple and orange juices, respectively, are shown in Fig. 1.

The shape of anodic polarization curve for iron, in apple juice as well as in malic and citric acid (BERKOVIĆ et al., 1992), shows uniform metal dissolution through entire range of the potential applied. Such a behaviour is typical for iron corrosion at pH below 4.5 (DELTOMBE et al., 1966). Simultaneously, as it can be seen from cathodic curve, cathodic depolarization reaction occurs as well.

Anodic polarization curves for tin shows the range of passive film formation at positive potential values. This is especially evident in apple juice and malic acid (BERKOVIĆ et al., 1992) at the potential value of 350 mV. At higher potential values, depending on type of juice analyzed, a limiting current density value is reached (POURBAIX & DE ZOUBOV, 1966; HURLEN et al., 1984; GIANNETTI et al., 1990).

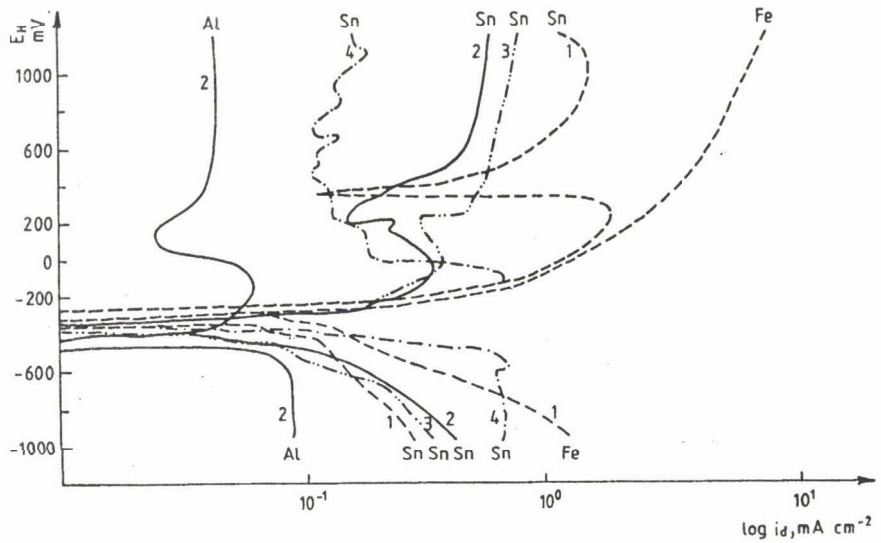

Fig. 1. Anodic and cathodic polarization curves of Sn, Al and Fe electrodes in fruit juices. 1: apple; 2: orange; 3: pineapple; 4: cherry

In case of cherry juice the limiting current density varies with potential which suggests that both formation and dissolution of a film occurs at the same time (BARD, 1978; YOUNG, 1961; WILEY, 1972; BERKOVIĆ et al., 1992). Anodic dissolution, producing bivalent tin, takes place at the potential above – 0.136 V forming protective hydroxide type films (GIANNETTI et al., 1990; BARD, 1978). Depending on prevailing fruit acid, participation of other ions in such reactions is also possible (HURLEN et al., 1984; GIANNETTI et al., 1990; BARD, 1978; YOUNG, 1961; WILEY, 1972; BERKOVIĆ et al., 1992).

At low pH value aluminium dissolve as Al^{3+} (a predomination limit of Al^{3+}/AlO_2 being at pH 5.07). Dissolved Al^{3+} ions may be involved in the formation of aluminium hydroxide as well as in the formation of various cathionic and anionic hydrocomplexes (NAGY & NICKDEL, 1986; DELTOMBE et al., 1966).

In Table 2 corrosion potential and corrosion current values are shown for the investigated samples. The results show the complexity of investigated media due not only to the presence of prevailing acid but also to its concentration. Furthermore, these parameters also depend on fruit maturity, type of fruit, processing technology, presence of additives (ascorbic acid) and final treatment of juices (ARVANITOYANNIS, 1990; SOUCI et al., 1981). The amount of acids as well as their ratio give total acidity of particular juice. Based on different degree of dissociation their influence on pH as on corrosion current/potential is also evident.

Table 2

Corrosion potential and corrosion current values of Sn, Fe and Al electrodes in fruit juices

Fruit juices	$-E_{corr.}$/mV			$i_{corr.}$/mA cm^{-2}		
	Sn	Fe	Al	Sn	Fe	Al
Apple	363	351	528	1.43	12.5	0.228
Cherry	272	334	512	0.295	10.8	0.342
Lemon	208	235	510	11.5	11.4	0.285
Orange	256	280	548	4.22	32.0	0.197
Grapefruit	331	335	603	1.78	6.77	0.378
Pineapple	372	325	585	1.16	8.9	0.156
Maracuja	324	311	538	1.36	3.42	0.024

Corrosion potential of aluminium are by about 200–250 mV more negative compared with those of tin and iron (−510 and −603 mV, respectively), while corrosion current values are very low (from 0.15 to 0.34 mA cm^{-2}). The lowest corrosion current for tin is recorded in cherry juice (0.296 mA cm^{-2}) and the highest in lemon juice. The highest corrosion current value (32 mA cm^{-2}) is obtained for iron electrode in orange juice. Apart from lemon juice, where corrosion currents for both, tin and iron, are identical (about 11 mA cm^{-2}) iron electrode shows much greater corrosion values (3 to 10 times greater). Corrosion current values, considering the investigated electrodes, increase in order as follows: aluminium, tin, iron, generally showing good agreement with pH and total acidity values.

Corrosion rates calculated on the basis of polarization measurements are in good agreement with those obtained directly from the weight loss method (Table 3). Lemon juice, with the lowest pH value, shows the highest corrosion. The amount of dissolved metals, after 28 days, agrees with the calculated corrosion rates.

Table 3

Corrosion rate and metal content data in fruit juices after 28 days of samples exposure

Fruit juices	Corrosion rate (mg cm^{-2} day^{-1})			Metal content (mg kg^{-1})		
	Sn	Fe	Al	Sn	Fe	Al
Lemon	21.28±1.8	22.81±4.5	0.428±0.11	7.52±1.6	10.92 ±7.1	0.42±0.11
Apple	16.55±2.8	18.35±8.2	0.023±0.01	10.10±2.5	12.058±7.2	0.02±0.01
Cherry	15.97±4.7	11.06±2.2	0.322±0.03	5.40±4.1	9.66 ±6.8	0.28±0.13

Average values and standard deviations of 30 samples

The average values and standard deviation of results of iron, tin and aluminium determination in fresh juices as well as in those stored for 12 and 18 months are represented in Table 4. According to these results it is obvious that packaging materials provide no satisfactory protection, especially for the lemon juice in tinplate cans, what was the case on our market with imported cans from Greece (SLUŽBENI LIST SFRJ, 1983).

Table 4

Metal content in fruit juices before and after storage

Fruit juices	Fresh juices (mg kg^{-1})			After storage (mg kg^{-1})		
	Sn	Fe	Al	Sn	Fe	Al
Lemon[a]	0.06±0.01	0.192±0.02	–	183±3.8	39±2.1	–
Pineapple[a]	0.08±0.01	0.122±0.018	–	59±14.2	13±6.6	–
Maracuja[b]	–	–	0.19±0.01	–	–	2±0.45

Average values from 30 samples and standard deviation (±)

[a] Storage time 12 months
[b] Storage time 18 months

3. Conclusion

The results obtained show that the investigated juice samples are moderately corrosive to Sn, Fe and Al. The shape of anodic curves shows passive film formation because the presence of carbohydrate and pectine inhibited corrosion processes on Al and Sn electrode, up to certain potential value, after which protective film dissolved. Some samples showed limiting current density. Iron continuously dissolved through the entire range of applied potential.

The composition of juice is complex and varies according to different factors, i.e. maturity index (acid: sugar ratio), climate conditions, soil composition, fertilizers and plant protection materials applied. The quality depends on the ratio of a particular fruit acid which reflect on pH and total acidity, the main causes of product corrosivity. Organic acids present in juices form complexes with metals also participate in corrosion reactions. In 100 g of juices mineral materials were also present, iron in the range of 0.2–0.38 mg (MCCANCE & WIDDOWSON, 1991; BELITZ & GROSCH, 1987), and 0.1–0.2 mg, respectively, carotene (17 mg) and some other materials which exert effects on corrosion parameters in the investigated juices. This effect is enhanced in the presence of oxygen.

The results also show that metal corrosion decreases in the following order: Fe > Sn > Al.

The most corrosive medium is lemon juice, while maracuja juice shows the lowest corrosivity.

According to metal concentration packaging materials provide no satisfactory corrosion protection after 12 and 18 months of storage.

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Literature

ARVANITOYANNIS, I. (1990): The effect of storage of canned juices on content of metals Fe, Cu, Zn, Pb, Sn, Al, Cd, Sb and Ni. *Nahrung, 34*, 141-145.

BARD, A. J. (1978): *Encyclopedia of electrochemistry of the elements*. Marcel Dekker, Inc., New York-Basel, p. 223.

BELITZ, H. D. & GROSCH, W. (1987): *Food chemistry*, 2nd ed., Springer-Verlag, Berlin, pp. 236-239.

BERKOVIĆ, K., CIKOVIĆ, N., GALIĆ, K. & PAVIĆ, M. (1992): Korozija kositra u vocnim sokovima. (Corrosion of tin in fruit juices.) *Proceedings of 10th Conference on Materials Protection and Industrial Finishing*. Zagreb, pp. 11-14.

DELTOMBE, E., VANLEUGENHAGNE, C. & POURBAIX, M. (1966): RT 4^2 CEBELCOR *Atlas of electrochemical equilibria in aqueous solutions*. Pergamon Press, Oxford, p. 168.

EWAIDAH, E. H. (1992): Studies on commercially canned juices produced locally in Saudi Arabia: Physiochemical, organoleptic and microbiological assessment. *Fd Chem., 44*, 103-111.

GIANNETTI, B. F., SUMODJO, P. T. A. & RABOCKAI, T. (1990): *J. appl. Electrochem., 20*, 672-676.

HURLEN, T., LIAN, H., ODEGARD, O. S. & VALAND, T. (1984): Corrosion and passive behaviour of aluminium in weakly acid solution. *Electochim. Acta, 29*, 579-585.

NAGY, S. & NICKDEL, S. (1986): Tin, iron and aluminium contents of commercially canned singlestrength grapefruit juice stored at varying temperatures. *J. agric. Fd. Chem., 34*, 588-593.

MCCANCE, R. A. & WIDDOWSON, E. M. (1991): *The composition of foods*. (5th rev. and ext. ed.), Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food, Richard Clay Ltd., Bungay, Suffolk.

POMERANTZ, Y. & MELOAN, C. E. (1971): Food analysis: Theory and practice. The Avi Publishing Company, Westport, Connecticut, pp. 145, 524.

POURBAIX, M. & DE ZOUBOV, N. (1966): RT 4^2 CEBELCOR *Atlas of electrochemical equilibria in aqueous solutions*. Pergamon Press, Oxford, p. 307.

SADLER, G. D., PARISH, M. E. & WICKER, L. (1992): Microbial, enzymatic and chemical changes during storage of fresh and processed orange juice. *J. Fd Sci., 57*, 1187-1191.

SLUŽBENI LIST SFRJ (1983): Pravilnik o kolicinama pesticida i drugih otrovnih tvari, hormona, antibiotika i mikotoksina koji se mogu nalaziti u ziveznim namirnicama 59, 1634. 13. studenoga. (Official paper SFRJ. Regulation of quantities of pesticide and other poisonous materials, hormones, antibiotics and mikotoxins which are contained in food, No. 59, 1634, 13th November.)

SOUCI, S. W., FACHMANN, W. & KRAUT, H. (1981): *Food composition and nutrition tables.* Wissenschaftliche Verlagsgesellschaft, Stuttgart, pp. 1002–1004.

UHLIG, H. (1977): *Corrosion and corrosion control.* S. Wiley & Sons, Inc., New York, McGraw Hill, pp. 323–329.

WEBER, G. (1987): Speciation of tin in lemon juice: An example of trace metal speciation in food. *Analytical chim. Acta, 200,* 79–80.

WILEY, A. R. (1972): Effect of tin ion complexing substances on the relative potentials of tin, steel and tin-iron alloy in pure acid and food media. *Br. Corros. J., 7,* 29–35.

YOUNG, L. (1961): *Anodic oxide films.* Academic Press, New York, pp. 193–210.

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METABOLISM OF OCTACOSANOL IN LIVER AND MUSCLE OF RAT

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Following oral administration into rats of ^{14}C-octacosanol, it was found that uptake of radioactivity in the liver and muscle was very low (0.08% of administered dose in liver; 0.26% in muscle) during early hours of the administration (30 min). The uptake in the two tissues increased markedly during the next 4–6 h in the liver (2.6–2.7% of the dose) and at 8 h in the muscle (1.8% of the dose). The radioactivity in these two tissues then disappeared sharply and reached a level of about 1% each in both the tissues at 24 h after the administration. The radioactivity in all other tissues examined (kidney, spleen, heart, lung and brain) was insignificant (less than 0.05% in each) at any time during this period. Of the radioactivity incorporated into liver at 4 h, 36.2% was recovered in the sterol fraction, 19.5% in the phospholipid fraction and only 3.4% in the triglyceride fraction. This indicated conversion of the fatty alcohol into a fatty acid followed by incorporation into phospholipids and sterols. Conversion of the fatty alcohols into an acid was also indicated from the detection of radioactivity in the expired CO_2 (2.3% of absorbed radioactivity) at this time period. The results are discussed in the light of the role of octacosanol in increasing muscle endurance in exercise.

Keywords: liver, metabolism, muscle, ^{14}C-octacosanol

In recent years, much interest has grown in the finding from many laboratories that physical motor endurance increases after octacosanol feeding (PASSWATER, 1982; KATAHIRA & SHICHIGO, 1984; SAINT-JOHN & MCNAUGHTON, 1986). Octacosanol, a long chain aliphatic alcohol [$CH_3(CH_2)_{26}CH_2OH$] that can be obtained from various foods, and such alcohols ingested as wax esters with some food can be adjusted to a suitable composition via fatty acid which may undergo various metabolic alterations. It is a substance which has been 'touted' as an ergogenic aid in many of gymnasiums and health clubs around the world and is being marketed as a sport supplement (CURETON, 1957). This was confirmed by a swimming endurance test on mice fed octacosanol extracted from sugar cane (SHIMURA et al., 1987). It

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was also found in the rat that the amount of voluntary exercise was significantly increased by supplementation of octacosanol in the diet (KABIR & KIMURA, 1991). It was earlier shown in a series of scientifically controlled experiments that some aspects of athletic performances such as increased stamina, endurance and vigor in health volunteers are improved upon feeding wheat germ oil (which is rich in octacosanol) in combination with physical training (CURETON, 1972). Basal metabolism, oxygen utilization reaction time and nerve function are also shown to be significantly improved (CURETON, 1972).

Mainly two lines of research have been pursued with octacosanol: its energy enhancing properties and its role in nerve physiology. However, no information is as yet available on the biochemical mechanism of the action of octacosanol. It would therefore be of interest to examine whether or not octacosanol is localized in the brain and fat tissues after oral administration. We have reported the biodistribution and metabolism of octacosanol after 7 days of a single dose administration in rats (KABIR & KIMURA, 1993). The present paper deals with some interesting findings on the biodistribution and metabolism of ^{14}C-octacosanol in the liver and muscle of young rats after short periods of administration.

1. Materials and methods

1.1. Preparation of administered [8-^{14}C]-octacosanol solution

One mCi(37 MBq) of [8-^{14}C]-octacosanol (specific activity 19.6 mCi/mmol, 726 MBq/mmol, Radiochemical Center, Amersham, UK) in a vial was diluted in 5.0 cm^3 tricaproylglycerol, so that about 200 μCi(7.4 MBq) were contained in 1.0 cm^3. Then some portion of that was diluted again in a fat mixture for oral administration, so that 2.0 μCi(0.074 MBq) were contained in 0.5 cm^3. The composition of the mixture was: cold octacosanol (Maypro Industries, Inc. New York), 100 mg; soybean lecitin, 4 g; stearoyl monoglycerol, 1 g; soybean oil, 2.5 g; and tricaproylglycerol, 2.5 g.

1.2. Experimental animals

Four week-old, male Wistar rats (purchased from Funabashi Farm, Japan) weighing about 65 g, were given [8-^{14}C]-octacosanol orally through a stomach tube and sacrificed at various time. The animals were acclimatized for at least 5 days on a stock laboratory diet before the start of the experiments. Food was removed from the cages of rats at 12 h before the administration of octacosanol. Three animals were killed each at 0.5, 1, 2, 4, 6, 8, 10, and 24 h after an administration of 2 μCi[8-^{14}C]--octacosanol in 0.5 cm^3 solution. All rats were killed by ethyl ether anesthesia and

blood was collected from the abdominal aorta in a disposable plastic syringe coated with heparin. Blood was centrifuged at 3000 r.p.m. for 15 min, and the plasma was stored in a vial at 0 °C for later analysis. The rats were then rapidly dissected.

1.3. Extraction and analysis

Liver, kidneys, spleen, heart, lungs, brain, gastrointestinal tract and a portion of leg muscles were quickly removed and washed in cold 0.9% NaCl. After blotting with a filter paper, their weights were recorded. The samples were homogenized in 10 volumes of chloroform-methanol (2:1, v/v) in a Potter-Elvehjem homogenizer. The homogenate was then filtered through Advantec No. 2 filter paper with washing of solvent. The chloroform-methanol extract was diluted to a known volume and a portion was taken in a counting vial and the radioactivity of the extract measured on an Aloka LSC–903 liquid scintillation counter (Packard Instrument Co., Illinois, USA) using toluene scintillator (PATTERSON & GREEN, 1965). The counts were corrected for quenching with external standards and computation was carried out using a digital computer.

1.4. Extraction of liver total lipid

The liver parameters were examined at 4 h. Total lipid was extracted from the liver tissue by the double extraction method of SUZUKI (1964), which is a modification of the original method of FOLCH and co-workers (1957). The liver was homogenized in 10 vol of chloroform-methanol (2:1, v/v) and the homogenate filtered through Advantec No. 2 filter paper, with repeated washing with chloroform--methanol (1:2, v/v) containing 5% distilled water. The two extracts were combined and chloroform was added to make the chloroform-methanol composition approx. 2:1 (v/v). The extract was washed overnight with 0.2 volume of 0.88% KCl. The upper phase was discarded and the interface was washed with few ml of fresh theoretical upper phase (FOLCH et al., 1957) which is chloroform-methanol-0.88% KCl (3:48:47, v/v) and then the upper phase containing salt was also discarded. The washed lower phase of the total lipid extract was used for TLC.

1.5. Fractionation of liver total lipids by thin-layer chromatography

The total lipid extract was evaporated under reduced pressure in a rotary flask evaporator and a small aliqout was taken in a test tube and evaporated to dryness under nitrogen. Then a known volume (0.5 cm^3) of hexane was added and spotted with standard lipids on a TLC plate coated with a 0.5 mm thick layer of Wako gel

B–0 (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The plates were developed with petroleum ether : diethyl ether : acetic acid (85:15:1, by vol).

1.6. Collection of expired $^{14}CO_2$ and excreta

The analytical procedure for this parameter was the same as reported in details previously (KABIR & KIMURA, 1993), except that instead of ACS–II counting scintillator, toluene scintillation counting solution was used and only 24 h samples were collected. In brief, soon after the administration of ^{14}C-octacosanol the animals were kept individually in a metabolic glass cage designed to trap expired CO_2 and to collect the excreta. Urine and feces were collected separately. The $^{14}CO_2$ expired was trapped for 24 h in large test tubes containing 1 *N* NaOH arranged serially. The radioactivity in NaOH solution was counted as described previously after neutralizing NaOH solution by adding an appropriate amount of HCl to the vial.

1.7. Extraction and analysis of radioactivity in feces and urine

These were done according to procedures reported previously (KABIR & KIMURA, 1993), except that instead of ACS–II counting scintillator, toluene scintillation counting solution was used and only 24 h samples were collected.

2. Results

Radioactivity from octacosanol in all tissues except for liver and muscle was insignificant at any time during the 24 h after a single oral administration. During this time, the highest amount of radioactivity was found in the liver, followed by muscle (Fig. 1). However, the radioactivity in the liver and muscle was very low (0.08% of administered dose in liver, 0.26% in muscle) during early hours of the administration (30 min). Subsequently there was a striking accumulation of radioactivity in the liver which peaked at 4–6 h after administration. About 2.6% of the administered dose and 25.6% of the absorbed radioactivity was present in the liver at that time. But within 24 h, the radioactivity disappeared to a level less than 40% of the maximum peak (Fig. 1). At the time when the radioactivity in the liver decreased, that in the muscle increased and reached a peak at 8 h after administration. About 1.8% of administered dose and 32.3% of the absorbed dose was present in the muscle at this time. The radioactivity in muscle then decreased. This implied that ^{14}C-octacosanol was possibly first taken up by the liver and then either remained intact or a metabolic product of octacosanol reached the muscle.

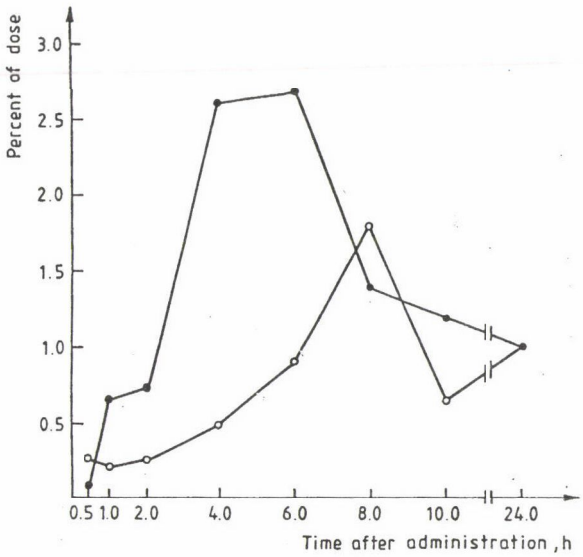

Fig. 1. Radioactivity in liver and muscle of rats at various time after administration of ^{14}C-octacosanol. Muscle radioactivity was calculated from an estimated muscle mass equal to 45% of body weight. ●—● : liver; o—o : muscle

To investigate in what from the radioactivity of ^{14}C-octacosanol was present in liver, we analyzed the lipids extracted from liver by TLC. It was found that radioactivity at 4 h after administration was mainly present in the sterol esters (36.2%) and phospholipid fractions (19.5%) as well as in free octacosanol (30.5%), but very little in the triglyceride fraction (3.4%) (Fig. 2). This indicated that octacosanol was converted into a carboxylic acid, which was then incorporated in sterol esters and phospholipids. The significance of this esterification is not clear.

The conversion of octacosanol into a carboxylic acid is also indicated from the recovery of radioactivity in the expired carbon dioxide. The expiration of $^{14}CO_2$ was at a maximum at 4–6 h (Fig. 3). After that time the expiration of $^{14}CO_2$ decreased and then remained constant. Only a small percentage of ^{14}C-octacosanol dose was expired as $^{14}CO_2$ (2.3%) within 24 h, which is about 22% of the accumulated radioactivity in the liver at the same period. About 24% of the administered dose was excreted in the feces and a small amount through urine over a period of 24 h (Fig. 4). As shown in Fig. 4, the total excretion of radioactivity through three different pathways within 24 h was about 27% of the administered dose.

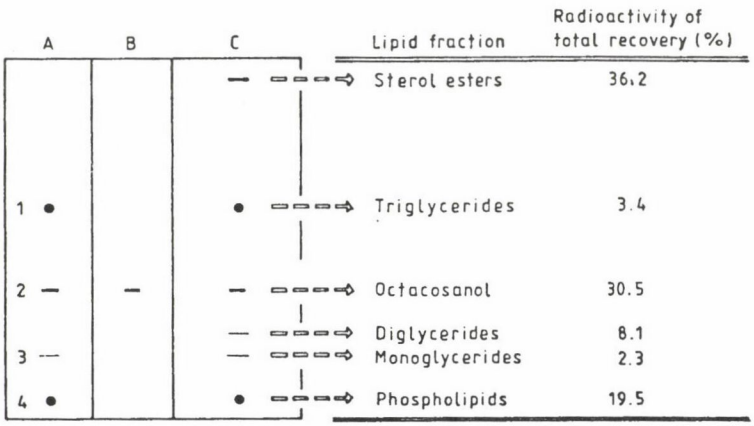

Fig. 2. Thin-layer chromatogram of liver lipids from rats 4 h after administration of ^{14}C-octacosanol Chromatogram A: authentic lipids; 1: tricaprylin, 2: octacosanol, 3: glycerol monostearate and 4: lecithin. Chromatogram B: octacosanol. Chromatogram C: liver lipids from ^{14}C-octacosanol administrated rat. Plate: Wako gel B–0. Developing solvent system was petroleum ether:diethyl ether:acetic acid (85:15:1). Total activity recovered from TLC plate: 95%

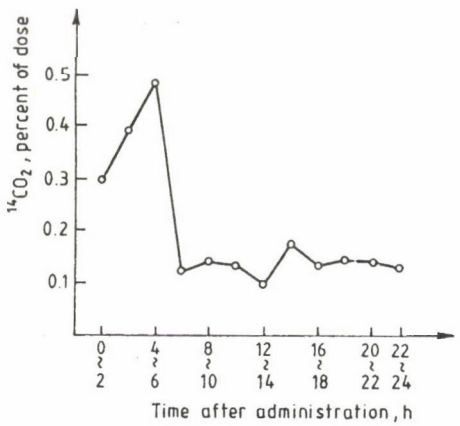

Fig. 3. The expiration of $^{14}CO_2$ over 24 h after administration of ^{14}C-octacosanol

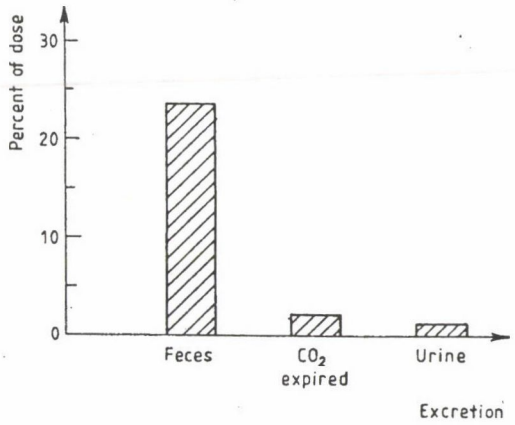

Fig. 4. The excretion of radioactivity through three different pathways over 24 h after administration of ^{14}C-octacosanol

3. Discussion

After oral administration of labelled octacosanol to rats with stomach tube, about 2.6% of the administrated dose was found in the liver at 4–6 h and 1.8% of the dose was recovered in the muscle at 8 h. Analysis of the liver lipids showed that 36.2% of the incorporated radioactivity was in sterol esters and 19.5% in phospholipids but very little (only 3.4%) in the triglycerides. This indicated that octacosanol was most probably converted in liver into a fatty acid which was then esterified with sterol and phospholipid. This is in line with NEPTUNE and co-workers (1960), who reported that both long- and short-chain fatty acids are incorporated into triglycerides, but only the long-chain acids are incorporated into phospholipids. However, the possibility that the newly formed acid was first incorporated into triglyceride and then transferred to phospholipids (and sterols) cannot be excluded (NEPTUNE et al., 1960).

The possibility that octacosanol was converted into a fatty acid is supported by the recovery of radioactivity in the expired $^{14}CO_2$. It is possible that this fatty acid was a source of energy by the process of β-oxidation. The other possibility is that all the free acid produced from octacosanol did not undergo a direct oxidation and part of it was stored in the fat pool of the adipose tissue. This is in accordance with the findings of NEPTUNE and co-workers (1960), who reported that the conversion of long-chain fatty acids to $^{14}CO_2$ constitute only a small fraction of the total CO_2 in rat diaphragm; almost all of it remains in the esterified form (NEPTUNE et al., 1959).

An interesting feature was the predominant accumulation of radioactivity in the liver and muscle within short periods of administration. The presence of radioactivity in liver was of particular interest since they are related to energy

metabolism and utilization. Whether octacosanol was converted into acid and then incorporated in other tissues especially in muscle cannot be answered from this study, although the data suggest that this is a possibility. It is also possible that the hormonal system might be altered by octacosanol or octacosanol itself acts as a hormone through influencing the central nervous system, because hormone-like activity of octacosanol was reported (LEVIN, 1963).

On the basis of the presence of large amount of radioactivity in the sterol ester fraction of liver, it was speculated that octacosanol possibly related to the changes in cholesterol metabolism. Further studies should, therefore, be quite interesting to investigate the effect of octacosanol on cholesterol metabolism.

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Literature

CURETON, T. K. JR. (1957): Science aids Australian swimmers. *Ath. J.*, *37*, 40–44.

CURETON, T. K. JR. (1972): *The physiological effects of wheat germ oil on humans in exercise*. Charles C. Thomas Publishing Co., Illinois, pp. 296–300.

FOLCH, J., LEES, M. & SLOANE, S. G. H. (1957): A simple method for the isolation and purification of total lipids from animal tissues. *J. biol. Chem.*, *226*, 497–509.

KABIR, Y. & KIMURA, S. (1991): Biochemical effect of octacosanol on voluntary exercise in rats. *Dhaka University Studies* (Part E), *6*, 77–85.

KABIR, Y. & KIMURA, S. (1993): Biodistribution and metabolism of orally administered octacosanol in rats. *Ann. Nutr. Metab.*, *37*, 33–38.

KATAHIRA, R. & SHICHIGO, I. (1984): Octacosanol. *Seiyakukojo*, *4*, 461–466.

LEVIN, E. (1963): Effects of octacosanol on chick comb growth. *Proc. Soc. exp. Biol. Med.*, *112*, 331–334.

NEPTUNE, E. M. JR., SUDDUTH, H. C. & FOREMAN, D. R. (1959): Labile fatty acids of rat diaphragm muscle and their possible role as the major endogenous substrate for maintenance of respiration. *J. biol. Chem.*, *234*, 1659–1660.

NEPTUNE, E. M. JR., SUDDUTH, H. C., FOREMAN, D. R. & FASH, F. J. (1960): Phospholipid and triglyceride metabolism of exercised rat diaphragm and the role of these lipids in fatty acid uptake and oxidation. *J. Lipid. Res.*, *1*, 229–235.

PASSWATER, R. (1982): Octacosanol: Its name really increased. *Health Q.*, *7*, 14–15.

PATTERSON, M. S. & GREEN, R. C. (1965): Measurement of low energy beta emitters in aqueous solution by liquid scintillation counting of emulsions. *Anal. Chem.*, *37*, 854–857.

SAINT-JOHN, M. & MCNAUGHTON, L. (1986): Octacosanol ingestion and its effects on metabolic responses to submaximal cycle ergometry, reaction time and chest and grip strength. *Int. clin. Nutr. Rev.*, *6*, 81–87.

SHIMURA, S., HASEGAWA, T., TAKANO, S. & SUZUKI, T. (1987): Studies on the effect of octacosanol on motor endurance in mice. *Nutr. Reps. int.*, *36*, 1029–1038.

SUZUKI, K. (1964): A simple and accurate micromethod for quantitative determination of ganglioside patterns. *Life Sci.*, *3*, 1227–1233.

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RELATIONSHIP BETWEEN HIGH MOLECULAR WEIGHT SUBUNITS OF GLUTENIN AND BREADMAKING QUALITY OF HUNGARIAN GROWN WHEATS

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HMW glutenin subunits spectra of 72 bread-wheat cultivars grown in Hungarian were studied using SDS-polyacrylamide gel electrophoresis.

The quality of varieties was determined based on Valorigraph test, glutenin quantity and quality, Hagberg's falling number and baking test.

In the 72 wheat cultivars 12 different permutations of HMW glutenin subunits were found. The cultivars have been evaluated by HMW glutenin subunit quality score (Glu–1 quality score). Highly significant correlations were found by statistical analyses of results. The highest linear correlation coefficients were obtained for the correlation of Glu–1 quality score Valorigraph value and baking test loaf volume ($r = 0.690$, $p < 0.001$ and $r = 0.573$, $p < 0.001$, respectively).

The evaluation method used can be a useful tool for screening test in breeding programs.

Keywords: wheat, glutenin, HMW glutenin subunits, breadmaking quality

HMW glutenin subunits are known as one of the key components determining functional properties of wheat flour. The nomenclature system of PAYNE and LAWRENCE (1983) based on the mobilites of HMW glutenin subunits of Chinese Spring on SDS page is internationally accepted. Significant relationships between the composition of HMW glutenin subunits and dough elasticity, valorigraph score and SDS test were found in several studies (PAYNE et al., 1981; BRANLARD & DARDEVET, 1985; CAMPBELL et al., 1987; PAYNE et al., 1987/a).

The Glu–1 score (PAYNE et al., 1987/b) has recently been widely used to estimate quality parameters based on HMW glutenin pattern. PAYNE and co-workers have attempted to rank all the alleles present at the GLU–A1, GLU–B1 and GLU–D1 loci in order of quality, and have introduced the concept of a quality score. In this system the individual subunits or subunit pairs are given scores between 1 and

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4, and these can be added to give overall scores for different cultivars. The subunits 5+10 (GLU-D1) always give better quality than the allelic pairs 3+12 or 2+12 (GLU-D1). The presence of GLU-A1 subunit (1 or 2*) is beneficial, too.

SDS PAGE separation of HMW glutenin subunits, Glu-1 scores as well as the results of several quality parameters of wheat varieties and breeding lines grown in Hungary were characterized in this study.

1. Materials and methods

1.1. Materials

Seventy-two wheat samples grown in 1987 and 1989 at Székkutas (Hungary) were studied. This collection of samples contained mainly Hungarian wheat varieties and breeding lines and some foreign varieties (for example: Jubileynaya 50, Bucsányi 20, Zagrebcsanka) cultivated in Hungary (Table 1).

Table 1

Baking properties of Hungarian grown wheats

Sample number	Wheat variety	Wet gluten content (%)	Wet gluten quality (mm h^{-1})	Water abs. capacity (%)	Valorigraph value	Falling number (s)	Loaf value (cm^3/360 g bread)	GLU-1 score
1.	ADRIANA	36.0	12.5	55.4	30.5	356	840	4
2.	ALFÖLD	34.5	5.0	64.8	75.6	420	1100	9
3.	BLC 2125	32.5	6.0	63.0	62.6	351	900	7
4. a	BUCSÁNYI 20	26.5	3.5	54.9	63.3	221	1040	7
5. c	DANKA	29.5	3.5	62.9	74.9	393	880	8
6. c	DERZSANKA	33.6	5.0	70.0	65.9	366	1030	8
7. b	FD-5	32.6	5.0	64.0	68.3	414	1050	8
8.	GK ÁBEL	29.0	2.5	65.9	64.2	409	880	7
9.	GK ÁRON	33.4	9.0	70.1	48.6	459	760	5
10.	GK BARNA	37.8	10.0	68.4	60.4	367	940	6
11.	GK BENCE	32.9	5.5	62.0	64.7	354	960	9
12.	GK CSANÁD	32.5	7.0	60.8	63.3	400	880	7
13.	GK CSŰRÖS	33.8	10.0	64.3	46.2	373	890	4
14.	GK GERGŐ	31.0	7.0	56.4	56.2	377	910	7
15.	GK ILMA	30.3	4.5	57.8	66.2	370	1090	9
16.	GK ISTVÁN	38.8	8.0	63.5	63.3	449	1080	7
17.	GK KALÁKA	31.4	8.0	62.0	67.0	412	890	7
18.	GK KALANGYA	29.4	5.0	63.9	56.4	402	750	7
19.	GK KINCSŐ	32.1	6.5	56.6	40.7	376	870	6
20.	GK ÖRSÉG	33.0	9.0	60.5	34.8	367	780	4
21.	GK ÖRZSE	37.1	10.0	58.5	47.0	368	880	6
22.	GK ÖTHALOM	32.2	3.5	62.7	64.2	382	1030	9
23.	GK PUSZTASZER	31.5	9.0	59.6	40.7	387	920	6
24.	GK SZŐKE	28.6	2.5	57.7	68.5	275	980	7
25.	GK ZOMBOR	34.3	9.5	62.5	55.1	386	920	4
26.	GK 1035	26.5	3.5	56.6	65.2	356	950	6

Table 1 (cont.)

Sample number		Wheat variety	Wet gluten content (%)	Wet gluten quality (mm h^{-1})	Water abs. capacity (%)	Valorigraph value	Falling number (s)	Loaf value (cm^3/360 g bread)	GLU-1 score
27.		GK 32-82	33.8	8.0	59.7	68.8	365	800	6
28.		GK 45-87	30.5	6.0	57.4	49.0	252	900	4
29.	d	JUBILEYNAYA 50	30.2	2.0	63.7	76.0	450	1145	9
30.	c	KORONA	32.1	9.0	67.4	49.0	374	1040	8
31.	c	LONJA	32.8	8.0	58.1	50.8	363	800	6
32.		MV 3	31.5	2.0	70.8	70.7	358	1040	9
33.		MV 9	33.5	2.5	63.8	74.6	440	1090	9
34.		MV 12	32.5	2.5	64.0	72.5	470	1100	9
35.		MV 14	39.7	14.0	65.6	63.5	370	860	6
36.		MV 15	32.9	7.0	63.6	71.6	415	1130	9
37.		MV 16	35.5	9.0	66.1	67.5	282	950	9
38.		MV 17	27.0	6.0	54.0	57.9	298	870	6
39.		MV 75-84	36.7	5.5	69.4	73.1	463	1150	9
40.		MV 08-85	34.5	6.5	66.6	76.8	377	1090	7
41.		MV 21-85	38.5	8.5	64.0	68.0	467	1100	6
42.		MV 03-86	39.4	12.0	72.9	49.3	460	940	5
43.		MV 06-83	35.7	5.0	63.1	57.3	298	1010	9
44.		MV 15-86	33.8	4.0	69.8	75.3	388	1050	9
45.		MV 107-86	31.6	10.5	60.3	51.4	309	930	6
46.		MV 04-87	35.0	7.0	64.7	73.1	387	970	9
47.		MV 32-87	34.8	8.0	65.2	54.6	360	950	9
48.		MV 37-87	32.5	6.0	65.6	66.2	424	850	9
49.		MV 10-88	36.0	8.5	67.0	62.6	451	990	9
50.		MV 12-88	29.0	5.0	62.3	58.3	329	840	9
51.		MV 23-88	26.9	6.5	55.7	46.0	301	870	4
52.		MV 109-88	30.4	2.5	64.9	73.1	486	970	9
53.		MV 119-88	27.5	4.0	64.0	73.6	371	760	6
54.		MV 130-88	30.0	3.5	62.6	68.3	302	930	9
55.		MV 213-88	32.8	4.0	63.3	77.4	406	940	9
56.		MV 218-88	30.0	7.5	64.0	51.4	365	730	4
57.		MVHB 26-87	33.8	7.5	57.5	58.3	200	990	8
58.		MVHB 47-87	29.0	6.0	61.1	50.9	260	920	8
59.		MVHB 48-87	30.7	6.5	59.6	54.4	293	960	7
60.		MVHB 69-87	30.0	6.0	60.2	58.5	342	920	9
61.		SZ 26-88	33.7	6.5	60.2	38.9	285	830	4
62.		SZ 146-88	27.3	3.0	58.0	55.8	366	1000	9
63.		SKOPJANKA	34.7	8.5	72.8	46.2	326	890	4
64.	c	ZAGREBCSANKA	34.5	7.5	58.6	49.6	392	950	6
65.	c	ZG 52-10	28.2	11.5	63.2	71.3	417	1030	9
66.	c	ZG 7057/79	38.3	10.5	60.6	44.4	376	970	4
67.	c	ZG 163/82	26.7	16.5	58.1	37.5	333	890	4
68.	c	ZG 213/83	39.0	12.0	65.5	52.0	353	910	5
69.	c	ZG 2008/85	35.4	8.5	62.6	46.0	390	810	6
70.	c	ZG 167/86	33.0	10.0	66.3	52.2	357	860	5
71.	c	ZG 291/86	33.4	4.5	66.2	67.8	423	920	4
72.		VITKA	31.0	1.0	56.3	62.0	359	910	7

a Czechoslovakian
b French
c Yugoslavian
d Russian

1.2. Methods

1.2.1. SDS-PAGE (Sodium dodecyl sulphate-polyacrylamide gel electrophoresis). Proteins were extracted from single kernels of wheat with SDS in a Tris-HCl buffer pH 6.8 as described by LAWRENCE and SHEPHERD (1980).

The composition of HMW glutenin subunits in 72 Hungarian bread-wheat varieties was determined by SDS-polyacrylamide gel electrophoresis according to LAWRENCE and SHEPHERD (1980).

1.2.2. Investigation of baking properties. Wet gluten quantity and quality, Valorigraph value, water absorption capacity, Hagberg falling number of samples were determined using the current HUNGARIAN STANDARD (1970; 1973; 1977) methods.

For the investigation of baking properties, the test method of HUNGARIAN STANDARD (1988) was used. The baking formula in this method does not contain any oxidizing agents.

2. Results

SDS PAGE patterns of HMW glutenin subunits of some investigated samples are shown in Fig. 1. Apparent molecular weights of HMW glutenin subunits were also determined using HMW Proteins KIT of Pharmacia standard (18.5-330 kD). As illustrated on Fig. 2, apparent molecular weight of HMW glutenin subunits was found between 95 kD-146 kD, comparable with the results of BUSHUK and NG (1988).

Subunits assignations of samples investigated using the system of PAYNE and LAWRENCE (1983) and Glu-1 scores (PAYNE et al., 1987/b) are shown in Table 2.

As it is marked in Table 2 some combinations of subunits not described previously were found:

20+9 - in Danka, Derzsanka, MV 107-86
7+8+9 - in FD-5, Vitka
7+9+20 - in MVHB 47-87

In some cases the apparent quantitative distribution of subunits was different from the generally observed distribution (for example the significantly weaker apparence of subunit 7 in MVHB 47-87). The sample sizes used in this study seemed to be too small for the precise determination of the biotype composition in MVHB 26-87 where, both 2+12 and 5+10 combinations were observed in the single seed analyses. Similar results were found in the case of GK 1035 where approximately 20% of the seeds investigated contained subunit 7 instead of 6+8 subunits.

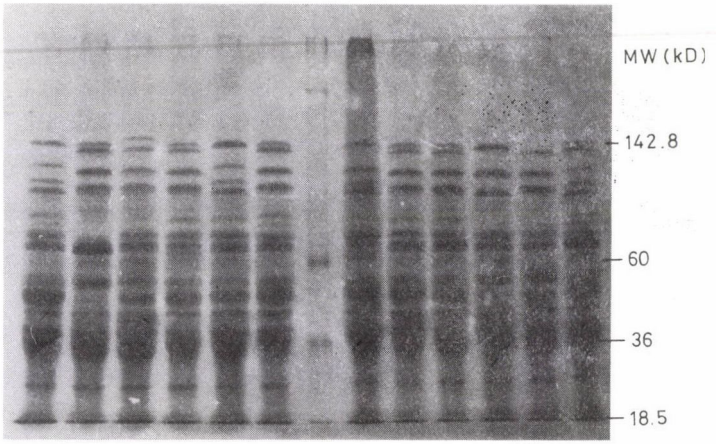

Fig. 1. SDS – PAGE electrophoretogram of glutenins of different wheat varieties
From left to right: ZG 163/82; ZG 52 – 10; GK BENCE; MV 75 – 84; MV 21 – 85; MV 06 – 83; STANDARD; CHINESE SPRING; JUBILEYNAYA 50; MV 12; GK ZOMBOR; GK ISTVÁN; GK ÖRZSE

Approximately one-third of the varieties and breeding lines used in this study was investigated in two seasons. Comparing samples from the two seasons, different subunit compositions were found in some cases (for example GK 1035, GK Szőke).

Correlation and linear regression methods were used to describe the relationships between the HMW glutenin composition and quality parameters (Table 3).

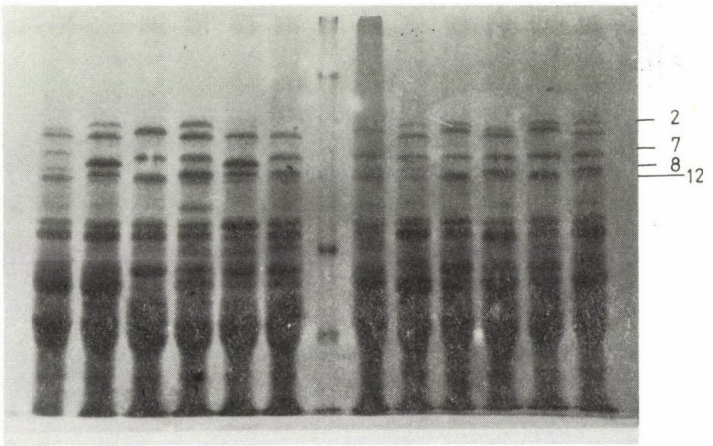

Fig. 2. SDS – PAGE electrophoretogram of glutenins of different wheat varieties
From left to right: ZG 213/83; DERZSANKA; MV 03-86; HV 15-86; MV 107-86; GK CSANÁD STANDARD; CHINESE SPRING; GK GERGŐ; GK PUSZTASZER; GK ILMA; GK BARNA; MV 3

The highest linear correlation coefficients were found for the relationships of Glu-1 score with Valorigraph value and loaf volume ($r = 0.690$, $p < 0.001$; $r = 0.573$, $p < 0.001$, respectively).

Table 2

HMW glutenin subunit compositions of Hungarian grown wheats

Sample number	Wheat variety	HMW glutenin subunits 1A	1B	1D	Glu-1 score
1.	ADRIANA	N	6+8	2+12	4
2.	ALFÖLD	2*	7+9	5+10	9
3.	BLC 2125	N	7+9	5+10	7
4.	BUCSÁNYI 20	N	7+9	5+10	7
5.	DANKA	1	20+9	5+10	8
6.	DERZSANKA	1	20+9	5+10	8
7.	FD-5	N	7+8+9	5+10	8
8.	GK ÁBEL	N	7+9	5+10	7
9.	GK ÁRON	N	7+9	2+12	5
10.	GK BARNA	1	7	2+12	6
11.	GK BENCE	1	7+9	5+10	9
12.	GK CSANÁD	N	7+9	5+10	7
13.	GK CSŰRÖS	N	7	2+12	4
14.	GK GERGŐ	1	7+8	4+12	7
15.	GK ILMA	2*	7+9	5+10	9
16.	GK ISTVÁN	N	7+9	5+10	7
17.	GK KALÁKA	N	7+9	5+10	7
18.	GK KALANGYA	N	7+9	5+10	7
19.	GK KINCSŐ	1	7	2+12	6
20.	GK ŐRSÉG	N	7	2+12	4
21.	GK ÖRZSE	1	6+8	2+12	6
22.	GK ÖTHALOM	2	7+9	5+10	9
23.	GK PUSZTASZER	1	7	2+12	6
24.	GK SZŐKE	N	7+9	5+10	7
25.	GK ZOMBOR	N	7	2+12	4
26.	GK 1035	1	7	2+12	6
27.	GK 32-82	1	7	2+12	6
28.	GK 45-87	N	20	2+12	4
29.	JUBILEYNAJA 50	2*	7+9	5+10	9
30.	KORONA	2*	6+8	5+10	8
31.	LONJA	1	20	2+12	6
32.	MV 3	1	7+9	5+10	9
33.	MV 9	2*	7+9	5+10	9
34.	MV 12	2*	7+9	5+10	9
35.	MV 14	1	7	2+12	6
36.	MV 15	2*	7+9	5+10	9
37.	MV 16	2*	7+9	5+10	9
38.	MV 17	2*	7	2+12	6
39.	MV 75-84	2*	7+9	5+10	9
40.	MV 08-85	N	7+9	5+10	7
41.	MV 21-85	2*	6+8	2+12	6
42.	MV 03-86	N	7+9	2+12	5
43.	MV 06-83	2*	7+9	5+10	9

Table 2 (cont.)

Sample number	Wheat variety	HMW glutenin subunits 1A	1B	1D	Glu-1 score
44.	MV 15-86	1	7+9	5+10	9
45.	MV 107-86	N	20+9	5+10	6
46.	MV 04-87	2*	7+9	5+10	9
47.	MV 32-87	2*	7+9	5+10	9
48.	MV 37-87	2*	7+9	5+10	9
49.	MV 10-88	2*	7+9	5+10	9
50.	MV 12-88	1	7+9	5+10	9
51.	MV 23-88	N	7	3+12	4
52.	MV 109-88	2*	7+9	5+10	9
53.	MV 119-88	2*	7	2+12	6
54.	MV 130-88	1	7+9	5+10	9
55.	MV 213-88	2*	7+9	5+10	9
56.	MV 218-88	N	7	2+12	4
57.	MVHB 26-87	2*	7	5+10; 2+12	8
58.	MVHB 47-87	N	7+9+20	5+10	8
59.	MVHB 48-87	N	7+9	5+10	7
60.	MVHB 69-87	2*	7+9	5+10	9
61.	SZ 26-88	N	7	2+12	4
62.	SZ 146-88	1	7+9	5+10	9
63.	SOKPJANKA	N	7	2+12	4
64.	ZAGREBCSANKA	1	6+8	2+12	6
65.	ZG 52-10	2*	7+9	5+10	9
66.	ZG 7057/79	N	6+8	2+12	4
67.	ZG 163/82	N	6+8	2+12	4
68.	ZG 213/83	2*	6+8	4+12	5
69.	ZG 2008/85	1	6+8	2+12	6
70.	ZG 167/86	2*	6+8	4+12	5
71.	ZG 291/86	N	7	2+12	4
72.	VITKA	N	7+8+9	5+10	7

Table 3

Linear correlation coefficients between baking quality and Glu-1 quality score

	Wet gluten content	Wet gluten quality	Water abs. capacity	Valorigraph value	Falling number	Loaf volume	Glu-1 score
Wet gluten content	1.000						
Wet gluten quality	0.453	1.000					
Water abs. capacity	0.461	0.058	1.000				
Valorigraph value	-0.079	-0.578	0.312	1.000			
Falling number	0.320	-0.031	0.473	0.326	1.000		
Loaf volume	0.155	-0.295	0.190	0.537	0.226	1.000	
GLU-1 score	-0.121	-0.530	0.180	0.690	0.175	0.573	1.00

3. Conclusion

Results shown indicate that in case of wheats cultivated in Hungary Glu-1 score may be a useful tool to predict quality parameters, therefore it can be used in breeding for selecting quality.

Literature

BUSHUK, W. & NG, P. K. W. (1987): Relationship between high molecular weight subunits of glutenin and breadmaking quality of Canadian wheats. – in: R. LÁSZTITY & F. BÉKÉS (Eds), *Proceedings of the 3rd International Workshop on Gluten Proteins*. World Scientific, Singapore, pp. 206–215.

BRANLARD, G. & DARDEVET, M. (1985): Diversity of grain proteins and bread wheat quality, Part II. Correlation between high molecular weight subunits of glutenin and flour quality characteristics. *J. Cereal Sci.*, *3*, 345–354.

CAMPBELL, W. P., WRIGLEY, C. W., CRESSEY, P. J. & SLACK, C. R. (1987): Statistical correlations between quality attributes and grain-protein composition for 71 hexaploid wheats used as breeding parents. *Cereal Chem.*, *64*, 293–299.

HUNGARIAN STANDARD (1970): *Lisztvizsgálati módszerek. A sikér vizsgálata.* (Flour testing methods. Gluten test.), MSZ 6369/5–70.

HUNGARIAN STANDARD (1973): *Lisztvizsgálati módszerek. A vízfelvevő képesség és a sütőipari érték vizsgálata.* (Flour testing methods. Determination of water absorption capacity and baking quality.), MSZ 6369/6–73.

HUNGARIAN STANDARD (1977): *Lisztvizsgálati módszerek. Amilolites állapot vizsgálata.* (Flour testing methods. Amylolytic condition test.), MSZ 6369/9–77.

HUNGARIAN STANDARD (1988): *Lisztvizsgálati módszerek. Sütésipróba.* (Flour testing methods. Baking test.), MSZ 6369/8–88.

LAWRENCE, G. J. & SHEPHERD, K. W. (1980): Variation in glutenin protein subunits of wheat. *Aust. J. Biol. Sci.*, *33*, 221–233.

PAYNE, P. I., CORFIELD, K. G., HOLT, L. M. & BLACKMAN, J. A. (1981): Correlations between the inheritance of certain high molecular weight subunits of glutenin and bread making quality in progenies of six crosses of bread wheat. *J. Sci. Fd Agric*, *32*, 51–60.

PAYNE, P. I., HOLT, L. M., HARINDER, K., MCCARTNEY, D. P. & LAWRENCE, G. J. (1987/a): The use of near-isogenic lines with different HMW glutenin subunits in studying bread-making quality and glutenin structure. – in: R. LÁSZTITY, & F. BÉKÉS (Eds), *Proceedings of the 3rd International Workshop on Gluten Proteins*. World Scientific, Singapore, pp. 216–226.

PAYNE, P. I. & LAWRENCE, G. J. (1983): Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1, and Glu-D1 which code for high-molecular-weight subunits of glutenin in hexaploid wheat. *Cereal Res. Com.*, *11*, 29–35.

PAYNE, P. I., NIGHTINGALE, M. A., KRATTIGER, A. F. & HOLT, L. M. (1987/b): The relationship between HMW glutenin subunit composition and the bread making quality of British-grown wheat varieties. *J. Sci. Fd Agric.*, *40*, 51–65.

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DETECTION OF IRRADIATION TREATMENT OF CINNAMON AND ALLSPICE USING PHYSICAL METHODS (VISCOMETRY, DSC, NIR)[a]

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The aim of the study was to extend the different physical methods for cinnamon and allspice based on the literature data and our earlier results obtained on irradiated black and white pepper and paprika.

The irradiation treatment of the samples with 0, 2, 4, 8, 16 kGy doses was carried out in a self-shielded Co–60 irradiator, type RH-gamma 30. The irradiated samples were investigated by three methods: viscometric, differential scanning calorimetric (DSC) and near infrared spectrophotometric (NIR) measurements.

The starch and saccharides content of the irradiated sample series were determined with standard methods.

The irradiation damage of the starch (18.1% in cinnamon and 16.1% in allspice) and the changes in the swelling capacity of the polysaccharides were investigated in a rotational viscometer type Rheotest 2 RV 2 (Dr = 437.4 s^{-1}).

The influence of the irradiation treatment on the heat gelatinization process of the starch was measured in a SETARAM type DSC micro calorimeter, determining the gelatinization temperature and heat.

The changes caused by the irradiation effect in the volatile oils, lipids, starch components of the samples were investigated on a SPECTRALIZER 1025 type spectrophotometer in the near infrared wavelength region between 1000 and 2500 nm.

Making a comparison between the 3 physical methods used in the identification experiments of both irradiated spices we can establish, that the apparent viscosity presented the highest differences between samples irradiated with different doses.

The storage time did not influence the apparent viscosity values. The identification limit by viscometry method was found at 2–3 kGy irradiation doses, by NIR spectrophotometric method at 4–5 kGy. With these two methods it was possible to distinguish and order correctly the irradiated samples.

The sensitivity of micro calorimetric DSC method was low. No significant changes was experienced after irradiation.

Keywords: cinnamon, allspice, irradiation, doses, identification, viscometry, starch, heat gelatinization, DSC, NIR spectrophotometry, NIR spectra, quality points

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Based on the JECFI (WHO/FAO/IAEA) recommendations, the highest irradiation dose used for the microbial decontamination of the spices and dry ingredients is 10 kGy.

The activity of the irradiation facilities is relatively easy to control with dosimetry by the authorities, but regarding international trade of spices, it is important to detect whether the final product was irradiated or not (HEIDE et al., 1987).

So the main task of the IAEA co-ordinated ADMIT research collaboration is to elaborate methods for the identification of irradiated foodstuffs.

Regarding interest areas of the specialists, and due to the fact, that there is no uniform identification method, ADMIT established 8 specialised research groups.

The aim of our group was to extend for cinnamon and allspice the different physical methods based on the literature data (HEIDE et al., 1987, FARKAS et al., 1990a) and our earlier results (FARKAS et al., 1990b; BARABÁSSY et al., 1990) obtained on irradiated black and white pepper and paprika (BARABÁSSY et al., 1992). These physical methods cover three fields: viscometry, microcalorimetry (DSC) and near infrared spectrophotometry (NIR).

1. Materials and methods

The two spices used in our investigation were: cinnamon originated from Vietnam and allspice originated from Mexico. In Table 1 the main components of both spices are given. The gel forming polysaccharides (starch) content is relatively high. Radiation damage of the starch and changes in the swelling capacity of all polysaccharides were investigated by viscometry and microcalorimetry (DSC). Changes caused by irradiation in the volatile oils, lipids and starch were investigated by near infrared (NIR) spectroscopy technique.

Table 1

Main components of cinnamon and allspice

Spice	Origin	Starch content (%)	Volatile oils (%)	Water content (%)	Other saccharides (%)
Cinnamon	Vietnam	18.1	3.7	11.5	46.8
Allspice	Mexico	16.1	2.8	10	20.4

For all investigations we used sets of similarly prepared samples. Cinnamon and allspice were ground, the fractionated on sieves to different sizes of the particles. Irradiation of the ground samples with 0, 2, 4, 8, 16 kGy doses were carried out in a self-shielded Co-60 irradiator, type RH-gamma 30.

After preparation of the samples the total saccharides content was determined with Boehringer enzymatic methods and the starch content according to HUNGARIAN STANDARD (1966).

Fifteen % Suspensions of pH = 13 (adjusted with NaOH) for viscometric measurements were prepared in two ways. In the first case the samples were heat gelatinised at 96 °C for 10 min, then cooled to ambient temperature for one h. In the second case no heat treatment was applied. From all suspensions we measured 3 parallel samples at ambient temperature and in each case we put 30 g of gel in the measuring cylinder.

The measurement of the apparent viscosity was carried out in a rotational viscometer type Rheotest 2RV2 with a velocity gradient of 437.4 s^{-1}.

For the micro calorimetric investigation we used a Setaram type DSC (differential scanning calorimeter) micro calorimeter instrument. 800 ± 10 mg mass of 30% spice suspension was measured in the sample holder and as reference the same mass of water in the other sample holder. The heating rate was: 1 °C min^{-1} between 25 and 95 °C temperature range.

For the NIR measurements we used a Spectralyzer 1025 type spectrophotometer. The wavelength region was between 1000-2500 nm. Thirty g of ground spice was placed in the sample holder, then the log(1/R) was measured where R is the reflectance of the sample. As reference halon was used. The data processing was performed on a PC using the software the NIR instrument was furnished with. The most characteristic wavelength was determined by multiple linear regression (MLR), and the standard error and correlation coefficient was determined as well. Using the qualitative evaluation by polar qualification system (PQS) method (KAFFKA & GYARMATI, 1990) the informations obtained from NIR spectra of the irradiated and control samples were compared.

2. Results and discussions

Comparing the starch content of the irradiated and control samples there are no significant differences as a function of irradiation (Table 2).

Table 2

Starch content of different irradiated samples

Doses (kGy)	Starch content (%)	
	Cinnamon	Allspice
0	18.1	16.1
2	15.2	12.9
4	17.8	10.2
8	20.5	8.2
16	18.3	10.5

2.1. Viscosity investigations

It was known from the literature and previous studies that the particle size and the suspension concentration influence the heat gelatinization process (FARKAS et al., 1990a, 1990b, BARABÁSSY et al., 1990) and the swelling capacity of the gel-forming polysaccharides. To establish the influence of these parameters for cinnamon and allspice, preliminary experiments with different particle sizes and suspension concentrations were carried out, as it is given in Table 3.

Table 3

The effect of concentration and particle size of heat gelatinised suspensions on apparent viscosity measured at the velocity gradient 437.4 s^{-1}

Cinnamon			Allspice		
Particle size (mm)	Concentration (%)	Apparent viscosity (mPas)	Particle size (mm)	Concentration (%)	Apparent viscosity (mPas)
< 0.16	10	8.2	< 0.5	5	1.1
	15	31.3		10	6.7
	20	28.9		15	20.2
0.16–0.25	10	4.5	0.5–0.8	10	0.3
	20	207.8		15	0.3
0.25–0.31	10	9.2	0.8–1.0	5	0.6
	15	9.8		10	1.8
	20	28.7		15	16.1

Statistical evaluation of the apparent viscosity values was performed using the Statgraph software program.

Based on these results further experiments were performed with 15% suspension concentration in both cases, particle size < 0.16 mm for cinnamon, and < 0.50 mm for allspice. The values of apparent viscosity of irradiated and control cinnamon samples prepared with the mentioned conditions were measured. Shear-stress curves on the first day after irradiation treatment are shown in Fig. 1.

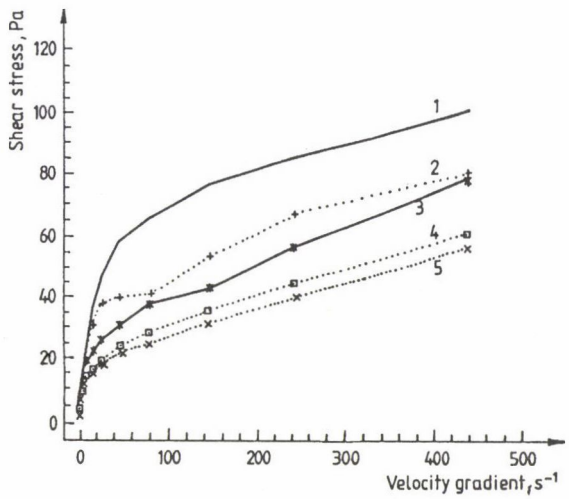

Fig. 1. Shear-stress curves of heat gelatinised cinnamon suspensions. 1 = 0 kGy; 2 = 2 kGy; 3 = 4 kGy; 4 = 8 kGy; 5 = 16 kGy

After 14 and 60 days of storage the same measurements showed practically similar data for the shear-stress values (Table 4). The samples were stored in polyethylene bags in dark room at 20–25 °C, the relative humidity was not controlled.

Table 4

Shear-stress values (Pa) of gelatinised cinnamon suspensions at the velocity gradient 437.4 s^{-1}.
Standard errors in parenthesis

Time (day)	Irradiation doses (kGy)				
	0	2	4	8	16
0	153 (0.0)	109 (0.1)	104 (3.3)	65 (2.3)	33 (0.3)
14	156 (2.3)	95 (2.0)	98 (1.6)	60 (3.3)	34 (0.3)
60	157 (6.6)	97 (0.3)	100 (1.6)	62 (0.0)	32 (0.9)

Figure 2 presents the shear-stress curves of heat gelatinised allspice suspensions on the first day after the radiation treatment.

Data measured on the 14th and 60th days are given in Table 5. No changes in the 60 days storage period were noted.

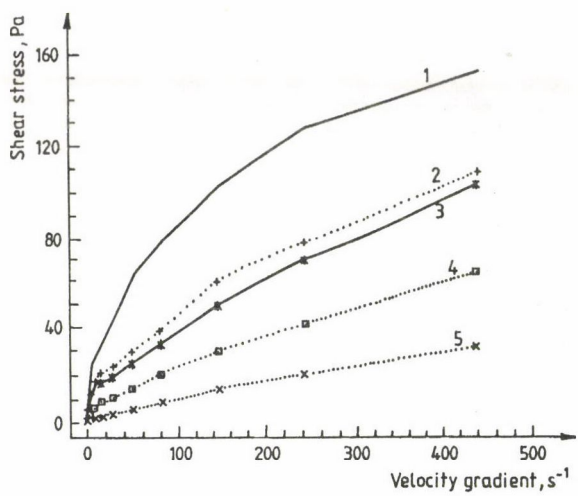

Fig. 2. Shear-stress curves of heat gelatinised allspice suspensions. 1 = 0 kGy; 2 = 2 kGy, 3 = 4 kGy; 4 = 8 kGy; 5 = 16 kGy

Table 5

Shear-stress values (Pa) of gelatinised allspice suspensions at the velocity gradient 437.4 s^{-1}.
Standard errors in parenthesis

Time (day)	Irradiation doses (kGy)				
	0	2	4	8	16
0	102 (2.0)	81 (4.1)	79 (0.0)	62 (0.6)	57 (0.7)
14	93 (2.6)	81 (2.6)	76 (1.7)	62 (0.0)	59 (2.7)
60	92 (1.6)	79 (0.0)	73 (0.0)	64 (1.6)	57 (1.6)

Both of the shear-stress diagrams show that the increase of the velocity gradient has an exponential character, which phenomenon is characteristic for the structural viscosity. The apparent viscosity values obtained from the heat gelatinised samples are given in the Table 6. With both spices during the total storage period (60 days), already the 2 kGy dose samples showed significantly reduced apparent viscosity in comparison with the control.

Table 6

Apparent viscosity (mPas) values of gelatinised cinnamon and allspice samples as a function of storage time at the velocity gradient 437.4 s^{-1}.
Standard errors in parentheses

Doses (kGy)	Apparent viscosity (mPas)					
	Cinnamon			Allspice		
	0 day	14 days	60 days	0 days	14 days	60 days
0	35 (0.0)	36 (0.5)	36 (1.5)	23 (0.5)	21 (0.6)	21 (0.4)
2	25 (0.0)	22 (0.4)	22 (0.1)	19 (0.9)	18 (0.6)	18 (0.0)
4	24 (0.7)	22 (0.4)	23 (0.4)	18 (0.0)	17 (0.4)	17 (0.0)
8	15 (0.5)	14 (0.7)	14 (0.0)	14 (0.1)	13 (0.0)	14 (0.0)
16	8 (0.1)	8 (0.1)	7 (0.2)	13 (0.2)	13 (0.6)	13 (0.4)

The apparent viscosity of the suspensions without heat gelatinization treatment was measured after pH adjustment. In this case the changes in the viscosity were caused by the swelling capacity of the polysaccharides. The results of these measurements are shown in Table 7.

Table 7

Apparent viscosity values (mPas) of non-gelatinised suspensions of spices.
Standard errors in parentheses

Spice	Doses (kGy)				
	0	2	4	8	16
Cinnamon	46.1 (2.6)	23.3 (0.0)	27.6 (1.1)	16.8 (0.0)	9 (0.1)
Allspice	28 (3.4)	22.4 (0.7)	20.7 (0.0)	18.9 (0.3)	18.1 (0.0)

For both spices irradiation caused significant changes in the apparent viscosity at 2 kGy dose already. The changes of the non-heat gelatinised suspensions of allspice are smaller than those of the heat gelatinised samples. The difference between the samples irradiated with 16 kGy doses and the control, in the case of cinnamon, was more than 500%, for allspice less then 100%. This is in good accordance with all polysaccharides content (Table 1, cinnamon 64.5%, allspice 36.5%) which determines the swelling capacity of these spices. Making a comparison between the heat gelatinised and non-treated samples, in the latter case the values of the apparent viscosity were slightly higher.

2.2. Micro calorimetric investigation

The heat gelatinization process of the starch of the cinnamon and allspice suspensions were studied using differential scanning micro calorimeter (DSC). The heat gelatinization of the starch is a well measurable endothermic process. Table 8 shows the results of the DSC investigations. The gelatinization temperature and the heat (enthalpy) did not change significantly after irradiation.

Table 8

DSC data of cinnamon and allspice samples

Doses (kGy)	Cinnamon		Allspice	
	Temp. of gelatinization (°C)	Enthalpy (mJ)	Temp. of gelatinization (°C)	Enthalpy (mJ)
0	63.3	300	82.2	390
16	63	312	86.7	261
30	62.9	322	–	–

2.3. NIR investigation

The spectra of the spice samples irradiated with different doses were taken in the near infrared wavelength region. Three parallel samples were studied from cinnamon (< 0.16 mm) and allspice (< 0.50 mm) ground products.

The NIR spectra obtained were evaluated using mathematical transformations, such as Savitsky-Golay and triangular smoothing, and second derivation.

The spectral data were taken at the 1st, 4th, 18th and 30th days after the irradiation treatment. The cinnamon data evaluated by multiple linear regression (MLR) are given in Table 9.

Table 9

Calculated doses obtained from the spectral data measured by the NIR spectrophotometer for cinnamon at different irradiation doses

Doses (kGy)	Calculated doses (kGy) by NIR			
	1 day	4 days	18 days	30 days
0	0.66	0.57	0.12	0.12
2	3.06	1.83	0.96	1.13
4	4.13	6.46	5.20	3.97
8	9.11	7.87	9.10	9.65
16	15.02	14.99	14.98	15.01
Standard errors	1.026	1.395	1.098	1.061

The samples were measured at different days and the most characteristic wavelength which was determined from the second derivative of the log(1/R) NIR spectra changed from case to case. For example on the first day it was at 2360 nm, which wavelength region is characteristic for the oil components, and on the 18th day it was at 1600 nm, characteristic for the starch component.

Figure 3 shows the second derivative of the log(1/R) spectra at the 18th day between the 1585 and 1625 nm wavelengths region.

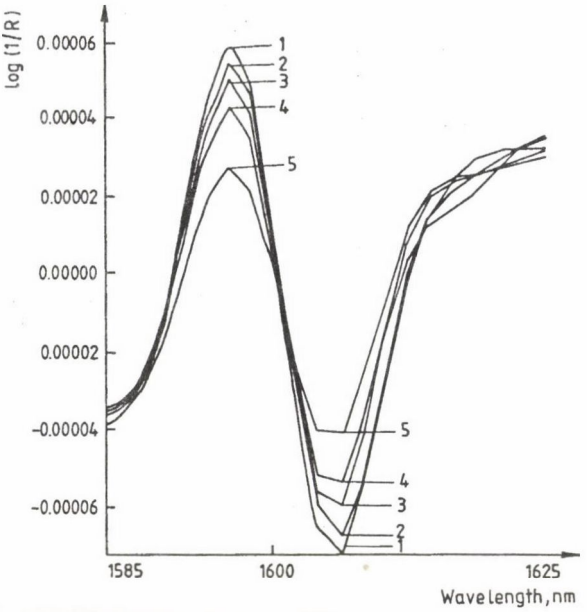

Fig. 3. Second derivate of the log(1/R) spectra of cinnamon on the 18th day as affected by the irradiation doses. 1 = 2 kGy; 2 = 0 kGy; 3 = 4 kGy; 4 = 8 kGy; 5 = 16 kGy

The allspice investigations on the 1st, 4th, 18th, 30th storage days showed a good correlation as well. The NIR-calculated data obtained from the spectral data and the true irradiation doses for the allspice are presented in the Table 10.

Table 10

Calculated doses obtained from the spectral data measured by the NIR spectrophotometer for allspice at different irradiation doses

Doses (kGy)	Calculated doses (kGy) by NIR			
	1 day	4 days	18 days	30 days
0	0.20	0.07	0.27	0.05
2	0.54	2.08	0.65	2.79
4	4.63	3.76	3.29	2.34
8	9.69	7.93	9.54	10.63
16	14.74	16.08	15.05	14.14
Standard errors	1.329	0.149	1.042	1.853

The characteristic wavelengths changed in the function of storage time as with cinnamon.

The log(1/R) spectra of the cinnamon and allspice were evaluated by polar qualification system (PQS) as well. The "quality points" of the samples obtained as the gravity points of log(1/R) spectra represented in polar diagram, concentrated all informations characteristic for the investigated sample. The centre of gravity of the polar spectrum can be expressed in a rectangular (Descartes) coordinate system. The arrangement of the quality points in two dimensions in shown in Fig. 4 (for cinnamon) and Fig. 5 (for allspice).

The quality points derived from the log(1/R) spectra for samples irradiated with different doses can be distinguished, namely the points in the quality plane are far from each other. Samples irradiated with higher doses are localised in the other part of the plane compared to the control samples. The distance between the points expresses the changes in the "quality" after irradiation treatment.

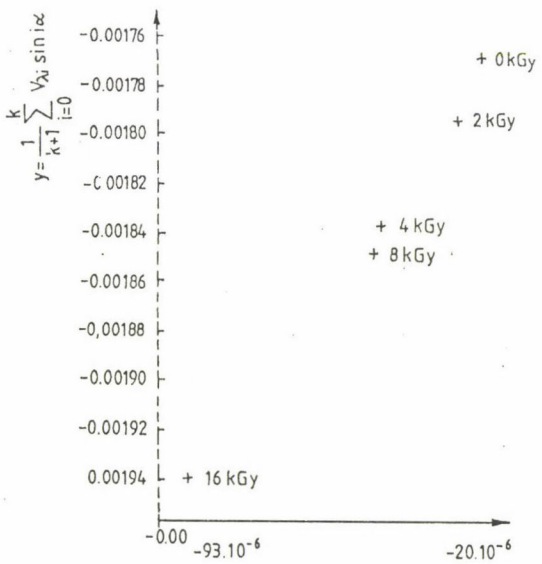

Fig. 4. The "quality points" of irradiated and control cinnamon samples determined by PQS method between 1560–1615 nm.

$$x = \frac{1}{k+1} \sum_{i=0}^{k} V_{\lambda i} \cos i\alpha$$

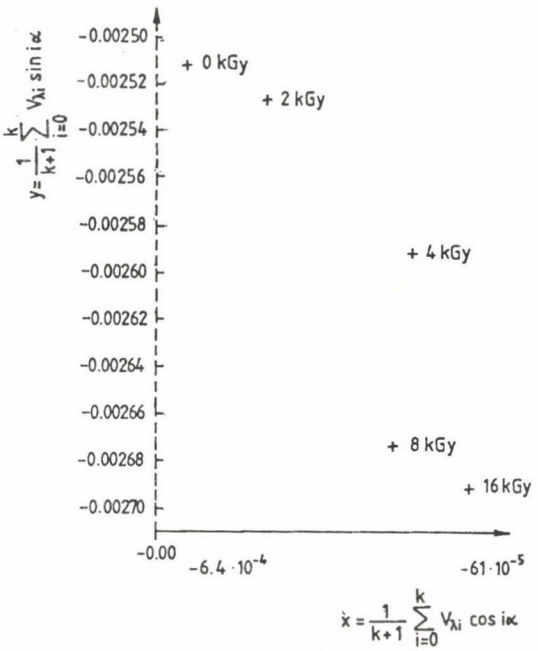

Fig. 5. The "quality points" of irradiated and control allspice samples determined by PQS method between 1185–1245 nm.

$$x = \frac{1}{k+1} \sum_{i=0}^{k} V_{\lambda i} \cos i\alpha$$

3. Conclusions

We can conclude that out of the 3 physical methods used in our investigations the viscometric measurement and the NIR calibration method were those which showed significant differences between the irradiated and control samples. With these two methods it was possible to order correctly the irradiated samples.

The identification limit by the viscometry method was at 2–3 kGy, by the NIR spectrophotometric method at 4–5 kGy.

For both investigated spices the changes in viscosity are caused by the effect of irradiation damage of the starch component – developed by the heat gelatinization treatment – as well as by the effect of irradiation to the swelling capacity of the other polysaccharides. Therefore it is possible to investigate the irradiated spices by viscometry with lower starch content if the swelling capacity of the other polysaccharides is relatively high.

Considering the NIR spectrophotometric measurements it can be established, that during the storage period the best characteristic wavelengths changed. In the

first days the best correlation was at the characteristic wavelengths of the oil components, later at the wavelengths characteristic for starch components.

The sensitivity of the micro calorimetric DSC method in comparison with the other two methods was lower. The relatively high irradiation doses caused only small detectable changes.

*

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Literature

BARABÁSSY, S., FARKAS, J., SHARIF, M. & KONCZ, A. (1990): Elaboration of a detection method for some irradiated dry foods and ingredients on the basis of radiation damage of their starch content. *IAEA Progress Report II.* Jachranka, Poland.

BARABÁSSY, S., FARKAS, J. & KAFFKA, K. (1992): Study of the gamma irradiation effect on paprika powder by NIRS. – in: MURRAY, I. & COWE, I. A. (Eds), *Making light work: Advances in near infrared spectroscopy.* VHC Ed., Weinheim, pp. 415 – 424.

FARKAS, J., KONCZ, A. & SHARIF, M. (1990a): Identification of irradiated dry ingredients on the basis of starch damage. *Radiat. Phys. Chem.*, *35*, 324 – 238.

FARKAS, J., SHARIF, M. & BARABÁSSY, S. (1990b): Analytical studies into radiation-induced starch damage in black and white peppers. *Acta Alimentaria*, *19*, 273 – 279.

HEIDE, L., MOHR, E., WICHMANN, G., ALBRICH, S. & BÖGL, W. (1987): *Viskositätsmessung, ein Verfahren zur Identifizierung strahlenbehandelter Gewürze?* ISH-Heft 120, Inst. für Strahlenhygiene, Bundesgesundheitsamt, Neuherberg.

HUNGARIAN STANDARD (1966): *Takarmányok tápértékének meghatározása.* (Determination of nutritive value of feedstuffs.) MSZ 6830 – 66.

KAFFKA, K. & GYÁRMATI, L. (1990): Qualitative analysis by near infrared spectroscopy. *Proc. of the 3rd Int. NIRS Conf. Brussels*, pp. 31 – 38.

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HYGIENIC-SANITARY INDICATORS FOR ICE CREAM SOLD AT THE RETAIL SALE

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Ice cream is a dairy product which favours the growth of microorganisms and can be exposed to contamination, during handling, while being sold at the retail market.

A total of 150 samples of ice cream, sold in retail from different non-industrial ice cream makers, were analysed. The chosen types were flavoured, with or without added ingredients (fruits, walnuts, etc.).

Forty seven % of the samples had mesophilic aerobic count in the range of $\geq 1 \times 10^5 - < 1 \times 10^6$ CFU $\times g^{-1}$. All samples were positive for total coliforms. The *Enterobacteriaceae* count was $1 \times 10^3 - 1 \times 10^4$ CFU $\times g^{-1}$ for the majority of the samples. Microbes belonging to the group D of *Streptococcus* genus were found in the range of $\geq 1 \times 10^1 - < 1 \times 10^2$ CFU $\times g^{-1}$ in 49% of the samples.

The presence of *Salmonella spp.*, *Shigella spp.* or enterotoxigenic *Staphylococcus aureus* were not detected in any of the samples.

Keywords: dairy product, hygienic-sanitary quality, ice cream, microorganisms

Ice cream is a foodstuff whose composition is sufficient to constitute an important part of diet, but also appropriate for the production, both quantitatively and qualitatively, of dangerous microbial growth, when the inhibitory action of cold ceases to function.

It may be a vehicle for germs pathogenic to man, owing to deficient pasteurisation of milk, contamination during production and preservation, and above all, to contamination whilst on sale (FRAZIER & WESTHOFF, 1978). The ice cream is favourable medium for the growth and survival of microorganisms, along with the exposure to environmental contamination during handling, to which ice cream is subjected whilst on sale (SÁNCHEZ et al., 1986).

Apart from checking the microbiological quality of the basic ingredients, it is especially important to control the bacteriological quality of products such as fruit, walnuts, and confectionary, which do not undergo thermal treatment before being added to the ice cream.

The chosen method of retail has an enormous influence on the degree of contamination to which the product is subjected. Ice cream sold in individual

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wrappings is less susceptible to contamination, the danger lies in the sale of ice cream in cones and other individual portions distributed by the retailers. In this case, there is a greater possibility of contamination depending on the method of sale and the personal hygiene of the handler (FERNÁNDEZ et al., 1988).

Hygiene at the retail outlet is one of the weakest links in the chain, as "soft ice cream", and that served in scoops, must invariably be handled. Personal hygiene, clean hands, clothes and healthy habits are of considerable importance, and the handlers should be prepared to use the best methods, both of storage and of distribution, regarding the individual portions of the product (IZQUIERDO et al., 1984).

AGUSTÍ (1990) is of the opinion that where ice cream mostly fails to meet the required standards in the retail, there is no significant difference between the non-pasteurised and pasteurised types. It indicates that contamination occurs after thermal treatment.

1. Materials and methods

A total of 150 samples of ice cream, sold in retail by different non industrial ice cream makers, were analysed. The chosen types were flavoured, with or without added ingredients. Out of the samples analysed, 97 were flavoured ice creams and 53 were ice creams with added ingredients.

The microorganisms studied were:

- Count of aerobic mesophile
- Total coliform count (Most probable number: MPN)
- *E. coli* count and identification
- Count of *Enterobacteriaceae* total
- Isolation and identification of *Salmonella spp.* and *Shigella spp.*
- Count and identification of enterotoxigenic *Staphylococcus aureus*
- Count of *Streptococcus* or group D

The techniques used were those stipulated in the Royal Decree 340/1987 (MINISTERIO DE RELACIONES CON LAS CORTES Y SECRETARIA DEL GOBIERNO, 1983) for the microbiological analysis of ice cream, and those recommended by I.C.M.S.F. (1983), National Centre of Nutrition (PASCUAL, 1982, 1989), Standard Methods for the Examination of Dairy Products (RICHARDSON, 1985) and by MOSSEL and MORENO (1985), for the microbiological analysis of foodstuffs.

The Duncan's multiple range test was used for statistical analysis (MILTON & TSOKOS, 1987).

2. Results

In none of the samples analysed was the presence of the pathogens *Salmonella spp.*, *Shigella spp.* or enterotoxigenic *Staphylococcus aureus* detected.

The mesophilic aerobic cell count was for the majority of the samples (47.0%) in the interval of $\geq 1 \times 10^5 - < 1 \times 10^6$ CFU $\times g^{-1}$, for both the flavoured ice creams (46.9%), and for those with added ingredients (47.2%), (Fig. 1).

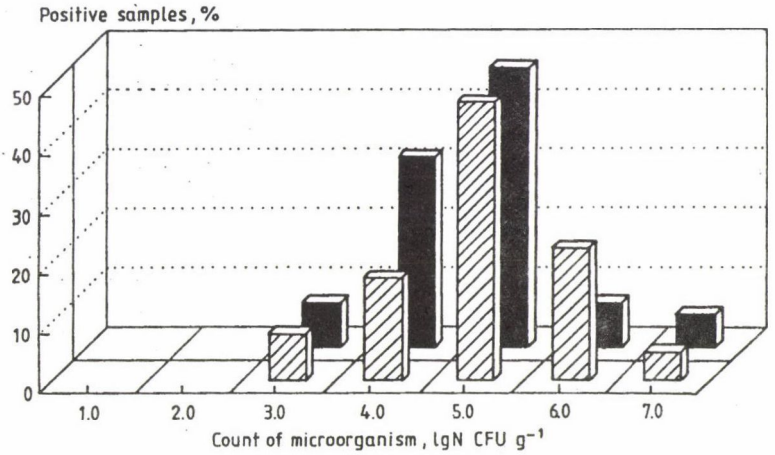

Fig. 1. Ice cream. Mesophilic aerobic cell count. ▨: Flavoured; ■: with ingredients

Concerning the total coliform count, all the samples analysed were positive, 89.4% giving a count equal to or higher than 2.400 CFU $\times g^{-1}$. These counts were the same for both the flavoured ice cream (89.8%) and for those with added ingredients (88.7%), (Fig. 2).

In case of *Enterobacteriaceae* total count, the greatest number of samples was in the interval of $1 \times 10^3 - 1 \times 10^4$ CFU $\times g^{-1}$, in both type of ice creams. It is important to point out that 5.7% of the ice cream with added ingredients showed a count of 10^6 CFU $\times g^{-1}$, against 2.0% of flavoured ice cream (Fig. 3).

In the study of *Streptococcus* group D, in 49% of the total number of samples, a count $\geq 1 \times 10^1 - < 1 \times 10^2$ CFU $\times g^{-1}$ was obtained. For flavoured ice cream it was 40.8%, and 64.1% for ice cream with added ingredients (Fig. 4).

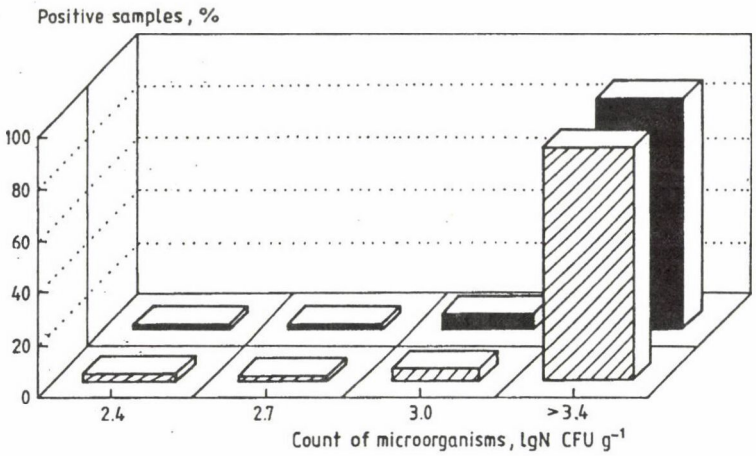

Fig. 2. Ice cream. Total coliforms. ▨: Flavoured; ■: with ingredients

3. Discussion

As regards the main average values ($CFU \times g^{-1}$) obtained in the different microbiological parameters analysed for flavoured ice cream, an average count of 9.3×10^6 for aerobic mesophiles, 2.2×10^3 for the total coliforms, 5.7×10^4 for *Enterobacteriaceae*, and 1.6×10^2 for the *Streptococcus* group D was obtained. In Table 1, these average values and their distribution, according to brand and flavour, are given.

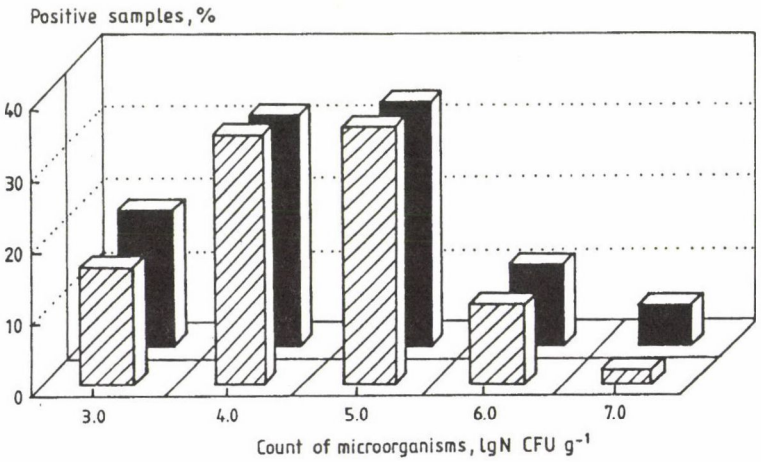

Fig. 3. Ice cream. Total *Enterobacteriaceae*. ▨: Flavoured; ■: with ingredients

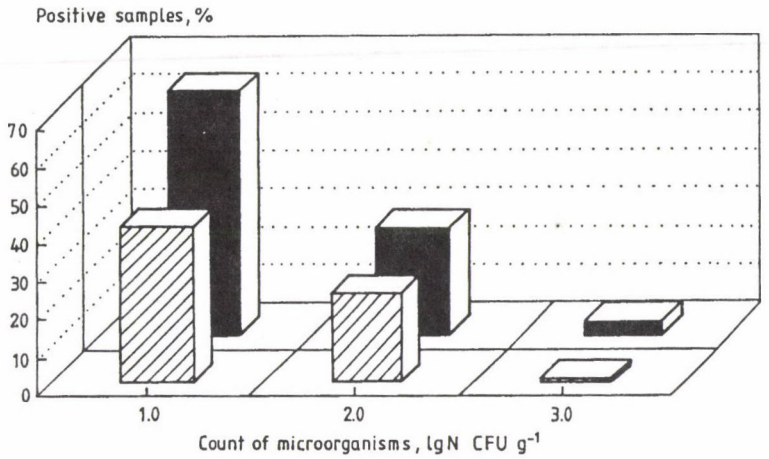

Fig. 4. Ice cream. *Streptococcus* group D. ▨: Flavoured; ■: with ingredients

The variance analysis of these average values does not show significant differences between brands and flavours. It is important to point out that there were significant differences ($p<0.05$) for the *Enterobacteriaceae* as regards flavour; pistachio flavour, with an average of 1.5×10^5 CFU $\times g^{-1}$, having the highest average count as opposed to vanilla, strawberry and chocolate, where no significant differences were observed.

For ice cream with added ingredients, the average counts (CFU $\times g^{-1}$) were for aerobic mesophiles 2.5×10^6, for coliforms 2.2×10^3, for *Enterobacteriaceae* 1.7×10^5 and for *Streptococcus* group D 2.5×10^2 (Table 2). In the variance analysis of these average values, significant differences were observed ($p<0.05$) only for the coliforms.

Table 1

Flavoured ice cream. Average values of the microbiological parameters
(CFU × g^{-1})

Microorganisms	Total	Mark				Flavour			
		A	B	C	D	Vanilla	Straw-berry	Chocolate	Pista-chio
Mesophilic aerobic total count	9.3×10^6	1.2×10^6	3.3×10^7	2.8×10^6	3.2×10^5	3.3×10^6	2.2×10^7	2.2×10^6	1.8×10^6
Total coliforms	2.2×10^3	2.3×10^3	2.0×10^3	2.3×10^3	2.2×10^3	2.1×10^3	2.2×10^3	2.2×10^3	2.4×10^3
Total *Enterobacteriaceae*	5.7×10^4	8.1×10^4	1.3×10^4	1.0×10^5	8.7×10^3	8.2×10^3	1.0×10^4	3.0×10^4	1.5×10^5
Streptococcus group D	1.6×10^2	1.6×10^2	2.9×10^2	1.1×10^2	7.7×10	9.6×10	1.1×10^2	3.0×10^2	5.8×10

Table 2

Ice cream with ingredients. Average values of the microbiological parameters (CFU $\times g^{-1}$)

Microorganisms	Total	Mark				With additions		
		A	B	C	D	Málaga	Turrón	Tutti-frutti
Mesophilic aerobic total count	2.5×10^6	1.8×10^5	8.5×10^6	1.5×10^6	1.4×10^6	2.0×10^6	6.2×10^6	9.9×10^4
Total coliforms	2.2×10^3	2.3×10^3	1.9×10^3	2.4×10^3	1.9×10^3	1.9×10^3	2.4×10^3	2.4×10^3
Total *Enterobacteriaceae*	1.7×10^5	2.1×10^4	2.8×10^5	3.9×10^5	1.4×10^4	1.2×10^5	4.9×10^5	3.0×10^3
Streptococcus group D	2.5×10^2	7.9×10	5.7×10^2	2.8×10^2	7.1×10	3.4×10^2	2.6×10^2	4.8×10

Table 3

Percentages of samples of flavoured ice cream suitable and unsuitable according to the microbiological reference limits

Microorganisms	Microbiological references values	Total	Mark					Flavour				
			A	B	C	D	Signif. (p)	Vanilla	Straw-berry	Chocolate	Pista-chio	Signif. (p)
Mesophiles	Suitable $\leq 2\times10^5$	38.8	9.2	13.3	7.1	9.2	N.S.	19.2	14.3	11.2	4.1	N.S.
	Not suitable $>2\times10^5$	61.2	19.4	9.2	17.3	15.3		18.4	16.3	18.4	8.2	
Total coliforms	Suitable $\leq 2\times10^2$	0.0	0.0	0.0	0.0	0.0	–	0.0	0.0	0.0	0.0	–
	Not suitable $>2\times10^2$	100.0	28.6	22.4	24.3	24.5		27.5	30.6	29.6	12.2	
Total *Enterobacteriaceae*	Suitable $\leq 2\times10^3$	29.6	7.1	12.2	3.1	7.1	$p < 0.01$	7.1	9.2	11.2	2.0	N.S.
	Not suitable $>2\times10^3$	70.4	21.4	10.2	21.4	7.3		20.4	21.4	18.4	10.2	
Streptococcus group D	Suitable $\leq 1\times10^2$	74.5	18.4	19.4	15.3	3.1	$p < 0.05$	19.4	24.5	20.4	10.2	N.S.
	Not suitable $>1\times10^2$	25.5	10.2	3.1	9.2	3.1		8.2	6.1	9.2	2.0	

Significance: – There are no significant facts
N.S. There are no significant differences

In Table 3, for each of the microbiological parameters studied, the percentages of the samples of flavoured ice cream which do or do not meet the microbiological reference values, are given.

As far as the aerobic mesophilic microorganism count is concerned, only 38.8% of the samples was below the value considered as acceptable ($\leq 2 \times 10^5$ CFU $\times g^{-1}$), and no significant differences between brand and flavour were observed.

The total count of aerobic mesophilic microorganisms was considered as an index of the sanitary conditions of the foodstuff. However, no clear correlation between the number of total germs and other microbiological parameters was observed; thus undertaking the study of the different hygienic-sanitary indicators is important. It represents an indicator for the microbiological quality, rather than a potential danger or deterioration of the foodstuff, and to obtain figures for ice cream under current conditions would be necessary (MATHUSEK et al., 1992; MISKIMIN et al., 1976).

For the total coliforms (M.P.N. $\times g^{-1}$), 100% of the samples exceeded the reference value, although the presence of *E. coli* was not identified at all.

In the *Enterobacteriaceae* total count, 70.4% of the total of the samples exceeded the reference value ($\leq 2 \times 10^3 \times g^{-1}$), and this percentage corresponded to 21.4% of brands A and C, 10.2% and 7.5% for brands B and D; a significant difference between the brands ($p < 0.01$) was observed, but not between the flavours. As regards the latter, the percentages of samples which exceeded this microbiological value were 20.4%, 21.4%, 18.4% and 10.2% for vanilla, strawberry, chocolate and pistachio, respectively.

These results were similar to those of other authors (COUSINS & MARLATT, 1990; IZQUIERDO et al., 1984; LLONA et al., 1984; MIGUEL et al., 1985; SÁNCHEZ et al., 1986) where the presence of *Enterobacteriaceae* and coliforms – and even fecal ones – was detected almost in all samples analysed.

The determination of *Streptococcus* group D of Lancefield seemed necessary, owing to its greater resistance to cold, and to the fact that it was a good indicator which did not need confirmation.

The percentage of samples showing a count higher than $1 \times 10^2 \times g^{-1}$ for the *Streptococcus* group D was 25.5% for all the samples. A significant difference was obtained ($p < 0.05$), amongst brands, with a percentage of unsuitability of 10.2%, 3.1%, 9.2% and 3.1% for brands A, B, C and D.

Table 4

Percentage of samples of ice cream with ingredients suitable and unsuitable according to the microbiological reference limits

Microorganisms	Microbiological references values	Total	Mark				Sig. ( )	With ingredients			Sig. ( )
			A	B	C	D		Malaga	Turrón	Tutti-frutti	
Mesophilic aerobic total count	Suitable $\leq 3\times10^5$	62.3	20.7	9.4	11.3	20.7	$p < 0.01$	28.4	11.3	22.6	$p < 0.01$
	Not suitable $> 3\times10^5$	37.7	5.6	15.1	13.2	3.8		26.4	11.3	0.0	
Total coliforms	Suitable $\leq 3\times10^2$	5.6	0.0	3.7	0.0	1.9	–	5.7	0.0	0.0	–
	Not suitable $> 3\times10^2$	94.3	24.4	20.7	24.5	22.6		49.1	22.6	22.6	
Total *Enterobacteriaceae*	Suitable $\leq 3\times10^3$	24.5	7.5	5.6	1.9	9.4	N.S.	9.4	0.0	15.1	N.S.
	Not suitable $> 3\times10^3$	75.5	18.9	18.9	22.6	15.1		45.3	22.6	7.5	
Streptococcus group D	Suitable $\leq 1\times10^2$	79.2	22.6	18.9	15.1	22.6	$p < 0.01$	45.3	11.3	22.6	$p < 0.01$
	Not suitable $> 1\times10^2$	20.7	3.8	5.6	9.5	1.9		9.4	11.3	0.0	

Significance: – There are no significant facts
N.S. There are no significant differences

For ice cream with added ingredients (Table 4), the aerobic mesophilic count gave favourable results, since only 37.7% of the samples exceeded the reference value, against 61.2% of flavoured ice cream. This did not happen with the total coliform count (M.P.N. $\times g^{-1}$), nor with the *Enterobacteriaceae* (unsuitable samples are in 94.3% and 75.5%, respectively). In case of flavoured ice cream, the presence of *E. coli* was not detected.

As far as the *Streptococcus* group D was concerned, 79.2% of the samples did not exceed the value $1 \times 10^2 \times g^{-1}$. Of the 20.7% considered as unsuitable, 3.8%, 5.6%, 9.5% and 1.9% corresponded to brands A, B, C and D, amongst which significant differences were obtained, both for brands and for flavours. For the latter, the percentage of unsuitable samples was 9.4% and 11.3% for "Málaga" and "Turrón" flavoured ice cream; "Tutti-frutti" did not exceed the reference value at any time.

From the analyses undertaken, the presence of added ingredients in ice cream sold in retail was not found to have significant effects on its microbial content.

As far as the presence of *Staphylococcus aureus*, *Salmonella spp.*, and *Shigella spp.* were concerned, the results showed negative in all the samples analysed. This corresponded to results found by POLO and co-workers (1978), IZQUIERDO and co-workers (1984), MIGUEL and co-workers (1985), SÁNCHEZ and co-workers (1986), FERNÁNDEZ and co-workers (1988) and AGUSTÍ (1990), who detected a slight presence of the former, perhaps due to the freezing process which destroys them. Pathogenic *Staphylococcus* can barely compete with lactic acid bacteria, thus their presence gives an indication of contamination by the handlers, although it is important to take into account that pasteurised foodstuffs constitute the ideal basis by far for the development of such *Staphylococcus* (UBACH et al., 1990). All of the samples analysed were considered to be unsuitable, the great majority having exceeded two or more microbiological reference values.

Likewise, according to the high percentage of positive samples, we consider it necessary to include the *Streptococcus* group D to the microbiological specifications for this type of dairy product.

Literature

AGUSTÍ, C. (1990): Carga microbiana an el helado. Origen y control. *Alimentación, equipos y techología*, *25*, 109–112.

COUSINS, D. L. & MARLATT, F. (1990): An evaluation of a conductance method for the enumeration of *Enterobacteriaceae* in milk. *J. Fd Prot.*, *53*, 568–570.

FERNÁNDEZ, A., DURÁN, A. & MAESTRE, J. (1988): Campaña de control de helados. Efectuada por el Laboratorio Municipal de Salud Pública de Elda. *Técnicas de Laboratorio*, *147*, 38–44.

FRAZIER, W. C. & WESTHOFF, D. C. (1978): *Food microbiology*. McGraw-Hill Book Company, New York, p. 512.

I.C.M.S.F. (INTERNATIONAL COMMISSION ON MICROBIOLOGICAL SPECIFICATIONS FOR FOODS) (1983): *Microorganismos de los alimentos 1.* Técnicas de análisis microbiológico. Acribia, Zaragoza, p. 431.

IZQUIERDO, J., GRIFOL, F. & LUCENA, F. (1984): Campaña de control microbiológico-sanitario de helados. *Alimentaria, 154*, 29–31.

LLONA, J., DEL VALLE, A., ZUBIAUR, L. A., DE LA FUENTE, I., ZILUAGA, J. M. & MAMOLAR, A. (1984): Control sanitario de helados fabricados, almacenados y extendidos en el municipio de Bilbao. *Comunicación a la IV Reunión Científica de Grupo de Microbiología de Alimentos de la SEM.*

MATUSHEK, M. G., CURIALE, M. S., MCALLISTER, J. S. & FOX, T. L. (1992): Comparison of various plating procedures for the detection and enumeration of coliforms in ice cream and ice milk. *J. Fd Prot., 55*, 113–115.

MIGUEL, A., PÉREZ, M. & UBACH, M. (1985): Estudio de la contaminación bacteriana de los helados artesanales de Palma de Mallorca. *Alimentaria, 160*, 73–77.

MILTON, J. S. & TSOKOS, J. O. (1987): *Estadistica para Biologia y Ciencias de la Salud*, Emalsa, Interamericana, División de McGraw-Hill, Madrid, pp. 289–350.

MINISTERIO DE RELACIONES CON LAS CORTES Y SECRETARIA DEL GOBIERNO (1983): REAL DECRETO 670 de la Presidencia del Gobierno por el que se aprueba la Reglamentiación Técnico-Sanitaria para la elaboración, circulación y comercio de helados. BOE núm. 78, de 2 de abril.

MISKIMIN, D. K., BERKOWITZ, K. A., SOLBERG, M., WERFRANKE, W. C., BUCHNANAN, R. L. & OLEARY, V. (1976): Relationships between indicator organism and especific pathogens in potentially hazardous foods. *J. Fd Sci., 41*, 1001–1006.

MOSSEL, D. A. A. & MORENO, B. (1985): *Microbiología de los alimentos. Fundamentos ecológicos para garantizar y comprobar la inocuidad y calidad de los alimentos.* Acribia, Zaragoza, p. 375.

PASCUAL, M. R. (1982): *Técnicas de Análisis Microbiológico de Alimentos y Bebidas.* Centro Nacional de Alimentación y Nutrición (CeNAM). Ministerio de Sanidad y Consumo, Madrid, p. 350.

PASCUAL, M. R. (1989): *Microbiología Alimentaria: Detección de Bacterias con significado Higiénico-Sanitario.* Centro Nacional de Alimentación y Nutrición (CeNAM). Ministerio de Sanidad y Consumo, Madrid, p. 440.

POLO, L. M., HERRER, A. & POZO, R. (1978): Identificación mediante la técnica de los anticuerpos fluorescentes de *E. coli* y *Salmonella* en helados de nata. *Anales de Bromatología, 30*, 151–156.

RICHARDSON, G. A. (1985): *Standard methods for examination of dairy products.* 15th ed. American Public Health Association, Washington, DC, p. 412.

SÁNCHEZ, J. C., MOLINA, R. & GONZÁLEZ, A. (1986): Control higiénico sanitario de los helados de venta a granel en Albacete. *Alimentaria, 97*, 93–98.

UBACH, M., MIGUEL, A. & PUIG, P. (1990): Comparación entre el método rápido-1 de la A.O.A.C. y el método oficial para el recuento de *E. coli* en helados. *Centro de Investigacion y Contol de la Calidad (C.I.C.C.), 6*, 30–33.

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ANTIOXIDANT ACTIVITY OF EXTRACTS AND ESSENTIAL OILS FROM TURKISH SPICES ON SUNFLOWER OIL

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Antioxidant effects of 35 methanol extracts and 20 essential oils from Turkish spices were tested in sunflower oil stored at 70 °C, by measuring peroxide values after regular intervals. Rosemary, Turkish sage and sumac extracts were found to be most effective in stabilizing sunflower oil, followed by wild thyme, black thyme. Cretan savory and marjoram in a decreasing order. Also rosemary and Turkish sage essential oils showed strong inhibitory effects, then marjoram and Greek oregano. The extracts exhibited high antioxidant activity compared with the essential oils. It appears that there was a relationship between the effect and the chemical composition of both extracts and essential oils, suggesting mainly their phenolic compounds. Sumac, due to its great activity competitive with rosemary and sage, had certainly potential as a new source of antioxidant substance.

Keywords: antioxidant activity, spices, extracts, essential oils, sunflower oil, peroxide value, sumac

Antioxidants commonly used in food products today are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). In recent years the safety of synthetic food additives, including the possible toxicity of these chemicals used as antioxidants has received increasing attention. So, there is need for other components to act as antioxidants and to render food products safer for mankind (BRANEN, 1975; KAHL & KAPPUS, 1993).

The most important natural antioxidants being exploited commercially are tocopherols, but unfortunately they are much less effective than BHA or BHT. The search for and development of other antioxidants of natural origin are, therefore, highly desirable (POKORNY, 1991; LOLIGER & WILLE, 1993). Spices used worldwide for culinary purposes have gained the interest of many research groups. Ground material or various extracts from such sources have been assessed so for as potent antioxidants in lipid systems (CHIPAULT et al., 1952; CHIPAULT et al., 1955, PALITZSCH et al., 1969; LOTTER, 1971; SAITO et al., 1976; ABDEL-FATTAH & EL-ZEANY, 1979; SZÁNTÓ-NÉMETH, 1980; KANDA & NAKAJIMA, 1981; HUANG et

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al., 1982; JI et al., 1992; AKGÜL & AYAR, 1993). The results of the studies were reviewed by some authors (HERRMANN, 1981; GERHARDT & SCHRÖTER, 1983; AKGÜL, 1989).

Among the spices extensively studied those obtained from the plants of Labiatae family appear to be extremely important. Today, rosemary is exploited on a commercial scale. The antioxidant activity of the spice and its extracts are attributed to the phenolic components (CHANG et al., 1977; BRACCO et al., 1981; WU et al., 1982; INATANI et al., 1983; HOULIHAN et al., 1985; CHEN et al., 1992).

In addition to ground spices, different extracts of spices were tested generally as antioxidants, however, very little is known about the extent to which essential oils contribute to the stabilizing activity of certain spices. These oils contain some phenolic compounds which can act as antioxidants (SEHER & IVANOV, 1976; FARAG et al., 1989a; 1989b; FARAG et al., 1990; LAGOURI et al., 1993). This is due to the strong typical flavour of the oil, which is a serious limitation. In certain foods, however, these oils may have a favourable effect.

In general, the ground spices or their extracts have been evaluated in lard and soybean oil. The chemical compositions of spices, differ due to several factors e.g. plant variety, climate, soil, cultivation practices, harvesting time, processing method. Therefore, and also for the exploitation of indigenous sources, this preliminary research work was conducted to study the effects of extracts and/or essential oils from 34 Turkish spices as antioxidants in sunflower oil.

1. Materials and methods

1.1. Plant material

The 34 spices, both fruit and leaf in case of dill and parsley, used in this research were given in Table 1. Of these, balm, capers, laurel, marjoram, mountain tea, mustard, myrtle, oregano, pickling herb, rosemary, sage, Cretan savory, sumac, wild thyme and black thyme were wild growing plants, and the rest were cultivated ones. The plants were purchase from miscellaneous districts of Turkey, and identified botanically at Biology Department of Selçuk University.

1.2. Preparation of extracts

The 35 plant organs, except for oregano due to insufficient sample, were dried at room temperature and ground to pass 0.5 mm sieve. Ground materials were extracted with pure methanol (E. Merck, Darmstadt, Germany) in a Soxhlet apparatus. This solvent was preferred because of being the most effective one already reported in suchlike works. The crude extracts were filtered and concentrated in rotary evaporator, and kept in sealed dark bottles under refrigerated and frozen conditions until used.

Table 1

Spice materials obtained extracts and/or essential oils

Common name	Botanical source	Plant family	Part used
Ajowan	*Trachyspermum ammi* (L.) Link	Umbelliferae	Fruit
Anise	*Pimpinella anisum* L.	Umbelliferae	Fruit
Balm, lemon	*Melissa officinalis* L.	Labiatae	Leaf
Basil, sweet	*Ocimum basilicum* L.	Labiatae	Leaf
Black Cumin	*Nigella sativa* L.	Ranunculaceae	Seed
Capers	*Capparis spinosa* L.	Capparidaceae	Flower Bud
Celery	*Apium graveolens* L.	Umbelliferae	Fruit
Chili	*Capsicum frutescens* L.	Solanaceae	Fruit
Coriander	*Coriandrum sativum* L.	Umbelliferae	Fruit
Cumin	*Cuminum cyminum* L.	Umbelliferae	Fruit
Dill	*Anethum graveolens* L.	Umbelliferae	Leaf, Fruit
Fennel, sweet	*Foeniculum dulce* Mill.	Umbelliferae	Fruit
Fenugreek	*Trigonella foenum-graecum* L.	Leguminosae	Seed
Hops	*Humulus lupulus* L.	Urticaceae	Flower
Laurel, bay	*Laurus nobilis* L.	Lauraceae	Leaf
Mahaleb	*Cerasus mahaleb* (L.) Mill.	Rosaceae	Seed
Marjoram, sweet	*Origanum majorana* L.	Labiatae	Leaf + Flower
Mountain Tea	*Sideritis tmolea* P.H. Davis	Labiatae	Leaf + Flower
Mustard, wild	*Sinapis arvensis* L.	Cruciferae	Seed
Myrtle	*Myrtus communis* L.	Myrtaceae	Leaf
Oregano, Greek	*Origanum vulgare* L. ssp. *hirtum* letswaart (Link)	Labiatae	Leaf + Flower
Parsley	*Petroselinum sativum* Hoffm.	Umbelliferae	Leaf, Fruit
Pickling Herb	*Echinophora tenuifolia* L.	Umbelliferae	Leaf
Rosemary	*Rosmarinus officinalis* L.	Labiatae	Leaf
Safflower	*Carthamus tinctorius*	Compositae	Flower
Sage, Turkish	*Salvia fruticosa*, Mill.	Labiatae	Leaf
Savory, summer	*Satureja hortensis* L.	Labiatae	Leaf
Savory, Cretan	*Satureja thymbra* L.	Labiatae	Leaf + Flower
Sesame	*Sesamum indicum* L.	Pedaliaceae	Seed
Spearmint	*Mentha spicata* L.	Labiatae	Leaf
Sumac	*Rhus coriaria* L.	Anacardiaceae	Fruit
Tarragon	*Artemisia dracunculus* L.	Compositae	Leaf
Thyme, wild	*Thymus serpyllum* L.	Labiatae	Leaf + Flower
Thyme, black	*Thymbra spicata* L.	Labiatae	Leaf + Flower

1.3. Preparation of essential oils

The 20 ground materials (the others were not used either due to their trace essential oil content or insufficient samples) were hydrodistilled for 3 h with a Clevenger apparatus. The oils were dried over anhydrous sodium sulphate, and kept in sealed dark bottles under cool conditions until used.

1.4. Sunflower oil

Refined oil without adding any antioxidant was kindly supplied by Paksoy Company in Adana city. Its peroxide number was 1.8 meq kg^{-1}. The oil was selected for its high degree unsaturation (80–90%) level and for being the most widely used as edible oil in Turkey.

1.5. Antioxidant activity measurement

The antioxidant activity of the extracts and essential oils was tested on sunflower oil and expressed as the decrease in the rate of peroxide formation. A calculated quantity of the extract or essential oil was added into sunflower oil, and the mixture was stirred. BHA-containing (Eastman Chem. Int., Switzerland) and control samples (without adding any antioxidant) were also prepared under the same conditions. All samples of 20 g each were incubated in 10×100 mm open beakers at 70 °C in the dark. The peroxide values of the samples were determined at definite time intervals according to the Method Cd 8–53 (updated 1992) of the American Oil Chemists' Society (A.O.C.S., 1989).

2. Results and discussion

2.1. Antioxidant activity of the extracts

Table 2 presents the antioxidant activity of the methanol extracts, compared with that of the BHA-containing and the control samples. Of the extracts, the most effective ones are also shown in Fig. 1.

After 7 days, all the extracts showed antioxidant effect in varying degrees on sunflower oil compared with the control test, except for only balm and mountain tea. Antioxidant activities of balm, coriander, dill (both fruit and leaf), fennel, fenugreek, hops, mountain tea, mustard, safflower and spearmint were slightly weaker than that of BHA. The most effective extracts were rosemary, sumac, summer savory, black cumin, sage, basil, anise, black thyme, marjoram, ajowan, Cretan savory, sesame and wild thyme, in descending order.

Until day 14 the extracts of marjoram, sumac, wild thyme, Cretan savory, black thyme, and especially rosemary and sage maintained their antioxidant activity, exhibiting a marked effect in comparison with BHA. The activity continued also until day 21, except for marjoram, however, after 28 days only three extracts had a persistent effect i.e. rosemary, sumac and sage, respectively.

Table 2

Antioxidant activity of spice extracts added to sunflower oil[a] stored in the dark at 70 °C

Extract (0.2%)	Peroxide value[b] (meq kg^{-1}) after days			
	7	14	21	28
Ajowan	27.3[c]	157.8	569.9	967.0
Anise	21.8	175.9	587.6	798.0
Balm	75.4	297.5	964.8	527.0
Basil	21.6	243.2	817.9	513.0
Black Cumin	19.7	279.5	411.8	797.0
Capers	44.2	202.6	852.4	985.0
Celery	41.4	191.8	668.2	.[d]
Chili	48.9	188.6	647.6	705.6
Coriander	55.9	282.4	670.3	341.0
Cumin	40.3	227.5	627.8	815.0
Dill (fruit)	62.4	267.6	728.4	613.0
Dill (leaf)	54.3	232.5	614.2	427.0
Fennel	50.5	232.5	814.7	–
Fenugreek	55.0	235.7	476.4	804.7
Hops	63.2	289.5	867.0	948.0
Laurel	39.5	197.5	657.8	797.0
Mahaleb	45.6	298.6	525.5	885.0
Marjoram	26.2	73.1	417.4	690.0
Mountain Tea	77.9	268.0	457.9	897.0
Mustard	65.0	228.7	785.0	544.7
Myrtle	40.0	137.0	892.6	502.0
Parsley (fruit)	46.8	186.0	881.9	632.0
Parsley (leaf)	41.1	169.5	697.0	–
Pickling Herb	39.5	179.6	442.7	–
Rosemary	8.2	31.6	51.6	69.5
Safflower	62.6	447.6	964.3	378.0
Sage	20.9	34.3	67.8	149.7
Savory (summer)	16.4	165.6	421.1	579.0
Savory (Cretan)	27.9	63.6	198.0	735.0
Sesame	29.1	137.1	696.9	467.0
Spearmint	67.4	185.9	395.6	517.0
Sumac	13.2	68.5	87.4	137.0
Tarragon	33.4	136.6	695.5	–
Thyme (wild)	31.4	65.8	157.6	667.0
Thyme (black)	22.0	57.4	184.4	592.5
BHA (0.02%)	49.2	497.0	1035.0	–
Control	73.0	567.0	943.0	–

[a] Initial (zero day) peroxide value of the oil was 1.8 meq kg^{-1}.

[b] Data are the average of two separate experiments.

[c] Each value is the mean of two determinations.

[d] Not tested due to the polymerisation of the oil.

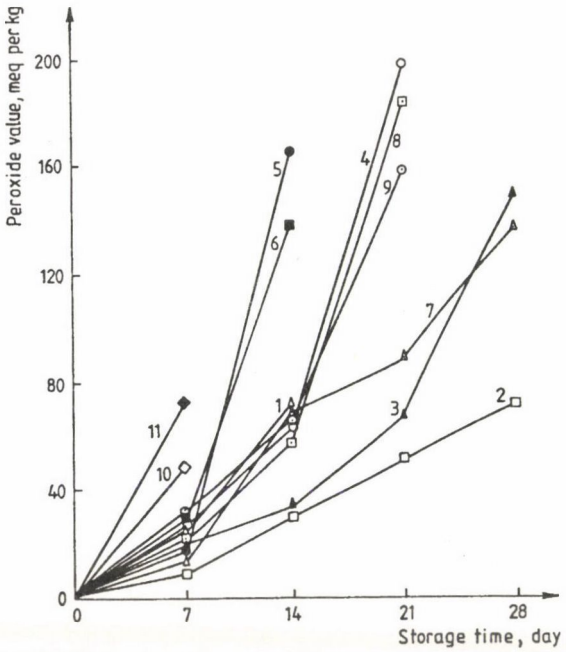

Fig. 1. Antioxidant activity of selected spice extracts. 1: marjoram; 2: rosemary; 3: sage; 4: savory, cretan; 5: savory, summer; 6: sesame; 7: sumac; 8: thyme, black; 9: thyme, wild; 10: BHA; 11: control

Pronounced antioxidant effect was obtained generally with the extract of the spices belonging to Labiatae family, in agreement to previous works (ECONOMOU et al., 1991; BANIAS et al., 1992). Overall strongest activity of rosemary was not surprising, because of various findings reported on its stabilizing effect (CHANG et al., 1977; BRACCO et al., 1981), and related active components such as carnosol, rosmanol, rosmariquinone, carnosic and ursolic acids etc. (WU et al., 1982; INATANI et al., 1983; HOULIHAN et al., 1985; CHEN et al., 1992). Some similar data were also found for sage (RUSSO, 1976; CHANG et al., 1977).

Sumac extract, however, presenting quite high activity comparable to rosemary and sage promises as a new source of natural antioxidant. Sumac fruit (pericarp) used as a flavouring agent in many foods in Turkey and some Middle East countries, was not so far evaluated for antioxidant properties, although its taste and aroma constituents were reported (AKGÜL, 1993; BRUNKE et al., 1993). The potent action of sumac extract is probably owed to the presence of high amount of organic acids (citric, tartaric, malic etc.) and/or tannin content, and further attempts should be made to establish the related compounds and their antioxidant effect, like in some other cases reported (HERRMANN, 1973; PAPADOPOULOS & BOSKOU, 1991; VEKIARI et al., 1993).

2.2. Antioxidant activity of the essential oils

The results and the most active oils are shown in Table 3 and Fig. 2, respectively.

Table 3

Antioxidant activity of spice essential oils added to sunflower oil stored in the dark at 70 °C

Essential oil (0.02%)	Peroxide value (meq kg^{-1}) after days			
	7	14	21	28
Anise	35.6	225.8	438.5	890.0
Basil	45.2	344.6	997.6	325.0
Celery	49.0	248.0	726.0	–
Coriander	59.0	314.8	750.9	–
Cumin	48.5	352.6	897.8	–
Dill (fruit)	67.1	293.2	845.9	–
Fennel	64.3	265.0	929.4	450.0
Hops	69.0	367.5	739.0	–
Laurel	43.5	163.0	711.0	–
Marjoram	33.1	77.3	478.4	770.0
Mustard	81.0	260.6	775.5	934.0
Oregano	34.0	80.2	264.8	836.0
Parsley (fruit)	52.3	209.3	917.5	544.0
Parsley (leaf)	60.8	179.6	945.6	527.0
Pickling Herb	45.1	197.6	583.5	–
Rosemary	25.5	47.2	79.8	100.7
Sage	29.0	69.4	54.6	217.0
Savory (summer)	22.0	203.4	599.4	420.0
Spearmint	75.4	317.0	461.7	–
Tarragon	39.0	164.0	567.0	780.0
BHA (0.02%)	49.2	497.0	1035.0	–
Control	73.0	567.0	943.0	

Footnotes as in Table 2.

On day 7, the oils showed some antioxidant effect, except for mustard and spearmint, compared with the control test. Coriander, dill (both fruit and leaf), hops, mustard, parsley leaf and spearmint oils were less effective than BHA. But all the oils exhibited greater persistent activity than BHA up to 21st day of storage. On day 7 the most active oils were those of summer savory, rosemary, sage, marjoram, oregano, anise and tarragon, respectively. The greatest antioxidant activities were exhibited by rosemary, sage, marjoram and oregano on day 14, however, by only rosemary and sage at days 21 and 28.

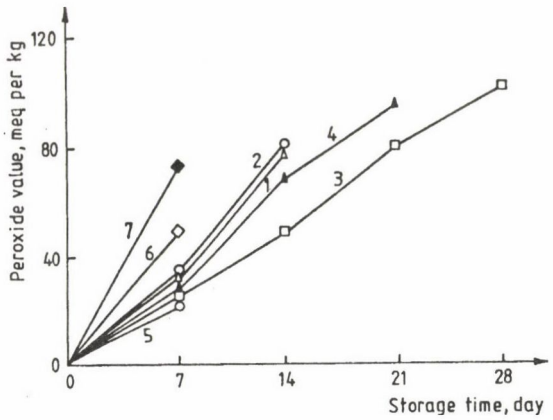

Fig. 2. Antioxidant activity of selected spice essential oils. 1: marjoram; 2: oregano; 3: rosemary; 4: sage; 5: savory, summer; 6: BHA; 7: control

Little is known about the extent to which the essential oils of spices may contribute to the antioxidant activity, although they have been extensively examined for their medicinal, culinary and antimicrobial properties (AKGÜL, 1993). SEHER and IVANOV (1976) reported antioxidant property of cumin essential oil. KRAMER (1985) has identified eugenol as one of the major antioxidants present in clove. Six spice essential oils and their major components were added to emulsified linoleic acid in aqueous media, and the antioxidant effect was reported in ascending order: caraway, sage, cumin, rosemary, thyme and clove (FARAG et al., 1989a). FARAG and co-workers (1989b) reported that clove and thyme essential oils exhibited antioxidant activity in refined cotton seed oil, with clove oil being superior to thyme oil. Also FARAG and co-workers (1990) recommended that the essential oils of cumin and especially thyme should be used to extend the shelf-life of butter during storage at room temperature. LAGOURI and co-workers (1993) examined the essential oils from four Labiatae herbs, having carvacrol and thymol as the main components, on lard at 35 °C, and reported high antioxidant effects compared with BHA.

All these results indicate that the antioxidant activity may be related the presence of carvacrol, thymol and eugenol in essential oils, and the higher effects is owed rather to the phenolic OH groups than to the terpene alcohols and ketones due to absence of aromaticity. The findings are in agreement with our data for oregano and marjoram, however, rosemary and sage essential oils had surprisingly higher persistent antioxidant activity, probably due to the composition and conditions of the experiment.

3. Conclusions

The results indicate that many spice extracts exhibited an antioxidant activity on sunflower oil at 70 °C. The most active ones were from Labiatae plants such as rosemary, sage, marjoram, thyme, savory and oregano. Essential oils from almost the same spices had high antioxidative effect compared with BHA. The extracts showed stronger activity than the essential oils. The novel finding was the potent antioxidant action of sumac extract similar to that of rosemary and sage. Further works should be made to establish the active components of sumac, and examine wether the addition of effective extracts and/or essential oils affects the flavour of various foods favourably, in relation to both the chemical composition and synergistic effect with synthetic additives.

Literature

ABDEL-FATTAH, L. E. & EL-ZEANY, B. A. (1979): Effect of spices on the autoxidation of fatty foods. *Riv. Ital. Sost. Gras.*, *56*, 441 – 443.

AKGÜL, A. (1989): Baharatlarin antioksidan özellikleri. (Antioxidant properties of spices.) *Doga Turk. J. Agric Forest.*, *13*, 11 – 24.

AKGÜL, A. (1993): *Baharat bilimi ve teknolojisi*. (Spice science and technology.) Turk. Assoc. Food Technol. Publ. No. 15, Ankara, Turkey, p. 451.

AKGÜL, A. & AYAR, A. (1993): Yerli baharatlarin antioksidan etkileri. (Antioxidant effects of Turkish spices.) *Doga Turk. J. Agric. Forest.*, *17*, 1061 – 1068.

A.O.C.S. (1989): *Official methods and recommented practices*. Vol. I, 4th edn., 2nd print. (including 1990 and 1992 additions and revisions). American Oil Chemists' Society, Champaign, Ill.

BANIAS, C., OREOPOULOU, V. & THOMOPOULOS, C. D. (1992): The effect of primary antioxidants and synergistics on the activity of plant extracts in lard. *J. Am. Oil Chem. Soc.*, *69*, 520 – 524.

BRACCO, U., LOLIGER, J. & VIRET, J.-L. (1981): Production and use of natural antioxidants. *J. Am. Oil Chem. Soc.*, *58*, 686 – 690.

BRANEN, A. L. (1975): Toxicology and biochemistry of BHA and BHT. *J. Am. Oil Chem. Soc.*, *52*, 59 – 65.

BRUNKE, E.-J., HAMMERSCHMIDT, F.-J., SCHMAUS, G. & AKGÜL, A. (1993): The essential oil of *Rhus coriaria* L. fruits. *Flavour Fragr. J.*, *8*, 209 – 214.

CHANG, S. S., OSTRIC-MATIJASEVIC, B., HSIEH, O. A. L. & HUANG, C.-L. (1977): Natural antioxidants from rosemary and sage. *J. Fd Sci.*, *42*, 1102 – 1106.

CHEN, Q., SHI, H. & HO, C.-T. (1992): Effects of rosemary extracts and major constituents on lipid oxidation and soybean lipoxygenase activity. *J. Am. Oil Chem. Soc.*, *69*, 999 – 1002.

CHIPAULT, J. R., MIZUNO, G. R., HAWKINS, J. M. & LUNDBERG, W. O. (1952): The antioxidant properties of natural spices. *Fd Res.*, *17*, 46 – 53.

CHIPAULT, J. R., MIZUNO, G. R. & LUNDBERG, W. O. (1955): Antioxidant properties of spices in oil-in water emulsions. *Fd Res.*, *20*, 443 – 448.

ECONOMOU, K. D., OREOPOULOU, V. & THOMOPOULOS, C. D. (1991): Antioxidant activity of some plant extracts of the family Labiatae. *J. Am. Oil Chem. Soc.*, *68*, 109 – 113.

FARAG, R. S., BADEI, A. Z. M. A., HEWEDI, F. M. & EL-BAROTY, G. S. A. (1989a): Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. *J. Am. Oil Chem. Soc.*, *66.*, 792 – 799.

FARAG, R. S., BADEI, A. Z. M. A. & EL-BAROTY, G. S. A. (1989b): Influence of thyme and clove essential oils on cotton seed oil oxidation. *J. Am. Oil Chem. Soc.*, *66*, 800 – 804.

FARAG, R. S., ALI, M. N. & TAHA, S. H. (1990): Use of some essential oils as natural preservatives for butter. *J. Am. Oil Chem. Soc.*, *68*, 188–191.

GERHARDT, U. & SCHRÖTER, A. (1983): Antioxidative Wirkung von Gewürzen. *Gordian*, *9*, 171–176.

HERRMANN, K. (1973): Phenolische Inhaltsstoffe als natürliche Antioxidantien. *Fette Seifen Anstrichm.*, *75*, 499–506.

HERRMANN, K. (1981): Über die Antioxidative Wirkung von Gewürzen. *Dtsch. Lebensm. Rdsch.*, *76*, 134–139.

HOULIHAN, C. M., HO, C.-T. & CHANG, S. S. (1985): The structure of rosmariquinone: a new antioxidant isolated from *Rosmarinus officinalis* L. *J. Am. Oil Chem. Soc.*, *62*, 96–98.

HUANG, J. K., WANG, C. S. & CHANG, W. H. (1982): Studies on the antioxidative activities of spices grown in Taiwan. II. *J. Chinese Agric. Chem. Soc.*, *20*, 61–66. (ref.: FSTA 9T507, 1983)

INATANI, R., NAKATANI, N. & FUWA, H. (1983): Antioxidative effect of the constituents of rosemary (*Rosmarinus officinalis* L.) and their derivatives. *Agric. biol. Chem.*, *47*, 521–528.

JI, C.-I., BYUN, H.-S., KANG, J.-H., LEE, T.-G., KIM, S.-B. & PARK, Y.-H. (1992): The antioxidative activities of spice extracts on edible soybean oil. *J. Korean Soc. Fd Nutr.*, *21*, 551–556.

KANDA, T. & NAKAJIMA, T. (1981): Natural antioxidants from herbs. *New Fd Ind.*, *23*, 36–40.

KAHL, R. & KAPPUS, H. (1993): Toxikologie der synthetischen Antioxidantien BHA und BHT im Vergleich mit dem natürlichen Antioxidans Vitamin E. *Z. Lebensmittelunters. u. -Forsch.*, *196*, 329–338.

KRAMER, R. E. (1985): Antioxidants in clove. *J. Am. Oil Chem. Soc.*, *62*, 111–113.

LAGOURI, V., BLEKAS, G., TSIMIDOU, M., KOKKINI, S. & BOSKOU, D. (1993): Composition and antioxidant activity of essential oils from oregano plants grown wild in Greece. *Z. Lebensmittelunters. u. -Forsch.*, *197*, 20–23.

LOTTER, G. (1971): *Über die antioxydative Wirkung von Gewürzextrakten und Gewürzextraktbestandeteilen in Schweineschmalz*. Vet. Med. Diss., München.

LOLIGER, J. & WILLE, H. J. (1993): Natural antioxidants. *Oils Fats Int.*, *9*, 18–22.

PALITZSCH, A., SCHULTZE, H., METZL, F. & BAAS, H. (1969): Untersuchungen über die Wirkung von Naturgewürzen, Gewürzextrakten, ätherischen Ölen, Extraktionsrückständen und synthetischen Antioxidantien auf den Abbau von Schweinefett und Modell-Lipiden. I. Wirkung von Naturgewürzen und Gewürzextrakten auf Schweinefett. *Fleischwirtschaft*, *49*, 1349–1354.

PAPADOPOULOS, G. & BOSKOU, D. (1991): Antioxidant effect of natural phenols on olive oil. *J. Am. Oil Chem. Soc.*, *68*, 669–671.

POKORNY, J. (1991): Natural antioxidants for food use. *Trends Fd Sci. Technol.* (September), 223–227.

RUSSO, J. R. (1976): Natural antioxidants from spices. *Fd Engng.*, *1*, 9–10.

SAITO, Y., KIMURA, Y. & SAKAMOTO, T. (1976): Antioxidant effect of some spices. *J. Jap. Soc. Fd Nutr.*, *29*, 404–411.

SEHER, A. & IVANOV, S. A. (1976): Natürliche Antioxydanten. 2. Mitt. *Fette Seifen Anstrichm.*, *78*, 224–228.

SZÁNTÓ-NÉMETH, É. (1980): Inhibition of rancidity of fats by paprika and tomato seeds. *Acta Alimentaria*, *9*, 173–187.

VEKIARI, S. A., OREOPOULOU, V., TZIA, C. & THOMOPOULOS, C. D. (1993): Oregano flavonoids as lipid antioxidants. *J. Am. Oil Chem. Soc.*, *70*, 483–487.

WU, J. W., LEE, M.-H., HO, C.-T. & CHANG, S. S. (1982): Elucidation of the chemical structures of natural antioxidants isolated from rosemary. *J. Am. Oil Chem. Soc.*, *59*, 339–345.

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ABSTRACTS
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STUDY OF PORE STRUCTURE OF IMMOBILIZED GLUCOAMYLASE

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Glucoamylase is the most popular enzyme used for industrial purposes in the conversion of partially hydrolysed or thinned starch to glucose syrups. It was observed that in soluble enzyme technology there is a higher glucose content than in immobilized glucoamylase technology. It means that in immobilized technology the reversion products were produced in higher concentration than that found in dissolved enzyme technology.

It is a well-known fact that the apparent reaction rate decreases during diffusion in porous media. The volume and structure of pores were determined by pulse response analysis and batch diffusion measurement. The porosity of the particles was determined from the first statistical moment of the elution curve for dextran-T10 (Mw. = 10 000) and for D-glucose. The effective diffusion coefficients and the tortuosity factors were determined from the decreasing of glucose concentration in the liquid phase under the diffusion by Laplace transformation.

The values of porosity, the effective diffusion coefficients, the tortuosity factors and rate of resynthesis reactions in case of some immobilized glucoamylase preparations were summarized in tables.

PRODUCTION OF IMMOBILIZED β-GLUCOSIDASE AND β-GALACTOSIDASE BY ONE-STEP FERMENTATION

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Cellulose, the organic matter permanently reproduced in the nature by photosynthesis, continuously provides us with the replacement of energy and nutrients, provided we are able to use profitable technology to convert in to the desired products. The first step of transformation is the glucose production by enzymes. The most often applied cellulase of *Trichoderma* origin does no contain β-glucosidase in high enough quantity, therefore using this enzyme for hydrolysis results in unsatisfactory parameters (rate of hydrolysis, obtained conversion, and composition of final product). These problems can be solved with the addition of β-glucosidase produced by other microorganism.

Another problem from energetic, nutritional and environmental pollution point of view is the break down of lactose present in the whey to monosaccharides. The common base of the two subjects is that both the β-glucosidase and the β-galactosidase are produced by fermentation of *Aspergillus phoenicis* strain QM 329. The fungus should grow in pellet form and the enzymes should remain inside the pellet. To meet these requirements the composition and pH (4.8-5.4) of medium should be appropriate. The microbiological pellets produced either in shaked flasks or in column fermentors are suitable for the use in different enzyme reactors. The dried pellets are storable, transportable, and keep 94-96% of their activity, however their reuse arises difficulties. The efflux of enzyme increases significantly, the determined half-life of 669 h is reduced to 30 h at the dried pellets. The cause of this drastic reduction of stability was studied and measures were taken to avert it.

PRODUCTION OF LACCASE ENZYME BY FERMENTATION AND ITS CLEANING

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There is an ever increasing number of research teams concerned with the study of the qualitative and quantitative composition of enzymes extracellularly produced by fungi causing white dry-rot. The production of the various members of the enzyme complex, their purification and enzymological description constitute a basic and primary prerequisite to revealing their operating mechanism, the synergic effects between the individual components, their substrate spectra, inducers and inhibitors. The knowledge of all these is required to develop those technologies that are based on the utilization of the special enzyme complexes of the various fungi causing white rot and of their individual components. In our research work we were interested in fermentative production of the laccase enzyme that is made by the fungi causing white rot. The laccase enzyme, as a polyphenoloxidase, may be of importance in decontaminating and neutralizing phenol-type environmental pollutants.

Different strains of *Lentinus edodes*, and *Pleurotus sajor-caju* strain were chosen to produce the enzyme in question.

A number of factors affect the variation in the activity of the laccase enzyme. Some of them are: the strain, the inoculant and the quality of the inoculum, the mode of breeding, the pH-value applied in the course of fermentation, the temperature, the carbon and nitrogen sources, and the presence of inducers and cofactors.

The Michaelis-Menten constant of the enzyme produced was determined in the course of enzyme-kinetic experiments.

Using ion-exchange chromatography fractionation for the enzyme of Pleurotus sajor origin, two laccase iso-enzyme fraction could be detected.

*

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FERMENTATION OF BIOLOGICALLY ACTIVE COMPOUNDS WITH FUNGI CAUSING WHITE ROT

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Out of the edible mushrooms, *Lentinus edodes* (Shiitake) is one of the most widespread species around the world, which is due to its characteristic aroma and chemical compounds that are isolated from it and that show a varied and favourable physiological traits (which effects of tumour suppression, cholesterol reduction and anti-viral influence). These bioactive substances were first isolated by Japanese researchers mostly from the productive organ, while the favourable physiological effects were observed mostly in animal tests and in human observations.

At the Bioengineering Department of Central Food Research Institute this fungus was multiplied in submerged liquid culture medium, as a result of which 1.2 – 1.5% mycelium yield was achieved using 10 dm^3 glass fermentor and a breeding period of 10 days. (Mixing: 100 – 150 r.p.m., aeration: 0.5 dm^3 per dm^3 min, temperature: 23 – 25 °C.)

Using this technology, considerable reduction could be achieved in the time required for mushroom production as compared to the traditional technologies. The mycelium pellets formed contain more aromatic substances than does the mycelium with filamentary structure.

Subsequently, in cooperation with co-workers from the University of Horticulture and Food Industry, investigation was made whether the mycelium also contains the two selected, bioactive compound groups, the eritadenine which characteristically reduces cholesterol-level in the blood (2,4-dihydroxiadenil-butyric acid) and the cyclic sulfur-bearing compounds (mostly lenthionine), which are typical aromatic components and are shown to have antibacterial effect as well.

Qualitative and quantitative determinations were carried out by IR, NMR, LC – MS and GC – MS systems. After the standard analytic study of synthesized eritadenine, the presence of dihydroxi-adenil-butyric acid could be detected in the biomass (in the pellets). The same method was applied by the Japanese researchers.

As to the other bioactive component group, prior to determination of the sulfur-containing compounds by means of GC – MS, adequate extractive method was selected (mixture of n-hexane and ethanol, 9:1). Using library identification, in case of scan-mode detection, lenthionine can be found in the mycelium and the productive body alike, along with other aroma components (octene 1 – 01, 1,2 – 4 tritiolan).

IMPORTANCE AND DESCRIPTION OF AROMATIC ALCOHOL OXIDASES (AAO) IN CASE OF FUNGI CAUSING WHITE ROT

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The special enzyme system of fungi causing white rot is already applied in several practical fields, such as:

- in paper manufacture for preparing high-quality paper-making crushed pulp, for paper-bleaching and for decomposing the chlorinated ligning compounds of sewage waters from paper-making;
- the decomposition of micro-pollutants (azo-dyes and pesticides) also belongs to those areas where the importance of white rot fungi is increasing;
- the manufacture of aromatic and scent substances (such as veratrilaldehyde) has also been given preference over the recent years;
- the extraction of lignocellulose-based by-products, the improvement of their digestibility and their use in mushroom farming (e.g. *Lentinus*, *Pleurotus* strains) are also subject to increasingly widespread application.

The efficiency of the above technologies depends mostly on the operation of the special enzyme system of the fungi causing white rot, and particularly of the lignin-peroxidase isoenzymes (LiP E.C. 1.11.1) and of the manganese-dependent peroxidase isoenzymes. The extracellular enzymes mentioned catalyze the decomposition of the aromatic ring in lignin.

Hydrogen peroxyde is required to make the enzymes function, operational. The H_2O_2, required to make the enzymes function, is released in the course of the action of glucose-oxidase, glioxaloxidase and of aromatic alcohol-oxidases (AAO).

The authors investigated the enzyme production of the strains *Pleurotus ostreatus* (MUCL 29527), *Pleurotus sajor-caju* (MUCL 29757) and *Trametes versicolor* (MUCL 28407); their AAO enzyme production features were investigated in shaken and static cultures.

In the course of the fermentation tests, *Trametes versicolor* produced no VAO enzyme. From the 7th day of the fermentation onwards, *Pleurotus ostreatus* and *Pl. sajor-caju* produce VAO enzyme as secondary metabolic product in ever increasing amount. The concentration of the enzyme with 72 000 Dalton mole-mass, with a pH-optimum at 6.4 and at pH-value of 3.9, attains 10 g l^{-1} and 5 g l^{-1} by the 20th day of fermentation.

The above work was supported by the financial grant of the Hungarian Scientific Research Fund (OTKA 1/3 5 – 375), for which the authors express their thanks.

INVESTIGATION INTO BIOSENSORS FOR CONTROLLING FERMENTATION PROCESSES

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Speedy development of biotechnology opens new possibilities for sensor technology, but at the same time new requirements arise for traditional instrumentation, too. Aim of the authors was to investigate the sensors applicable for measuring concentration of substrates during fermentation processes, and to develop sensors for determining carbohydrates (e.g. glucose, maltose) and alcohols.

One of the most important criteria for sensors used in biotechnological processes is that they should resist sterilisation. To solve this problem an on-line sensor system used in by-pass technology was developed. It consists of an enzyme-reactor and an electrochemical detector applied for measuring the product of enzyme-reaction. The enzyme or enzymes are immobilized on a protein membrane. Because of the short response time and the linear relationship between concentration and electrochemical signal the amperometric technique was chosen for detection. Pt working electrode and Ag/AgCl reference electrode were used. The sensor was examined in a flow injection system to ensure the small quantity of sample, the continuous washing the system, increasing the life-time of sensor.

For the measurement of various substrates the proper quality and quantity of enzymes, the technique of enzyme immobilization was chosen. The effect of pH, temperature, flow rate was examined on both the enzyme reaction and on the electrochemical reaction. Statistical parameters of the sensor system in different matrix (e.g. foods, fermentation broth) were determined by means of standard solutions. The biosensors developed for measuring maltose and galactose were successfully applied for controlling fermentation processes. The biosensors developed for measuring glucose and alcohol were used for testing the quality of juices and beer. Results obtained by biosensors were checked by other accepted chemical methods, too. High correlation values of the methods proved that the biosensors open up a new prospect in the field of automatization and quality assurance.

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DISTRIBUTION OF PROTEOLYTIC ENZYME ACTIVITY AMONG THE MAIN PROTEIN FRACTIONS OF SACCHAROMYCES STRAINS

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Our investigations on *S. cerevisiae* baker's yeast and *S. carlsbergensis* brewer's yeast have shown, that activities of several specific proteases are influenced by glucose concentration of the medium and aeration intensity as well. Soluble and cell wall bounded proteinase activities of these yeasts are seriously different concerning their pH-activity profile and SDS-IEF protein print (Yeast, 8, 579, 1992; Acta Alimentaria, 22, 193, 1993). In the present work two collection strains *S. cerevisiae* CBS 1395, *S. pastorianus* CBS 1503 and two *S. cerevisiae* auxotrophic mutants CB 67 and CB 89 were investigated. Measured proteinase activities were: caseinase, hemoglobin splitting activity, Azo-caseinase activity, distribution of Azo-caseinase activity among the Osborne protein fractions. Yeast was grown at 0.1 and 1.0% glucose concentrations and two aeration intensities: 100 l h^{-1} and 500 l h^{-1} respectively.

S. pastorianus showed the highest caseinase activity under fermentative conditions. Increase in aeration resulted in the decrease of activity. Azo-caseinase activity was detected in the fractions 1, 2, 4, 5 and 6. Fractions 1+2 represent 40–60% of the total activity. At higher aeration level proportion of fraction 1 increased.

S. cerevisiae also showed the highest caseinase activity under fermentative conditions. Maximum Azo-caseinase activity was detected at 1% glucose and 500 l h^{-1} aeration level. Activity was distributed between the fractions 1, 2, 4, 5 and 6, proportion of fractions 1+2 was in the range of 68–100%. At high aeration Azo-caseinase was mostly in fractions 1+2.

CB 67 auxotrophic mutant differed tremendously from the original strain, as it showed hemoglobin activity and Azo-caseinase activity was also at a higher level. Fractions 1+2 contained 90–100% of the total activity, at 1% glucose content ratio of the two fractions was 1:1 and 1:2, while at 0.1% glucose content activity appeared mainly in fraction 1. In both substrates activity-maximum was at 0.1% glucose and 500 l h^{-1}, which decreased at higher sugar concentration.

CB 89 showed highest protease activity at 0.1% glucose and 100 l h^{-1} aeration, fractions 1+2 represent 70–95% of the total activity.

Results stated that both proteinase activity level and distribution the activity are different at fermentative and respirative metabolism, respectively.

NITRIFICATION AND DENITRIFICATION IN FLUIDIZED BED SYSTEMS

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The elimination of nutrients i.e. the nitrogen and the phosphorous is the aim of the waste water treatment development. Waste waters with high nitrogen content cause problems also in certain fields of food industry (meat- and poultry processing, canning), especially when discharged into rivers or other natural water bodies. In activated sludge systems however the biological nitrogen removal – especially in case of plants originally not designed for this task – requires significant land area and financial investment.

Our team has remarkable experience in development and utilisation of fluidized bed bioreactors. The rate of denitrification accomplished at industrial size (50 m^3/d) for drinking water denitrification reached 11 kg NO_3-N/m^3d. Pilot scale nitrification was operated at a rate of 1-3 kg NH_4^+-N/m^3d for both waste and drinking water. It seemed desirable to join these processes – both having a rate an order of magnitude higher than the equivalent activated sludge processes – in a single unit, this way further simplifying the system.

With appropriate reactor shape, carrier size and with the control of the redox conditions in the reactor one can create two zones having very different characteristics in one single fluidized bed*. A reactor of this type (with 20 l fluidized bed volume) was operated for three months. The total nitrogen removal rate accomplished was 0.8-1.2 kg N/m^3d. A new simple hydrostatic pressure method was used to monitor biofilm thickness in the fluidized bed.

* Patent pending

PRODUCTION AND APPLICATION OF MICRO ELEMENT ENRICHED YEASTS

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Under adequate circumstances yeasts are able to take up micro elements and incorporate them into organic compounds in their cells. In most cases the amount of the accumulated element is multiple of the normal level in the yeasts.

These new natural micro element resources are more suitable for and favourable to both human and animal organism. Not only baker's yeast (*Saccharomyces cerevisiae*), but yeast for feed (*Candida utilis*) can accumulate and build in micro elements in to the cell. These "biotransformed" micro elements show better absorption quotients, are less toxic, have a pleasant taste and are easier to portion in dosages and to package. Another benefit is the protein of full value and high vitamin content of the yeast.

Micro element enriched yeasts and their water-soluble extracts can be utilized as additives in all areas of food industry. The first developed food product is a special bread for medicinal purpose, supplied with selenium-yeast.

Another field of application is the production of paramedicinal preparations. In Hungary and in some other European countries Biorex Corp. has recently introduced a capsule under the trade name Epasel, which contains selenium-yeast, polyunsaturated fatty acids (EPA, DHA) and vitamin E. This product combines favourable effects helping decrease the development of certain cardiovascular diseases. A similar product is prepared by Vireco Ltd. under the trade name Kondi. This product contains zinc-yeast vegetable lecithin and vitamin E. This mixture can improve general physical condition especially in young children, pregnant or suckling mothers as well as women taking contraceptive pills. It can reduce the toxic effect of heavy metals coming from the polluted environment.

ENZYMATIC MODIFICATION OF FOOD PROTEINS

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The ability of proteinases to catalyse peptide bond hydrolysis and synthesis has an old history and this area has also a recent interest. The enzymatic protein tailoring for food and medical uses is, however, of particular importance.

One of the basic questions of food quality and food safety is the structure and functional activity relationships of proteins.

An enzymatic technique called EPM (enzymatic peptide modification) has been elaborated for designed modification of proteins and peptides. In our recent study we apply the EPM reaction to have the following products of special nutritional character:

- special amino acid enriched peptides for improvement of the biological value
- methionine enriched products in order to increase the content of the methylating agent in the modified protein chains
- proteins and peptides of reduced allergenic character
- tailored proteins for particular uses.

THE PROCYANDINE CONCENTRATION OF HUNGARIAN WINES

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This report gives an improved method and analytical results analyzing the phenolic composition – including the procyanidine composition – of the Hungarian grape varieties and wines. This work is a part of the research project supported by OTKA (T 5212).

Both the quantity and the composition of phenolic compounds in grape play an important role in the wine quality. Presence of these compounds is interesting from respect of stability and sensory properties (colour and taste).

In recent publications their physiological and defensive effects against some heart and cardio vascular diseases have also been emphasized.

The form of these compounds are different, some of them (like catechin and its stereo isomer from, epicatechin) are monomer types, belonging to the flavonoid

group, the others – to our knowledge the procyanidine – exist in dimeric and trimeric form.

The results of this study are:

– A reliable analytical procedure was developed for the separation and determination of procyanidines from the different parts of cluster and from wines.

– The concentration level of the procyanidines in the different parts of the cluster was found in the following order:

must < skin < pedicel < seed

– Generally higher procyanidine concentration was determined in the red grapes. There was significant difference among the grape varieties. The interspecific grape varieties seem to have higher procyanidine concentration.

– Since the procyanidines are mostly located in the solid parts of the cluster, the amount of these in wine is strongly influenced by the vinification technology with special regard to skin-contact.

– There were no significant differences among the production area.

PHYSIOLOGY OF L-MALIC ACID FERMENTATION BY *SCHIZOSACCHAROMYCES POMBE* AND STRAIN IMPROVEMENT FOR ENOLOGICAL PURPOSES

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Certain *Schizosaccharomyces pombe* strains are able to degrade L-malic acid into ethanol by the malo-alcoholic fermentation pathway. Metabolic route of this fermentation is well known, but the physiological circumstances, which affect its rate were not intensively studied.

Selected strains of *Schiz. pombe* were subjected to a detailed analysis aiming to determine the main physiological factors which influence the growth of strains in wine as well as the L-malic acid fermentation rate. From the results of the determination of ethanol tolerance, specific growth rate and L-malic acid fermentation rate it was concluded that growth and L-malic acid degradation are mainly influenced by the pH and ethanol tolerance of the strains. The limiting ethanol concentration for growth was 12–13% (v/v) but this decreased the specific

growth rate by 30–50% even in the case of the most ethanol tolerant strains and the malic acid fermentation rate was similarly influenced.

Auxotrophic mutants of *Schiz. pombe* strains which differed in the malic acid fermentation rate, ethanol tolerance and H_2S production were hybridized and improved by protoplast fusion, genetically stable hybrids were produced.

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DETERMINATION OF THE PHYSIOLOGICAL FACTORS WHICH EFFECT THE FLOCCULATION CHARACTER OF *SCHIZOSACCHAROMYCES POMBE*

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Spontaneous formation of macroscopic aggregates of yeast cells is termed flocculation. This feature is important in the brewing industry using *Saccharomyces cerevisiae* strains and also in other yeasts (e.g. *Kluyveromyces bulgaricus*). In these cases flocculation takes place in the late or early exponential phase of growth, respectively. Lectin molecules which develop on the cell surface are involved in the flocculation, because they show high affinity for cell wall carbohydrates (mainly for mannans) as receptors.

A *Schizosaccharomyces pombe* strain was selected in our laboratory which is able to ferment L-malic acid and shows a flocculation character at the beginning of the late exponential growth phase. The flocculent fenotype is expressed only when the ethanol concentration is minimum 5% (v/v) in the culture medium and in the presence of Ca^{2+} ions.

Yeast cells can flocculate in aerobiosis and also in anaerobiosis, when the ethanol induction happens in the early exponential phase of growth. EDTA causes reversible disintegration of aggregates because it binds Ca^{2+} ions.

The presence of Ca^{2+} ions is a prerequisite for the flocculation but Mg^{2+}-, Na^{+}- and K^{+} ions can substitute it. To determine the chemical feature of the surface macromolecules involved in the flocculation, the cell aggregates were treated with proteinase-K, trypsin, α-chimotrypsin, lyticase and novozyme. All these enzymes caused irreversible deflocculation after a few minutes treatment. Heat treatment of flocculated cells caused reversible (55 °C, 10 min) or irreversible (70 °C, 10 min) deflocculation.

On the bases of these results it seems highly probable that lectin type protein molecules are involved in the flocculation in this case, too. Because lectins have high affinity for sugars, the deflocculating effect of different sugars, sugar-derivates and polysaccharides was studied. The result shows that a galactose specific lectin is responsible for the flocculation of this *Schizosaccharomyces pombe* strain.

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COMPARATIVE STUDY ON ACTIVE DRY YEASTS IN LARGE-SCALE VINIFICATION

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The influence of starter strains of *Saccharomyces cerevisiae* on the chemical composition and quality of wines has been widely studied in lab-scale or pilot-scale fermentation but poorly investigated in large-scale experiments under standard conditions. The objective of the present study was to compare three commercial dry yeasts (products of LALLEMAND) in respect to their impact on wine quality.

Well homogenized white must (Sauvignon blanc), was filled into 40 hl volume steal fermenters and inoculated with the same quantity of the different starters rehydrated in standard way. Fermentation with indigenous yeasts was also carried out as a control. Alcoholic fermentation was conducted under temperature control at 15 °C. Young wines soon after the completion of the fermentation were evaluated by chemical and sensory analysis with special regard to their aroma composition. Aroma compounds were determined by capillary GC using FID and also by capillary GC–MS technique. Glycerol and higher alcohols were measured by packed column GC using FID and organic acids by HPLC with UV detector.

The analytical results showed that the various strains of the same species considerably influenced the aroma composition of the Sauvignon wine. Quantitative differences in the secondary aroma compounds among the different strains were more pronounced than the qualitative diversity of these compounds.

However, the analytically detectable differences could not be confirmed by sensory evaluation. Paneling the samples by a trained jury no significant differences were perceived either in taste or in odor and flavor.

From the results it was concluded that to choose an appropriate starter strain for practical use one should rely on the technological properties and the data of sensory evaluation rather than on the results of chemical aroma analysis.

EFFECT OF CARBONIC MACERATION ON SOME PARAMETERS OF HUNGARIAN RED WINES

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Carbonic maceration is a special vinification technology widely used in the South-European countries, primarily for red winemaking. Wines made by this technology have a special fruity character. Another advantage of the process is that the wines produced by carbonic maceration need shorter maturing and are ready for bottling earlier than the traditional red wines.

Red winemaking in CO_2 atmosphere is based on complex biochemical processes which have not been known exactly. Contrary to the traditional red winemaking, in which the main process is the alcoholic fermentation by yeasts in the crushed grape and diffusional occurrences relating to it, in case of carbonic maceration the technology uses chemical and enzymatic processes which occur in the impact grape berries in anaerobic conditions under CO_2. Nowadays the consumers prefer the light, fresh wines with fruity character. This tendency can be noticed in the consumption of red wines as well. The carbonic maceration is suitable for the production of these kind of wines.

This technology has not been studied and practiced in Hungary before. First time in domestic relation this study subjected the process of carbonic maceration to an examination. The main purpose was to investigate the applicability of red grape varieties which are widespread in Hungary for carbonic maceration.

The effects of carbonic maceration technology on the composition of rose and red wines were investigated under various conditions. Different treatments were compared with traditional red winemaking. Series of experiments were organised in 1992 and 1993 with six grape varieties (Zweigelt, Kékfrankos, Blauburger, Cabernet franc, Biborkadarka and Kékoporto). The sound and mature grape clusters were held in sealed tanks for 7–9 days at different temperatures in CO_2 atmosphere.

The investigation of the suitability of different grape varieties predominating in Hungary showed that the grape varieties which have not distinctive varietal character can be used well for carbonic maceration (e.g. Zweigelt, Kékfrankos).

The changes in acidity and other compounds under carbonic maceration modify the character of new wines. The enzymatic malic acid degradation, which is characteristic to this special technology, could also be observed in the experimental wines. The wines produced by this special process reached the acid stability earlier than wines made by traditional method, since the special process induces malo-lactic fermentation.

Some differences are well observable in the colour values as well. Due to the characteristic of bluegrape varieties growing in Hungary they are only capable of achieving the colour quality of rose wines if cold MC technology is used. In case of warm conditions the wines have different colour values than the traditional red wines. They are less red, but their colour intensity and tone are acceptable in general.

The cold carbonic maceration is able to produce good quality rose with special flavour. The classic carbonic maceration produces rich bouquet red wine with special fruity character which shows faster maturing. If the carbonic maceration is combined with short skin maceration we can produce more full-bodied wine with more complex fragrance. The wines made by carbonic maceration are suitable first of all for blending, but in the authors' opinion the wines produced in this way are appropriate for consumption without blending, too.

EFFECT OF SOME TYPES OF BENTONITE ON THE METAL COMPOSITION OF WINE

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Bentonite is a widely used fining agent in the wine technology. It is especially effective in removing heat-sensitive proteins, but it may be added in combination with other fining agents to accelerate clarification process. In spite of strict wine and quality assurance regulations it has some disadvantages. Bentonite fining of wine leads to a change in metal composition.

It is important for two main reasons:

- some heavy metal e.g. lead, mercury, cadmium, arsenic, nickel, chromium are toxic in extremely low concentration.
- the stability of wines depends on the relationships between several components which include metals, proteins and various colloids. Increasing concentrations of iron, copper, aluminium, calcium in wine result in an enhanced potential for instability.

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The present study is a part of the research project "Investigation of some theoretical relationship between stability and composition of wine" (OTKA T 007588).

One of the purposes of this work was to investigate the metallic composition of wines before and after bentonite fining by testing some types of bentonite regularly used in Hungary. For metal determination from wine the inductively coupled plasma emission spectrometry (ICP – AES) was used.

To achieve reliable results first we had to adapt ICP – AES technique to wine analysis. The main part of the methodological study was to solve the calibration question of the ICP – AES method at wine analysis, because the physical properties of wine (viscosity, nebulization effect, effect of organic compounds on spectral and emission interferences etc.) are different from sample to sample.

From this research it was found:

– The ICP – AES technique was suitable in metal analysis of wine.

– Bentonite products, depending on the type, form, quantity and quality of bentonite cause significant increase in concentration of some metals (mainly Al, Ca, Na, Fe).

– Some of the toxic heavy metal content of wine (Cd, Cr, Ni, As, Pb, Hg) either or not treated with bentonite was lower than the determination limit which is far below the international specifications.

EFFECT OF PRESERVATION TREATMENTS ON BACTERIA

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The aim of food preservation is to reduce the activity of spoilage microorganisms causing shelf-life reduction of food, and to inactivate or kill the pathogen microbes. The effectivity of preservation treatments is influenced by the composition and viability of the surviving microflora. The spoilage of foods is caused mostly by bacteria and bacterial spores. Therefore, the study into the growth conditions, the effect of treatments, and the environmental conditions playing role in the survival and activity of microorganisms is of great importance.

The effect of ionizing radiation on Gram-positive and Gram-negative bacteria was studied. The radiosensitivity of the latter is greater than that of the Gram-positive bacteria, thus their activity, i.e. in case of meats, can be reduced even by relatively small doses (1 – 2 kGy). The effect of ionizing radiation can be further increased by combination with oxygen depletion (vacuum, CO_2-atmosphere), refrigeration, addition of NaCl and/or heat treatment. Investigating the increase of

shelf-life of meat it was concluded, that the irradiation, over the cell count reduction, changed the propagation time of the sublethally injured surviving microflora, too. The lag-phase of the irradiated Gram-positive and Gram-negative bacteria is different, indicating their varying repair-capacity. When this repair-mechanism is inhibited by the presence of sodium chloride or by heat treatment following irradiation, relatively small radiation doses will already be effective. Data indicate that the microbes surviving irradiation treatment have smaller growth rate.

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THE EFFECT OF ANTIMICROBIAL TREATMENTS ON THE LAG-PHASE OF *YERSINIA ENTEROCOLITICA*

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The number of cases caused by food-borne diseases are increasing continuously world-wide. The micro-ecological factors should be considered seriously in food processing because of their determining role in the growth of pathogens and other bacteria in any food system.

We carried out model experiments to study the microbiological safety of some chilled foods inoculated with psychotrophic pathogens such as *Yersinia enterocolitica*.

The growth of this bacteria was detected by an impedimetric method with Malthus instrument at an incubation temperature of 25 and 10 °C close to the optimal and at suboptimal temperature representing cold storage, respectively. Some characteristics of the growth kinetics were analysed such as generation time and lag-phase which could be calculated based on the change of detection time of treated and untreated populations.

The antimicrobial treatments were: heat treatment, irradiation and frozen storage. The last two were combined and applied also in a reverse order.

The lag-phase of *Yersinia enterocolitica* treated by any of these factors was always longer than that of the untreated one as shown in Table 1. Heat treatment had the strongest effect on this part of the growth phase. After a certain reduction in number of bacteria the heat treated population had longer lag-phase than the irradiated one so the former one had a more damaging effect to the bacteria. Suboptimal temperature had great influence on the increase of the lag-phase.

The D_{10} value of the examined population was 47 Gy and 87 Gy when irradiated in frozen state. The sequence of these treatments had an additional (increasing) effect on the lag-phase of *Yersinia enterocolitica*. When it was irradiated after frozen storage the number of survivors was higher than in case of irradiation followed by frozen storage.

Table 1

The effect of antimicrobial treatments on the lag-phase (h) of Yersinia enterocolitica *at different incubation temperatures*

Treatment	25 °C	10 °C
No	3.0	14.4
Freezing (−20 °C)	4.25	–
Irradiation (50–400 Gy)	4.56	–
Freezing & irradiation	4.55	16.1
Irradiation & freezing	4.78	18.35
Heat treatment	5.6	46.86

VIABLE BACTERIA COUNT IN FERMENTED MILK PRODUCTS AND THEIR CHANGING DURING THE SHELF-LIFE

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The health-promoting qualities of fermented milks depend on the viable starter cell count. Therefore, in the majority of European countries have already established standards are for the viable count at the sell-by-date of these products and the introduction of such a standard is going to be prepared in the European Union. Present study deals with the storage-induced changes in the populations of viable starter organisms and contaminants in some Hungarian yoghurt varieties untill the sell-by-date or up to the end of palatability.

The main component of viable starter bacteria counts of the examined natural and flavoured yoghurts was the *Streptococcus salivarius* ssp. thermophylus which was present in number exceeding the 10^8 c.f.u. cm^{-3} and hardly decreased in the samples stored at 4–5 °C while in the samples stored at 14–16 °C, the c.f.u. decreased significantly. The c.f.u. of *Lactobacillus delbrüeckii ssp. bulgaricus* was mostly lower by 1.5–2 orders of magnitude and showed a more intensive decrease during storage.

From the point of view of the microbial contaminants (yeasts and moulds), the flavoured yoghurts were more perishable.

At the sell-by-date, 90% of the yoghurts stored both at 4–5 °C and at 14–16 °C met the standards planned by the European Union considering the viable microbial count ($>10^8$ c.f.u. cm^{-3}) but the remaining 10% also fulfilled the specification of minimum level (10^7 c.f.u. cm^{-3}) proposed by the International Dairy Alliance. However, the *Streptococcus Lactobacillus* ratio should be decreased.

The recent results, in comparison with examinations performed in previous years, verify distinct advance concerning product quality, the total viable starter bacteria count of yoghurt varieties and microbiological stability and, furthermore, keeping quality.

The examinations may present a basis for the tasks of adaptation to the regulations of European Union and for maintenance or improvement of competitiveness of Hungarian products.

COMPARISON OF NUTRIENTS FROM PIGS OF VARIOUS AGES KEPT IN NATURAL (FREE RANGE) AND LARGE-SCALE FARMING

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A new pig genotype of Hungarian Big White (75%) and mangalica (pigs with curly bristles, 25%) was developed having more resistance against diseases. The experimental group of this genotype was kept in an isolated farming system, under natural circumstances. The feed and supplements were free of antibiotics, sulfonamides and yield increasing hormones. The control group was the same pig genotype kept in the traditional large-scale farming conditions.

In the first part of the experiment the pigs were slaughtered at the bodyweight of 120 kg, and at the age of 7 and 8 months, respectively. The protein content of pork from "free range" pigs was higher and the cholesterol level was lower as compared to the control animals kept in large-scale conditions – this is favourable from the nutritional viewpoint.

The linoleic acid level and the superoxid dismutase (SOD) activities were lower, zinc and in some cases copper level were higher in the pork of the "free range" pigs.

In the second part of experiment only the "free range" pigs were examined. Two groups were compared: first group: bodyweight of 120 kg, age of 7–8 months; second group: bodyweight of 160–180 kg, age of 12–13 months.

In the pork of the second group as compared to the first one the protein content was lower and the fat content was considerably higher.

The intensity of lipid-peroxidation (level of TBA-reactive substances) connected with the higher fat content increased. Parallel with the increase of fat content the linoleic acid level reduced.

In the pork of "overweight" pigs the thiamin level referring to the protein content was significantly higher. In all cuts from the new genotype the zinc level was higher as compared to that in the traditional genotype of Hungarian Big White – this was proved by parallel experiments carried out in 3 consecutive years.

POSSIBLE RISK FACTORS OF THE HEALTHY NOURISHMENT

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The rapid rise of environmental damage endangers the whole society, nowadays our conservation of humans and their environment cannot be treated separately. The wide-ranging use of chemical substances has exercised the greatest influence perhaps on the natural elements of the environment and indirectly on the health of humans.

The extended use of chemical substances gives rise to several toxicohygenic problems; mainly to those, that can be followed up in the soil-, vegetable-, animal-, human food chain.

The quality control of basic healthy food like fibre-containing fruits and vegetables, oil seeds, fish and poultry, is of growing importance.

Some data in the literature and also our laboratory results call our attention to the fact that some risk factors may be present in the foodstuffs of animal and plant origin, i.e. nitrite, nitrate, pesticide compounds, toxic metals and mycotoxins.

It is necessary to control the risk factors systematically to protect our health and to guarantee the quality of foodstuff according to the hygienic regulations.

INVESTIGATIONS OF STRUCTURAL AND FUNCTIONAL PROPERTIES OF PLANT PROTEINS

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In the modern food science the protein research activity is aimed at to discover the relationship between the molecule-structure and functionality and at the application of the results of investigation.

The paper is presenting new data on chemical, structural and physico-chemical properties of soya and pea proteins.

The aim of the research activity was:

– to evaluate the native protein components of plant protein sources;

– to compare the characteristic properties of native and treated plant proteins;

– to evaluate the analog properties of different plant protein sources and their components;

– to investigate the specific properties of plant proteins and their fractions measured by different methods;

– to analyse the specific properties of different varieties of plant protein sources;

– to improve the method of characterisation of plant protein sources;

– to establish relationship between the structure and functionality. The methods were used as follows: fraction-separation and isolation, SDS – PAGE electrophoresis, measurements of conductivity, turbidimetry and hydrophobicity, DSC analysis.

The results of experiments were compared to literature data.

THERMODYNAMIC AND STRUCTURAL INCOMPATIBILITY IN FOOD PROTEIN SYSTEMS

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The growing demands concerning the quality of food products, the increase of use of different protein concentrates and isolates in the food processing, the development of new – protein rich – food products stimulate the basic research

work connected with the properties and changes of protein systems occurring in different foods.

A deeper knowledge of thermodynamics of such systems, the study of molecular interactions may be a basis for the further improvement or development of processing of protein-containing foods and also for the development of new food products. It is also a prerequisite to the product ion of new protein rich foods with in advance calculated (predicted) properties.

Water – protein 1 – protein 2 and also water – protein – polysaccharide ternary systems were investigated. The study included all the parameters influencing the occurrence of thermodynamic incompatibility and also investigation of conditions of incompatibility (such as equilibrium of entropic and enthalpic effects, interactions between polymer 1 – polymer 2, polymer 1 – solvent, polymer 2 – solvent).

It was found – on the basis of results of food systems investigated – that the most important factors playing role in thermodynamic incompatibility are temperature, pH, ionic strength and quality and concentration of polymers. In experiments the following components were used: casein, gliadin, glutenin, alginate, dextran and pectin.

The structural incompatibility of myosin-plant protein (soy-protein-, sunflower seed protein-isolates and vital gluten) systems was also studied. Model experiments were made and also pilot plant experiments. It was found that not only the individual properties of proteins play role in the occurrence of structural incompatibility but also the kinetic of protein denaturation during thermotropic gelation. It was also found that the "two state" models of denaturation process have limited applicability in describing the whole process. Experiments to elaborate a "multi-state" model for more complex food proteins (consisting of different subunits or domains) started and their results will be published in near future.

QUANTIFICATION OF CHOLESTEROL OXIDATION DERIVATIVES

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A large number of compounds can be formed during the oxidation of cholesterol due to free radical reactions in the presence of air. Numerous oxidation products are suspected to have atherogenic, cytotoxic, mutagenic effects and to be involved in cholesterol biosynthesis by the inhibition of 3-hydroxy-3-methylglutaryl-

coenzyme A-reductase. Hydroxy, carbonyl- and epoxy derivatives are the most frequently occurring products during processing and storage of food.

There are some difficulties in separation of cholesterol and cholesterol oxides because of differences in orders of magnitude (mg/100 g cholesterol, mg/1000 g cholesterol oxides).

The aim of this study was to develop a rapid, simple method for the quantification of some cholesterol oxides separated by thin-layer chromatography and eluted from the adsorbent.

The enzymatic reaction of cholesterol with cholesterol oxidase based on the oxidation of hydroxyl-group at C−3 position is widely used for quantification of cholesterol. It was suspected that not only the cholesterol, but also its oxidized products could be the substrates of cholesterol oxidase and the reaction might take place in a similar way. To prove the above hypothesis four cholesterol oxides often appearing in foods during processing and/or storage were tested: 7α-hydroxycholesterol, 7β-hydroxycholesterol, 7-ketocholesterol, cholesterol-5α,6α-epoxide.

Statistical analysis showed a linear relationship between the amount of oxysterols in the range of 1 − 12 μg and the optical density measured at 500 nm after development of colour. Measurements were carried out with evaporated standard solutions and with standards and samples eluted from the silicagel adsorbent. Recoveries were found to be satisfactory. Since the slopes of 7α-hydroxycholesterol, 7β-hydroxycholesterol, 7-ketocholesterol are very close to each other a summarized regression line could be elaborated. Because of the difference in slopes in the case of cholesterol-5α,6α-epoxide individual calibration line had to be used.

This method can be applied for the quantification of oxysterols obtained from the non-saponifiable lipid fraction and separated by TLC even when small amounts of several samples containing large amounts of cholesterol are present at the same time.

ANALYSIS OF RAW MATERIAL SUITABLE TO PRODUCING FOOD RICH IN INULIN AND FRUCTOSE

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The inulin and the fructose are important ingredients of the up-to-date nourishment. In the natural sources the fructose can be found in polymer format, as inulin which is suitable both for normal food and health-food production.

Based on the Hungarian and international literature, the existence and quantity of inulin in certain plant varieties were analysed. The conclusion that can explicitly be drawn is, that Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Cichorium intibus*) are the most suitable raw materials to extract inulin from. The presentation is dealing with the analysis of components of Jerusalem artichoke.

The major part of edible carbohydrate of the Jerusalem artichoke tuber is the inulin, that is of high value in itself, but it can also be hydrolysed to fructose, moreover, its mineral content is extremely high.

The objective of our work is the analysis of various varieties of Jerusalem artichoke for their suitability for food processing.

The following varieties cultivated under similar agrotechnical conditions were analysed:

Bárdi 3, Büki 20, Gyöngyvér, Rika, Sugárka, Waldspindel. The place of origin of the analysed samples: NÖMI Agrobotanical Centre, Tápiószele. The investigated characteristics are shown in Fig. 1.

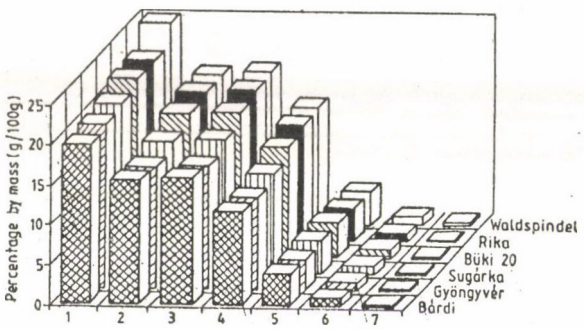

Fig. 1. Average values of main components of the investigated various varieties of Jerusalem artichoke tubers. 1: Extract content; 2: inulin content; 3: total sugar content; 4: fructose content; 5: glucose content; 6: ash content; 7: reducing sugar content. Time of harvest and analysis: November, December, January, March

The extract content directly affects the economy of processing. Major part of the valuable extract content in the tuber is the inulin (inulin = total amount of sugar – direct reducing sugar). The most important factor of selecting the proper variety is the fructose content. Out of the investigated 6 varieties the Waldspindel, Rika and Büki 20 have the highest values of fructose and inulin. We recommend the above-mentioned three varieties to produce inulin and dried materials, or a concentrate rich in fructose and minerals.

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PARAMETER ESTIMATION OF MULTIVARIATE LINEAR FUZZY REGRESSION

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The author showed a possible generalization way for Otto and Bandemer's univariate fuzzy regression method. For developing the multivariate fuzzy regression method n-dimensional geometric conceptions were used instead of integral formulation. Hyperellipsoid supports were chosen for characterizing the uncertainty of measurement points, then the membership functions belonging to the points were defined by hyperparaboloids with those supports as their bases. Because the derived surface tends to be rough with many sharp peaks local optimum searching methods such as gradient methods, simplex method, etc., can only find a local optimum with high probability which can be far the global one. That is why the fuzzy regression procedure needs global optimum searching method. The author recommends genetic algorithm rather than grid method or simulated annealing. The algorithm seems to be rather complicated, even with genetic algorithm as global optimum searching method. In higher dimensions it is, however, very difficult to find outliers, because of the masking effects and there is no relatively simple multivariate robust procedure. The described procedure really a computer-intensive one, but its features are listed hoping the reader will come to realize that the advantages outweigh the drawbacks.

- It is robust, i.e., it is insensitive to outliers emerging from mistakes in measuring by either humans or instruments,
- it saves its robustness in higher dimensions too,
- no distinction between 'dependent' and 'independent' variables to the effect that all variables can be vague, suffer from measurement errors, which is the general case,
- it takes the heterscedasticity into consideration, i.e., all measurement points have own membership function denoted their uncertainty.

One of the natural application of the described method is for calibration of data measured by near infrared (NIR) spectroscopy instrument, because data of NIR determinations need multivariate treatment and outliers can occur. In the near future it would be also intresting to investigate the applicability of the procedure for clustering NIR-data and to compare the results with other clustering techniques.

INFLUENCE OF THE MODIFIED ATMOSPHERE PACKAGING ON THE QUALITY CHANGE OF SOME MODEL FOODS

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In the frame of the research project sponsored by OTKA the possibilities of shelf-life extension of some model foods were investigated. The selected models are important from nutritional aspects. Modified atmosphere packaging methods were used. The project involved the packaging material tests, the modified atmosphere optimization, the quality changing determination of stored foods and the shelf-life prediction.

In the last year of the program the storability of various green salads were investigated regarding their vitamin C and β-carotene contents. The possibility of shelf-life extension of the biologically valuable wheat-germ was also examined, using various gas atmospheres. Out of the products of animal origin the extension of shelf-life of the so-called farmer ham as model was examined as a function of packaging material, gas composition and duration of the storage. The relationship between quality changing and packaging was determined by the results of physical, chemical and sensory tests and, depending on products, microbiological investigations.

From our research results it can be stated that the modified atmosphere does not influence essentially the enzymic changes and their rate. The rate of quality change in connection with the oxidation could be reduced but its rate basically depends on the products therefore general conclusion between the reaction rate and the atmosphere in packaging could not be established. The hygienic state of the investigated products, considered as of model did not change. Defining correlations between the chemical reaction rate, effecting quality change and the atmosphere inside the packaging needs further researches.

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APPLICATION OF NON-DESTRUCTIVE METHODS FOR QUICK QUALITY CONTROL OF COLOUR PIGMENTS

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In order to determine the stability of colour pigments of grape non-destructive methods have been used: near infrared spectroscopy, photoacoustic spectroscopy and colorimetry.

Two different extraction methods were used to extract natural pigments of grape. The pigment powder was gained by vacuum-drying of the concentrated extracts. Stabilization of pigments was obtained by the addition of two different carriers: maltodextrine and arabic gum.

The stability of the samples were investigated under different conditions: UV--irradiation and heating over 50 °C.

The samples were compared by tristimulus colorimetry the surface colour difference of samples was defined by CIELAB method.

NIR and PAS techniques were applied for optical measurements. Raw spectra were processed by different pretreatment transformation (2OFD, FFT). PCA algorithm was used to classify and monitoring the change of quality during UV--irradiation and heating treatment.

METHODICAL PROBLEMS OF PAH MEASUREMENTS

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The difficulties of PAH measurements derive from two basic problems. The first is the extremely huge mass of isomers belonging to the same ring number that means the identical molecular weight. The high structural similarity cause troubles in the resolution even if capillary gas chromatography is used for the separation. The small variation of retention times might leed to misidentification and the failure of identification causes the complete uselessness of quantitative determination.

The other problem is the very complicated and many-step sample preparation procedure that spoils both the sensitivity and reproducibility of the measurements. The low sensitivity is a consequence of the low volatility of the PAH compounds too.

Components having 350 °C boiling point or above cannot be evaporated in measurable quantities in split/spitless mode. Thus some highly carcinogeneous compounds like benz(a)anthracene, benz(a)pyrene or dibenz(a,h)anthracene are not detectable in the desirable 1 μg/kg range. This lack of sensitivity cannot be overcome by raising the sample quantity, because it grows the amount of the "artefact" components and the chaos of the identification, as well.

At our department both problems could be eliminated by applying a GC–MSD instrument equipped with cool on column injection system in Selected Ion Monitoring mode.

EVALUATION OF THE PHYSICOCHEMICAL PROPERTIES AND COMPOSITION OF THE HONEYS OF DIFFERENT BOTANICAL ORIGIN

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Hungary is traditionally a major honey-producing and exporting country. To keep our market position and satisfy the growing qualitative requirements modern analytical methods are needed.

According to the Hungarian standards the quality of the honey is to determined by its water, naturally occurring reducing sugar, HMF and ash content, besides pH and amylase activity.

The identification of honeys of different botanical origin is carried out by their pollen analysis and sensory evaluation. The determination of botanical origin by pollen analysis is confronted with some fundamental problems, like

- the countings are relatively inaccurate,
- the number of "marking pollen grains" in the nectar depends on the anatomy of flowers, the weather and on many other conditions.

The aim of our work was to search for significant parameters in distinguishing honeys.

The honey samples were provided by the Lukács et al. Laboratorium B.T. Budapest. The sensory assessment and pollen analysis were carried out by them. We measured the sugar composition by HPLC, the electrical conductivity, the free and lacton acids, the concentration of macro and micro elements and the prolin content of the samples. The results show that floral honeys and honeydew honeys can be distinguished by the measured parameters. Among the floral honeys acacia honey

differs from the others by its high fructose content, fructose/glucose ratio, prolin and total element content besides free and total acids.

The methods used may serve as additional tools in specifying samples.

SUPERCRITICAL FLUID EXTRACTION OF SPICE OLEORESINS

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Spices are aromatic plant materials used for the flavouring and colouring of foods and beverages. They consist of rhizomes, leaves, flowers, fruit and other parts of plant. The quality of the finished products depends on the physical and sensory properties of spices. Commercially used spices are available in whole, broken, rubbed or ground form. Commercial production of oleoresins began in the 50s, and the list of available oleoresins quickly expanded to meet the needs oaf the food industry.

The spice oleoresins are defined as liquid, semi-solid or solid residues obtained by solvent extraction and possessing the full organoleptic character of natural spices. The advantages associated with the use of oleoresins are: consistency of flavour and aroma, sterility, stability in storage, reduced storage space and economy. Extraction with carbon dioxide (CO_2) is more efficient method of separation than the conventional extraction with organic solvents.

Carbon dioxide is an ideal solvent because it is non-toxic, readily available and easily removable from products. CO_2 solvent allows low-temperature processing and there is no degradation of aroma components. Oleoresins obtained by supercritical fluid extraction (SFE) more closely resemble the flavour and aroma of the natural spice than do that of the steam distilled or solvent extracted equivalents.

Rhizome of sweet flag (*Acorus calamus* L.), leaves and flowering tops of thyme (*Thymus vulgaris* L.) and fruit of parsley (*Petroselinum crispum* Mill.) were extracted with carbon dioxide in high pressure apparatus with 5 L extractor vessel volume. Fractionations of extracts were carried out by releasing the separation pressure at two stages to effect separation of the dissolved components into aroma and flavour fraction and fatty-waxy fraction. The SFE was compared to conventional steam distillation (essential oils) and to Soxhlet extraction with hexane or alcohol (fatty fractions).

Examinations of the volatile constituents of the oils resulted in conclusion that distilled and SFE products contain essentially the same components. Although, the relative proportion of various components is extremely different in the products. The relative amounts of oxygenated compounds, the main flavour components, are higher in the SFE products.

The fatty acid composition is only slightly different for extracts obtained by SFE and hexane extraction.

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INVESTIGATION ON BIOGENIC AMINE CONTENT OF APPLES AT DIFFERENT VEGETATION STAGES IN DEPENDENCE OF SOIL NITROGEN SUPPLEMENTATION

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Aim of the work was to study biogenic amine content of the variety JONNEE at three nitrogen supplementation levels during the whole vegetation period. Samples were taken from twigs and pistils at falling of the leaves and budding in case of fruits six weeks after petal fall (green apple stage), at harvest and at the end of storage. Investigations have extended for three years. In the samples putrescine, spermidine, agmatine and spermine could have been detected. Putrescine had the highest concentration in the green apple stage, spermidine and agmatine showed maximum in the pistils. These later ones showed lower concentrations in the green apple status especially agmatine which decreased to 1/10 of the spring pistils level. At the time of the harvest in the ripened apple practically only agmatine was detectable, in the stored apple trace amounts of putrescine, spermidine and agmatine were found. Poliamine level of the different vegetation stages varied from year to year. Between biogenic amine and nitrogen fertilisation of the soil no correlation could have been shown.

The detected changes are in accordance with the role of di- and poliamines in the plant cell considering the new scientific results concerning the importance of poliamines in the biosynthesis of ethylene and its function of ripening.

PROCESS OF CHANGES OF TEXTURE DURING BLANCHING

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The softening of texture after blanching of different commodities at different methods and time-temperature parameters was analysed. The change of breaking force and relaxation time of vegetables was determined. The results showed that the process of changes of texture during blanching at different vegetables can be approximated by minimum two-three phases and minimum three basic patterns can be identified: an initial transition phase, followed by a faster and slower softening phase. The two softening phases can be described by consecutive first order kinetics. Rate constants were calculated.

The two consecutive reactions approach was applicable for potatoes, peas, carrots. While breaking force studies indicated mostly the two later phases, relaxation studies mostly showed a temporary increase of elasticity before the softening has started. Potatoes showed a significant increase of elasticity at the early stage. The results prove that a detailed analysis based on frequent sampling of the initial period is necessary for the interpretation of the mechanism of texture changes in different vegetables during blanching. The kinetical approach helps to compare and forecast changes at different process conditions.

STRUCTURAL CHANGES ARISING DURING FREEZING OF FRUITS AND VEGETABLES AND THEIR CONSEQUENCES

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During freezing the texture of fruits and vegetables is transformed from soft to hard. The phase transformation of water to ice starts in the surface layers. Mechanical stresses can arise due to changes in volume associated with liquid to solid transformation and from temperature gradients within the product.

The results of the investigation support the assumption that the freeze-cracking of fruits and vegetables is mainly related to stresses arised by volumetric increase and non-uniform contraction may have a secondary effect only.

The expansion caused by ice formation is clearly reflected by the size changes of the specimens, which start close to the freezing point and are finished after the freezing of the bulk. The minimum of cracking resistance and the finishing period of the static cracking is closely associated with the end of the phase of the rapid volumetric changes.

Larger specimens produce larger volumetric increase and higher occurrence of cracking. If the core is hard – consists of stone, or hollow these volumetric changes and the cracking phenomenon are smaller.

PROTEASE INHIBITORS IN PEAS CULTIVATED IN HUNGARY

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In the West European countries the consumption of peas both for human food and for animal feeding has been increased in the last 10 years.

The protein content of peas is about 25%, it is lower than in soybean, but its starch content is a valuable energy source. The antinutritive compounds in pea are present in a relatively lower concentration than in soybean. However, on the basis of animal feeding experiments it was established that in case of piglets the feed should not contain more pea than 15–20% and for chicken 30% of the daily diet without negative effect on the digestive system.

The increasing consumption of pea in the future depends on the reduction of antinutritional factors.

Our aim was to determine the protease inhibitor activity (i.e. trypsin and α-chymotrypsin) of peas grown in Hungary to select the low activity varieties and to study the biochemical properties of protease inhibitors. Our results show that the mass of the peas grown in Hungary has 2–6 U mg^{-1} trypsin and 0.8–2.5 U mg^{-1} α-chymotrypsin inhibitor activity. On the basis of our results using 38 pair of data, a significant linear relationship was established between the trypsin and α-chymotrypsin activity.

CHANGES IN THE NUTRITIONAL VALUE INDICES OF THE TOMATO FRUIT AS AFFECTED BY THE PRODUCTION SITE: GLASSHOUSE OR FIELD

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At our Department the changes in the nutritional value indices of vegetables for fresh comsumption, as affected by different production methods, have been thoroughly studied recently. In this field the available data are rather scarce, as measurements have been made mainly with processed products, or in some instances, with raw material for processing.

Tomato is one of the most important vegetable species in our research work. Fresh tomatoes are on the market all the year round, however, the ware is of very different origin in the different seasons. Home produced tomatoes come from glasshouses in autumn, early spring and late spring, and subsequently from heated and unheated plastic houses. They are followed by tomatoes from early outdoor production and finally from field production. The technologies of the enumerated production methods vary, and accordingly, the varieties used are very different, too, and the crop produced is diversified as regards the nutritional value. The assortment has been complemented by the increasing choice of imported tomatoes.

In contrast to European quality standards, Hungarian consumers mean by quality the colour and the taste of the tomato, and there is a lot of misconception regarding the other quality factors, i.e. mineral constituents, vitamins. The universal belief is that forced tomatoes are flavourless, however they are worthful as primeurs. Hungarian tomatoes are thought better than those from abroad.

Our research work has been aimed at clearing this sphere of problems by a series of objective measurements.

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DETERMINATION OF ACETONE IN COW'S MILK

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Milk acetone is usually determined to detect ketotic state in dairy cows. However, due to its potential toxicity, the knowledge of milk acetone concentrations raises other considerations as well.

Method: Milk sample is clarified with Carrez reagents, elementary iodine is added to the filtrate, then the monoiodoacetone formed is extracted into *n*-hexane.

Before gas chromatography the excess iodine is removed with β-cyclodextrin and the organic phase is analysed by GC using an electron capture detector. The peak corresponding to monoiodoacetone is regarded as the analytical signal. The area of the peak corresponds to the quantity of monoiodoacetone.

Instrument: CHROMPACK CP 900 gas chromatograph equipped with a ^{63}Ni EC detector. Column: 50 m × 0.25 mm fused silica capillary coated with 0.25 μm CP-SIL8 CB (CHROMPACK). The composition of coating: 5% phenyl- and 95% methyl silicone gum, chemically bonded. Temperatures: injector 250 °C, oven 100 °C for 4 min, then heated with 100 °C min^{-1} up to 280 °C. Carrier gas: nitrogen (60 kPa). Detector gas: nitrogen (30 cm^3 min^{-1}). Injected volume: 1 μl.

The retention time of the iodoacetone is about 7.2 min. The calibration curve is linear between 0 and 3×10^{-4} mol dm^{-3}. The detection limit is 5×10^{-7} mol dm^{-3} acetone in the clarified filtrate, calculated from the triple of the standard deviation of the blanc solution and from the slope of the curve.

The relative standard deviation was 7.2% in case of a sample containing 1.87×10^{-4} mol dm^{-3} acetone (n = 7).

The developed method might be considered as the inverse of the method proposed by HASTY (1971) for determination of iodine.

Literature

HASTY, R. A. (1971): A gas chromatographic method for the microdeter-mination of iodine. Microchim. Acta (Wien), 348-352.

METHODS FOR DETERMINATION OF D-AMINO ACID CONTENT OF FOODS AND FEEDS

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Three methods were reviewed for determination of D-amino acids from foods and feeds. First a method of ion exchange column chromatography was developed for the determination of D-amino acids in the form of alanyl- and 2-sulfonic acid alanyl diastereomeric dipeptide. The protein containing amino acids were separated in an LKB automated amino acid analyser, then the single amino acids were transformed into alanyl- and 2-sulfonic acid alanyl dipeptide. The alanyl- and 2-sulfonic acid alanyl dipeptides were easily separated from one another and from the initial amino acids; the accuracy of the determination was satisfactory.

Secondly a reverse phase chromatographic method was reviewed for separation and determination of D-amino acids after derivatization with 1-(9-fluorenyl)-ethyl--chloroformate. Highly fluorescent diastereomers of amino acids were obtained without racemization within 4 min at room temperature. The extraordinary advantage of the procedure is given the enhanced reliability of peak identification using both enantiomers of the reagent (+FLEC, -FLEC). Derivatization with the other enantiomer of the reagent changes the elution order of the diastereomeric derivatives. Another advantage is the possibility to determine the retention of the derivative of an amino acid enantiomer which is not present in the sample, by chromatographing its enantiomerically related derivative. The secondary amino acids were separated at a lower pH and could be determined without interferences from primary amino acids. Cis and trans modification of D- and L-hydroxy proline could be perfectly separated by this method. The racemization of free amino acids and proteins during liquid and gas phase hydrolysis was determined.

A third method was reviewed for the separation and determination of D-amino acids after derivatization with o-phthalaldehyde (OPA) and optically active 2,3,4,6--tetra-O-acetyl-1-thio-β-glucopiranoside by reversed phase liquid chromatography. The reaction between the reagent and the amino acids was complete in a few minutes at room temperature, and the derivatives were quite stable. It could be stated that the selectivity of the diastereomers formed – with the exception of lysin and ornithine – was especially good. The fluorescence excitation and emission maximum of the derivatives were 342 and 410 nm, respectively. The detection limits for the majority of

amino acids were 2 pmol in case of fluorescence detector and 1 pmol using electrochemical detector. The extent of racemization occurring during gas and liquid phase hydrolysis of ribonuclease was also determined by the method reviewed.

EFFECT OF DIRECT ULTRAVIOLET LIGHT ON FREE RADICAL REACTIONS IN EDIBLE FATS

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The authors investigated free radical reactions generated by ultraviolet light during 20 min and 6 h treatment in soy oil, sunflower oil, olive oil, margarine and lard. The control samples had no treatment.

Lipidperoxidation characteristics – chemiluminescence intensity, peroxide number, conjugated dienes, thiobarbituric acid reactive substances (TBARS) –, fatty acid composition, vitamin E and oxidized cholesterol derivatives were measured.

Ultraviolet light treatment during 20 min had no effect on lipid-peroxidation characteristics, fatty acid composition and levels of vitamin E in every samples but small amounts of oxidized cholesterol derivatives were observed in lard.

Significant expansion of lipidperoxidation was found after 6 hours treatment in every samples. The number of free radicals measured by chemiluminometer increased to tenfold level in consequences of ultraviolet light. Vitamin E decreased considerably in the oils and margarine.

Degree of changes in lipidperoxidation characteristics was influenced by the fatty acid composition and vitamin E content in fats. For example soy oil has about 7% of linolenic acid ($C_{18:3}$) an ability to oxidize hundred times faster than oleic acid ($C_{18:1}$) and the other oil samples without linoleic acid. In spite of this fact soy oil did not show an extremely high lipidperoxidation capacity induced by UV light because the half amount vitamin E content originally present remained in oil after 6 h treatment.

Strong significant correlations were observed between chemiluminescence intensity and peroxide number, chemiluminescence intensity and TBARS, peroxide number and TBARS in various edible fats (0.99; 0.64 – 0.92; 0.62 – 0.99), respectively.

FORMATION OF STORAGE PROTEINS OF WHEAT DURING THE DEVELOPMENT OF GRAIN

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The development of grain of two Hungarian wheat varieties (MVM, MV-21) were studied from 6th days after anthesis till mature seed. Eight samples were harvested at different stages of maturity in 1992, 1993.

The main aim of research work was to study the changes of nitrogen containing compounds (protein, amino acids) in this period of seed development.

The storage proteins (gliadin, glutenin) were separated by gel electrophoresis and HPLC.

The changes of compounds in stems, leaves and grains giving a positive ninhydrin reaction were also studied in the period after anthesis and the quantity of free and peptide bound amino acids were determined, too.

Summarising it was found that the different types of storage proteins are accumulated in different periods.

During the development of grain first the low molecular weight proteins were detected (under 40 kD) as early as the 9th day after anthesis. From the 14th day after anthesis the high molecular weight proteins appeared, too (over 200 kD).

In the wheat seeds the typical gliadin pattern (depending on the wheat varieties and weather-conditions) was developed by the 9th-20th day after anthesis, the difference from the mature grain was observed only in the intensity of a few bands. The final pattern has evolved by the 27th-30th day after anthesis.

The typical glutenin subunits were extracted as early as the 20th day after anthesis depending on the wheat varieties and the weather-conditions. Difference from the mature grain was observed only in the intensity of a few bands. The final pattern was detected by the 27th day after anthesis.

The quantity of ornithin merits particular attention. It was present in high concentration after flowering in both wheat varieties. Later rapid decreasing of ornithin concentration was detected.

To investigate the influence of nitrogen quantity on the wheat proteins two different dosage were applied.

Enhanced N fertilizing sped up the production of both the gliadin and glutenin by four days comparing to the normal conditions. The increase of protein content due to N fertilizing was not durable. The difference in protein content of mature grains was not significant.

COMPOSITION OF SOW'S MILK AND ITS ALIMENTARY VALUE

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Total solids, fat content and fatty acid composition, fat soluble vitamins (A, D_3, E, K_3) and vitamin C content, macro and micro element, protein content, protein fractions, amino acid composition and biological value of colostrum and milk of 10 Danish large white, 10 Danish duroc and 10 Norwegian landrace sows were determined. It was established that the total solids (23.5-24.7%) and fat content (5.2-5.4%) of the first colostrum increased by the 48-72 h of lactation (total solids: 27-28%, fat content: 12.7-13.1%), afterwards decreased by the end of lactation (18.5-18.7% and 6.2-6.8%). The fat of sow's milk - similarly to mother's milk - contained (at the limit of identification) saturated fatty acids with 4-12 C number only in very low concentration. The sow's milk contained significantly more unsaturated fatty acids than the cow's milk. Especially great differences were found in the case of linolenic acid, from which the sow's milk contained significantly more than the cow's milk.

The sow's milk contained more ash (0.8428%), calcium (1965 mg/kg), phosphorus (1510 mg/kg), zinc (6.49 mg/kg), iron (2.44 mg/kg) and copper (1.34 mg/kg), and less potassium (748 mg/kg), sodium (387 mg/kg) and magnesium (111 mg/kg) than the cow's milk, while in the manganese content there was no difference between the two species. The potassium, sodium, iron and copper content decreased, the calcium and phosphorus content increased during the lactation.

Contents of vitamins A, D_3, E, K_3 and C of colostrum (1.61, 0.015, 3.69, 0.092, 68.4 mg/kg) were found - except for vitamin K_3 - one and half - twice higher, than that of normal milks (0.92, 0.0094, 2.53, 0.089, 45.3 mg/kg). The sow's milk contained twice or three times more vitamins than the cow's milk.

The total protein content of the first colostrum (16.5-16.8%) was approximately three times higher than at the end of lactation (5.8-5.9%). All of the protein fractions decreased during lactation with the except of casein - which reached its maximum between 24-72 h of lactation with a value of 3.5-3.7%, and non protein nitrogen - which increased from the beginning of lactation (0.400%) to the end of lactation (0.460-0.480%).

While the amino acid content of colostrum and milk - similarly to the change of total protein - increased during the lactation, up to that time most of the essential amino acids (threonine, cystine, valine) decreased, but the non essential glutamic acid and proline increased. This is why the biological value of the colostral protein was approximately 10% higher during the first five days of lactation (118-119) than between the 10-60th days of lactation.

There were no significant differences among breeds in the composition of their colostrum and milk samples.

Acta Alimentaria, Vol. 24 (1), pp. 129–131 (1995)

BOOK REVIEWS

Lebensmittel-Lexikon

A. TAUFEL, W. TERNES, L. TUNGER and M. ZOBEL (Eds)

N. Behr's Verlag GmbH Co, Hamburg, 1993, 2 Vols, 1774 pages

In 1993 the Behr's Verlag GmbH, Hamburg (Germany) published the 3rd, revised, up-to-dated edition of the Lebensmittel-Lexikon, edited by A. TAUFEL, W. TERNES, L. TUNGER and M. ZOBEL. Beyond them 27 experts took part in compilation of the book.

The encyclopaedia consists of two volumes. The first volume includes entries from A (Aal) to K (Kwiekbrot) and the second one from L (Lab) to Z (Zytosäure). The size of the volumes are 852 and 922 pages, respectively. Both volumes begin with the same preface, which is followed by the instructions for use with examples, the abbreviations and the list of symbols. At the end of the second volume data on recommended energy-consumption according to age-groups (7 pages) and the recommended quantity of food components per day are tabulated. The two volumes together contain 13 000 entries.

The encyclopaedia is dealing with the latest basic knowledge on foods and their composition. Each entry gives information on the definition of the concept, the properties, the characteristics, the scientific name, the composition, the origin, the role in human nutrition, the significance in dietetics, the processing and the use. It gives full details of components, additives and foreign materials (residues). Moreover it informs on their chemical, physical and food technological characteristics, on their occurrence, production, application, significance and physiological effects.

Summing up: the encyclopaedia could be recommended for those all dealing with praxis, teaching, research and development. It could be employed in the domains of agronomics, botanics, food industry, gastronomy, public catering, public health, household, food chemistry, food trade and food technology.

I. VARSÁNYI

Food packaging and preservation

M. MATHLOUTHI (Ed.)

Blackie Academic & Professional, an imprint of Chapman & Hall, Glasgow, 1994.
275 pages

This book is the second edition of Food packaging and preservation, Theory and Practice, first published in 1986 by Elsevier Applied Science.

Thirty-two contributors summarize in 13 chapters the current research and the actual needs about various topics in food packaging.

The publication discusses the possibilities and importance of new packaging methods. It deals with the new trends in food packaging such as the microwavability of packaged foods or their direct

control by NMR imaging, as well as the effect of irradiation and the role of trehalose for food preservation.

The chapters in this book are presentations at international symposia (IFTEC, The Hague /NL/, Conditionment alimentaire; 2 défis: Innovation et environment, Pouzauges /France/) as well as other papers.

The book includes detailed discussion of the preservation of packaged food and the increase of shelf-life require knowledge of the permeability of plastic film to O_2, CO_2 and water vapour, as well as migration and flavour retention. These aspects are described with reference to the structure of the polymer itself and from analytical aspects, too.

Several chapters relate to permeability characteristics of plastic packaging materials. Attention is also given to using plastic packaging materials to aid in establishing modified atmosphere conditions for cheese, fruits and vegetables. Also considered in detail is the influence of packaging materials on the response of foods to micro-wave heating, to ionizing radiation and to spatial mapping of water and fat molecules by nuclear magnetic resonance.

Finally, recycling, reuse and disposability of plastic packaging materials are covered in several chapters.

Food packaging and preservation is a new multidisciplinary speciality. It deserves to be treated as science, which is what the contributors aim to do in this book.

The chapters are as follows:

The first chapter deals with the permeability and structure in polymeric packaging materials.

The second chapter outlines the use of

alternative fatty food simulants for polymer migration testing,

and compares the results from migration measurements into fats and oils with those in alternative fatty food simulants.

The 3rd chapter discusses the food flavour and packaging interactions.

In the 4th chapter the microwavability of packaged foods is discussed.

The 5th chapter summarizes the effects of irradiation of polymeric packaging material on the formation of volatile compounds.

The 6th chapter deals with package coating with hidrosorbent products and the shelf-life of cheeses.

The 7th chapter discusses the application of trehalose – a multifunctional additive for food preservation.

In the 8th chapter recent results of the packaging of fruits and vegetables are covered.

The 9th and 10th chapters deal with the bio-packaging: the technology and properties of edible and/or biodegradable materials of agricultural and bacterial origin.

The 11th chapter outlines the NMR imaging of packaged foods.

The 12th chapter summarizes the recycling, reuse and disposal of food packaging materials: a UK perspective.

The final (13th) chapter deals with the influence of light transmittance of packaging materials on the shelf-life of milk and dairy products.

The reader can find updated references at the end of each chapter.

Finally, a well-constructed index helps the users in looking for different topics.

Readers with an interest in food packaging will find the subject matter of this book timely, representative of some of the best work in the field of food packaging, and of considerable value.

J. MONSPART-SÉNYI

Packaging and the environment: alternatives, trends and solutions

S. E. M. SELKE

Technomic Publishing Co. Inc., Lausanne-Basel, 258 pages

It is a new and revised edition of the author's book on the correlations between the packaging and the environment. The book also covers legislative approaches to remedying solid waste-related impacts in the United States. It contains after the Foreword and Preface 16 chapters, together with a list of abbreviations (Appendix) and an Index on 241 and 243 pages. The overwhelming majority of examples and data are from the everyday praxis of the United States. All the chapters have a list of references from the latest years.

The first chapter is Introduction and it summarizes some of the environmental concerns as the functions of packaging, the quantity of packaging, the packaging disposal options, litter, and other concerns. In Chapter 2 the author examines the role of packaging in depletion of scarce resources and then considers the influence of packaging on pollution of water and air, including ozone depletion and the greenhouse effect (Chapter 3). Most of the remainder of the book deals with the most serious environmental impact of packaging today – its role in solid waste. The book focuses on the roles of various waste disposal options, such as landfill (Chapter 5), waste reduction (Chapter 6), incineration (Chapter 7), composting (Chapter 8) and overview of recycling (Chapter 9), recycling of metals, glass, paper, plastics (Chapters 10–13), and it examines potential for degradable packaging to alleviate these problems (Chapter 14). Next they discuss legislative approaches to remedying solid waste-related packaging impacts in the United States (Chapter 15), and finally the role of environmental issues in packaging design (Chapter 16).

The declared purpose of this book is to investigate the role of packaging in various environmental problems and look at possible solutions. The content covers the expectations and it can be useful in the environment protection as well as in the package design. It gives basic considerations to understand the role of packaging in environmental problems and solutions.

I. VARSÁNYI

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Continued from back cover

Continued from back cover

RECENTLY ACCEPTED PAPERS

Composition of sow's colostrum and milk. Part I and II

CSAPÓ, J., CSAPÓ-KISS, ZS. & MARTIN, T.G.

Comparison of some components from pigs of different body mass kept in natural conditions

DWORSCHÁK, E., BARNA, É., CZUCZY, P., GERGELY, A., HÓVÁRI, J., KALTENECKER, J., KONTRASZTI, M., LUGASI, A., NESZLÉNYI, K. & RADNÓTI, L.

The occurrence of trans-resveratrol in Slovenian red and white wines

VRHOVSEK, U., EDER, R. & WENDELIN, S.

NOTICE TO CONTRIBUTORS

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Symbols. Units, symbols and abbreviations should be used according to SI (System International) rules. Unusual abbreviations must be explained in the text or in an appendix.

Figures and tables. Illustrations must be cited and numbered in the order they appear in the text. All line drawings should be submitted as clear, glossy, black and white originals or photographs of good quality. The author's name and the title of the paper together with the serial number of the figure should be written on the back of each figure. Figures and Tables, each bearing a title, should be self-explanatory and numbered consecutively. The number of measurements (n), their mean values ($\bar{x}$) and standard deviations ($\pm s$) should be indicated. To prove the objectivity of conclusions drawn from the results mathematical statistical methods must be used. Quantitative evaluation of data is indispensable.

References. The reference list (Literature) should be in alphabetical order as follows:

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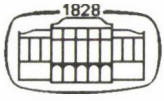

AKADÉMIAI KIADÓ
BUDAPEST

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COMPOSITION OF SOW'S COLOSTRUM AND MILK
I. PROTEIN CONTENT, AMINO ACID COMPOSITION AND BIOLOGICAL VALUE

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Protein content, protein fractions, amino acid composition and biological value of colostrum and milk of 10 Danish Large White, 10 Danish Duroc and 10 Norwegian Landrace sows were determined. The total protein content of the first colostrum (16.65%) was approximately three times as great as that at the end of lactation (5.83%). All of the protein fractions decreased during lactation, with the exception of casein, which reached its maximum between 24–72 h of lactation with a value of 3.4–3.6%, and non-protein nitrogen, which increased from the beginning of lactation (0.41%) to the end of lactation (0.47%). Significant differences were observed between free amino acid contents of colostrum and milk. Colostrum contained less acidic and hydroxy, and more basic amino acids than did milk. When amino acid concentrations were expressed as g AA/100 g protein, most of the essential amino acids (threonine, cystine, valine) decreased, while the non-essential glutamic acid and proline increased. This explains why the biological value of colostral protein was approximately 11% higher during the first five days of lactation (118–129) than that of milk protein on the 10–60th day of lactation. There were no significant differences among breeds or interaction between breeds and sampling dates relative to composition of colostrum and milk samples.

Keywords: sow's colostrum and milk, protein content, protein fractions, free amino acids, amino acid composition, biological value

As early as 1892, ERLICH observed that the main protein fraction of colostrum – the globulins – was responsible for development of passive immunity in new-born animals. Many publications have appeared on this subject (SPEER et al., 1959, PAYNE & MARSH, 1962; LECCE et al., 1962; HARDY, 1965; BUTLER, 1971; WERHAHN et al., 1981; KLOBASA et al., 1981, 1986). Many researchers measured differences among individual animals and studied the influences of stage of lactation, age of sow, feeding regime and season on milk production and milk composition (NEUHAUS, 1961; ONDERSCHEKA, 1969; RERAT & DNEE, 1975).

KOVÁCS and co-workers (1967) examined the composition of sow's colostrum during lactation, and established that, to the 6th day of lactation, the pre-albumin did

not change but the fractions of α- and β-globulins increased a little. On the other hand, the quantity of γ-globulin decreased rapidly after parturition. JENSEN (1978) and JENSEN and PEDERSEN (1979) examined the IgG, IgA and IgM composition of colostrum, while the IgG content of colostrum and the changes in its concentration were studied by FRENYÓ and co-workers (1981). According to INOUE (1981) the environment, age, feeding and number of sows on the farm scarcely influenced the IgM content of colostrum, while vaccination had a significant influence upon it. KLOBASA and co-workers (1987) found that the total protein content of the first colostrum was two to two and half times higher, whey protein content five times higher, and non-protein nitrogen content lower than that of normal milk. The largest constituents of whey protein were IgG and IgM. HENNIG and ANKE (1966) obtained similar results when they examined the total protein and NPN content of sow's milk.

KOVÁCS (1961) examined milk production and milk composition of Large White sows and established that the digestible protein content of colostrum on the first day of lactation was 10.02%. Comparable values for milk samples drawn on the 12th and 34th days of lactation and at the end of lactation were 4.40, 5.18 and 6.47%, respectively. BEREZVAI and RÁKÓCZI (1966) compared composition of milks from Hungarian White and Cornwall sows, and established that the colostrum of Cornwall sows contained more milk protein than the colostrum of Hungarian Large White sows. KLAVER and co-workers (1981) compared composition of milks from sows which were in good and poor body condition, while WHITE and co-workers (1984) compared composition of milks from sows which consumed fructose and dextrose. The conclusions were that feeding did not significantly influence milk composition. NOBLET and ETIENNE (1986), however, found significantly higher protein content in milk of sows consuming higher energy level food that in milk of sows consuming lower energy level food. In all of the above citations, stage of lactation had a significant effect on milk composition.

The amino acid composition of sow's milk and milk protein was described by ELLIOTT and co-workers (1971), DUEE and JUNG (1973), DOURMAND and co--workers (1991). GURR (1981) compared the amino acid compositions of sow's and mother's milks, and established that the protein of mother's milk contained less glutamic acid, methionine, tyrosine, lysine and histidine and more cystine and tryptophan than did sow's milk. Especially conspicuous was the dissimilarity in the ratio of methionine to cystine. The differences in contents of other amino acids were negligible when comparing sow's and mother's milk. Recently KING an co-workers (1993) studied the effect of dietary protein concentration on the amino acid composition of sow's milk. It was established that the dietary protein level significantly affected milk yield, and gave higher milk protein output but did not affect the proportions of amino acids in milk.

1. Materials and methods

Milk samples of 10 Danish Large White, 10 Danish Duroc and 10 Norwegian Landrace sows were collected and analysed. Sows were milked on the following schedule: colostrum and milk samples (50–100 cm^3) were taken directly after parturition and on the 12th, 24th and 48th h and on the 3rd, 5th, 10th and 20th days of lactation and finally between 45–60 days of lactation by hand milking. The milkings were performed by two qualified pig keepers. During the experiment, pigs suckled their mothers. Only the first colostrum was taken before the pig could suckle. After first colostrum, great care was taken to completely milk all of the mammary glands so that the sample would be representative of milk consumed by the pigs. The pigs were separated from the sow approximately 10 min prior to milking and milk let down was initiated by a warm water wash of the udder. Since time following last nursing by the pigs was not controlled, that factor could influence concentration of samples. However, the effect of such variability on data analysis would be to make the tests of significance conservative (i.e. to find fewer differences than actually exist).

Milk samples were frozen and stored at −25 °C. At time of analysis, the samples were thawed in water at +35 °C and blended. Total protein content and protein fractions of colostrum and milk were measured by the Kjel-Foss nitrogen analyser (protein content = N% × 6.38). Separation of protein fractions was performed as described by CSAPÓ (1984). The amino acid composition of milk protein was measured by automatic amino acid analyser (type: LKB 4101). Protein was hydrolysed by 6 mol dm^{-3} HCl, the sulphur-containing cystine was determined in the form of cysteic acid and the tryptophan content of milk protein was determined by the barium hydroxide hydrolysis method (CSAPÓ, 1982; CSAPÓ & CSAPÓNÉ, 1986). The biological value of milk protein was calculated by the method of MORUP & OLESEN (1976) on the basis of amino acid composition.

The composition of cow's milk was determined in samples from 32 cows by the same methods as were used for sow's milk. The cows were under summer feeding conditions, based principally on grass. The sample consisted of 17 Holstein-Friesian sired crossbred cows (62.5% Holstein-Friesian, 25% Jersey and 12.5% Hungarian red spotted) which were in the second or third lactation and 15 Hungaro-Friesian cows in the first lactation (CSAPÓ, 1984; CSAPÓ et al., 1994).

The data were analysed by analysis of variance using a model which contained main effects of breed and time of collection plus the interaction of breed and time. Differences among times were tested by the method of ranked means.

2. Results and discussion

Data for each trait were analysed by analysis of variance with main effects of breed (2 d.f.) and time of collection (8 d.f.) plus the breed by time interaction effect in the model. Neither breed nor interaction were found to represent a significant ($P > 0.25$) source of variance for any of the traits. Therefore, the results reported here describe only the time trends.

2.1. Contents of protein and protein fractions of colostrum and milk

The concentrations of protein and protein fractions and their changes in sow's colostrum and milk to the 60th day of lactation are shown in Table 1. Table 2 shows the distribution of protein fractions expressed as percentages of total protein. The free amino acid contents of sow's colostrum and milk expressed as mg free amino acid/100 g milk and g free amino acid/100 g free amino acid are shown in Table 3. Amino acid contents of sow's colostrum and milk expressed as g/100 g fluid are shown in Table 4, while the amino acid composition of milk protein expressed as g/100 g protein are shown in Table 5. Table 6 contains the biological value of milk protein calculated by the method of MORUP and OLESEN (1976) based on the amino acid composition.

The total protein content of colostrum immediately after parturition averaged 16.65%. This value decreased to 7.97–9.35% in transition milk (48th to 72nd h of lactation) and to 5.75% in milk on the 10th day of lactation, which is characteristic of normal milk. Due to the fact that the true protein content was calculated by substraction of NPN (non-protein nitrogen) from total protein, the changes in true protein coincided closely with those of total protein.

There were large changes in whey protein and true whey protein content over time. These two components, respectively, decreased from 15.16 and 14.75%, measured immediately after parturition, to 8.55 and 7.93% at 24 h, and to 3.0% and 2.65% at 10 days, which is characteristic of normal milk. The casein content of colostrum was 1.48% immediately after farrowing, increased to 3.57% at 48 h and afterwards declined to 2.75% at 5 days of lactation. Corresponding changes in the NPN content were much smaller; NPN content of the first colostrum did not differ significantly from the NPN content of the milk milked in the middle or at the end of lactation.

The distribution of protein fractions, expressed as percentages of total protein (Table 2), also changed over time. The true protein content decreased from 97.5% for first colostrum to 95.5% at 48 h, and to 91.8% for milk at 45–60 days of lactation. Due to decreasing total protein content, the proportion of NPN increased over time. The proportion of total protein in the casein fraction increased rapidly following

parturition (8.9% at parturition to 28.2% at 24 h and to 44.0% at 5 days). Whey protein and true whey protein declined as a proportion of total protein (91.1% at farrowing, 71.8% at 24 h and 51.7% at 20 days for whey protein). These changes are related to the transition from colostrum to milk. Most of the changes observed occurred during the first 24 h after parturition, and the remained of the changes from colostrum to milk occurred gradually over the first five days. The changes between the compositions of colostrum and milk were not as great as those observed in ruminant animals. Milk from ruminants has much higher casein content than that from sows (CSAPÓ, 1984; CSAPÓ et al., 1994).

Table 1

Means and standard deviations of contents of protein and protein fractions of sow's colostrum and milk (g/100 g milk)

Protein fractions	Time after parturition									Cow's milk[c]
	hours					days				
	0	8–14	20–28	44–52	68–76	5	10	20	45–60	5–270
Total protein										
Average[a]	16.65	13.05	11.91	9.35	7.97	6.25	5.75	5.55	5.83	3.33–4.13
S.D.[b]	2.56	2.16	2.43	1.82	1.57	1.08	0.64	0.45	0.49	
True protein										
Average	16.23	12.66	11.50	8.93	7.54	5.81	5.32	5.11	6.36	3.18–3.97
S.D.	2.33	2.04	1.88	1.90	1.45	0.96	0.56	0.34	0.38	
Whey protein										
Average	15.16	10.43	8.55	5.78	4.79	3.50	3.07	2.87	3.01	0.723–0.791
S.D.	1.56	1.43	1.02	1.12	0.96	0.61	0.55	0.26	0.35	
True whey protein										
Average	14.75	10.15	7.93	5.36	4.35	3.06	2.65	2.43	2.54	0.573–0.632
S.D.	1.48	1.34	0.97	1.02	0.65	0.53	0.48	0.24	0.29	
Casein										
Average	1.48	2.63	3.36	3.57	3.19	2.75	2.68	2.68	2.81	2.61–3.34
S.D.	0.11	0.23	0.31	0.31	0.24	0.21	0.16	0.16	0.16	
NPN × 6.38										
Average	0.41	0.40	0.41	0.42	0.44	0.44	0.43	0.44	0.47	0.150–0.159
S.D.	0.05	0.06	0.05	0.05	0.07	0.06	0.05	0.04	0.06	

[a] Mean of samples from 30 sows. (10 Danish Large White, 10 Danish Duroc and 10 Norwegian Landrace)

[b] Standard error of mean = 0.1826 S.D.; 95% confidence range = mean ±0.3578 S.D.

[c] From our earlier investigations based on milk samples from 32 cows

Table 2

Distribution of protein fractions expressed as percentages of total protein

Protein fractions	Time after parturition									Cow's milk[b]
	hours					days				
	0	8–14	20–28	44–52	68–76	5	10	20	45–60	
Total protein[a]	100	100	100	100	100	100	100	100	100	100
True protein	97.5	97.0	96.6	95.5	94.6	93.0	92.5	92.1	91.9	95.5–96.1
Whey protein	91.1	79.9	71.8	61.8	60.1	56.0	53.4	51.7	51.6	19.2–21.7
True whey protein	88.6	77.8	66.6	57.3	54.6	49.0	46.1	43.8	43.6	15.3–17.2
Casein	8.9	20.2	28.2	38.2	40.0	44.0	46.6	48.3	48.2	78.3–80.9
NPN × 6.38	2.5	3.1	3.4	4.5	5.5	7.0	7.5	7.9	8.1	4.0–4.4

[a] Mean of samples from 30 sows. (10 Danish Large White, 10 Danish Duroc and 10 Norwegian Landrace)

[b] From our earlier investigations based on milk samples from 32 cows

These results, relative to decrease in total protein and changes in NPN and whey protein contents, are in good agreement with the results of KLOBASA and co-workers (1987). The value of 16.65% for the total protein content of first milked colostrum was slightly higher than that reported by LUCAS and LODGE (1961). In this study we obtained a higher whey protein ratio and a lower casein ratio than that measured by SALMON-LEGAGNUER (1965). The results for NPN content of colostrum and milk were very similar to those reported by LUCAS and LODGE (1961). No reports were found which compared protein fractions of colostrum and milk, or described changes in protein fractions from first colostrum to the end of lactation.

2.2. Free amino acid composition of colostrum and milk

The free amino acid contents of colostrum and milk are shown in Table 3. The ratio of free amino acids are expressed as mg/100 g milk and as g/100 g free AA. The total free amino acid content of milk was 18% higher than that of colostrum. The free amino acid contents of colostrum were significantly higher than those of milk in studies on mare's colostrum (CSAPÓ-KISS et al., 1995). There were significant differences between acidic and basic amino acids. Milk contained more (69.59 mg/100 cm^3) acidic and hydroxy amino acids (aspartic acid, threonine, serine,

glutamic acid), and less (5.9 mg/100 cm^3) basic amino acid (lysine, histidine, arginine), than colostrum, which contained 28.96 mg/100 cm^3 acidic and hydroxy amino acids and 13.8 mg/100 cm^3 basic amino acids. Significant differences were observed between colostrum (0 to 72 h) and milk (5 to 60 days) in valine, methionine, isoleucine, tyrosine and phenylalanine contents. Colostrum contained three to eight times as much of these amino acids as did milk. Milk contained significantly more aspartic acid, serine and glutamic acid than did colostrum. No comparable data on the free amino acids were found in the literature.

Table 3

Free amino acid contents of sow's colostrum and milk

Amino acid	Free amino acid content			
	mg free amino acid/100 g milk		g free amino acid/100 g free AA	
	colostrum	milk	colostrum	milk
Aspartic acid	4.31	12.18	5.0	11.8
Threonine	3.50	6.54	4.1	6.3
Serine	5.51	24.32	6.4	23.5
Glutamic acid	15.63	26.55	18.2	25.6
Proline	6.91	3.81	8.1	3.7
Glycine	4.64	10.21	5.4	9.9
Alanine	6.92	7.33	8.1	7.1
Cystine	0.99	1.02	1.2	1.0
Valine	4.41	0.85	5.1	0.8
Methionine	3.50	0.89	4.1	0.8
Isoleucine	1.60	0.47	1.9	0.5
Leucine	7.51	0.99	8.8	1.0
Tyrosine	3.14	1.42	3.7	1.4
Phenylalanine	3.41	0.93	4.0	0.9
Lysine	4.52	1.03	5.3	1.0
Histidine	2.43	1.35	2.8	1.3
Arginine	6.85	3.74	8.0	3.6
Totals	85.78	103.63	100.2	100.2

[a] Mean of samples from 3 Danish Large White, 3 Danish Duroc and 3 Norwegian Landrace sows representing first drawn colostrum

[b] Mean of samples from 3 Danish Large White, 3 Danish Duroc and 3 Norwegian Landrace sows representing milk samples between 45–60 days of lactation

2.3. Amino acid composition of colostrum, milk, colostrum protein and milk protein

The amino acid content of colostrum and milk (Table 4) declined from farrowing to 45–60 days in a manner which parallelled the change of total protein content. This means that each amino acid decreased, without exception, from initial

colostrum to final milk. Threonine content declined from 0.96 g/100 g colostrum to 0.24 g/100 g milk and the corresponding change for glutamic acid was 3.01 to 1.30. The ratios of final to initial values for the individual amino acids ranged from 0.22 to 0.44. The comparable ratio for total protein was 5.83/16.65 = 0.35. When the amino acid (AA) composition was expressed as g AA/100 g protein, the changes were much less apparent (Table 5). Threonine decreased from 6.5 to 5.4 g/100 g protein, valine decreased from 5.1 to 3.8 g/100 g protein while glutamic acid increased from 18.1 to 22.1 g/100 g protein, and proline increased from 9.1 to 11.1 g/100 g protein. The sum of five essential amino acids (threonine, valine, cystine, tyrosine and lysine) decreased from 22.9 to 20.1 g/100 g protein, while the total of two non-essential amino acids (glutamic acid and proline) increased form 27.2 to 33.2 g/100 g protein. In general, the majority of essential amino acids in milk protein decreased, while the non-essential amino acids increased or showed no changes during lactation.

ELLIOTT and co-workers (1971) reported that the amino acid composition of milk collected from sows remained relatively stable during lactation despite quite large variations in the level of dietary protein consumed by sows. The diet influenced only glutamic acid content of milk. Sows which consumed a low protein diet had a higher proportion of glutamic acid in their milk. This observation, related to the stable amino acid composition of milk protein during lactation, seems to be contradictory to results reported here. However, ELLIOTT and co-workers did not investigate the critical period of the first three days after parturition, when the largest changes occurred in this investigation. The lysine content of milk protein in this experiment was between 6.9–7.2 g/100 g protein between 10 and 60 days of lactation, which agrees with the results of ELLIOTT and co-workers (1971) and KING and co-workers (1993). Higher levels of lysine were reported by DUEE and JUNG (1973) and DOURMAND and co-workers (1991) (7.59 and 7.24 g lysine/100 g protein, respectively). No references were located which reported data on the amino acid composition of colostrum milked immediately after parturition. Data for the amino acid composition of milk protein, with the exception of glycine, alanine and histidine, agree with the results of KING and co-workers (1993). In the case of some amino acids, these results differed from results of ELLIOTT and co-workers (1971), DUEE and JUNG (1973) and DOURMAND and co-workers (1991).

Table 4

Amino acid composition of sow's colostrum and milk relative to time after parturition
(gAA/100 g milk)

Amino acids	Time after parturition									Cow's[a] milk
	hours					days				
	0	8–14	20–28	44–52	68–76	5	10	20	45–60	
Aspartic acid	1.29	0.99	0.94	0.76	0.64	0.49	0.45	0.45	0.47	0.26
Threonine	0.96	0.69	0.64	0.47	0.47	0.25	0.24	0.24	0.24	0.15
Serine	1.09	0.84	0.75	0.59	0.48	0.36	0.31	0.29	0.33	0.16
Glutamic acid	3.01	2.48	2.23	1.76	1.47	1.37	1.28	1.24	1.30	0.77
Proline	1.51	1.16	1.13	0.90	0.78	0.67	0.63	0.61	0.66	0.32
Glycine	0.52	0.39	0.36	0.28	0.24	0.16	0.13	0.13	0.13	0.06
Alanine	0.74	0.56	0.51	0.40	0.30	0.22	0.16	0.16	0.16	0.10
Cystine	0.30	0.22	0.21	0.16	0.12	0.09	0.09	0.09	0.09	0.02
Valine	0.84	0.66	0.60	0.43	0.37	0.26	0.22	0.22	0.22	0.16
Methionine	0.29	0.22	0.20	0.17	0.14	0.12	0.10	0.10	0.10	0.06
Isoleucine	0.40	0.32	0.29	0.24	0.25	0.19	0.17	0.17	0.17	0.14
Leucine	1.65	1.32	1.23	0.98	0.80	0.66	0.57	0.56	0.57	0.29
Tyrosine	0.66	0.54	0.45	0.38	0.33	0.24	0.23	0.23	0.22	0.15
Phenylalanine	0.74	0.57	0.52	0.40	0.31	0.22	0.22	0.21	0.20	0.16
Lysine	1.05	0.89	0.73	0.58	0.50	0.39	0.42	0.40	0.39	0.27
Histidine	0.35	0.28	0.26	0.20	0.18	0.13	0.13	0.13	0.12	0.10
Tryptophan	0.26	0.21	0.19	0.14	0.13	0.09	0.10	0.10	0.10	0.05
Arginine	1.01	0.74	0.66	0.52	0.47	0.34	0.32	0.31	0.32	0.11

[a] From our earlier investigations based on milk samples from 32 cows

2.4. The biological value of milk protein

The biological value of milk protein was calculated by the method of MORUP and OLESEN (1976) based on amino acid composition (Table 6). The biological values of colostrum milked between farrowing and the fifth day of lactation were only slightly lower (extremes: 118.0 and 129.5) than the maximum of the method (140). This was due to very high essential amino acid (especially threonine and cystine) contents. During hours 0 to 52, the biological value of milk protein increased from 118.0 to 129.5, and later decreased to 106.4–108.6 due to the reduced quantities of essential amino acids near the end of lactation. From the 10th to the 45–60th days, the biological values of milk protein averaged 107.5. This is a very high biological value compared with that of cow's milk which was 80.2 based on the data in Table 5. These differences can be explained by the higher proportion of whey protein and higher quantities of essential amino acids, especially threonine, in sow's milk. No comparable data were found in the literature.

Table 5

Amino acid composition of sow's colostrum protein and milk protein relative to time after parturition (g AA/100 g protein)

Protein fractions	Time after parturition									Cow's milk[a]
	hours					days				
	0	8–14	20–28	44–52	68–76	5	10	20	45–60	protein
Aspartic acid	7.8	7.6	7.9	8.2	8.0	7.9	7.9	8.0	7.9	7.8
Threonine	*5.8*	*5.3*	*5.4*	*5.1*	*5.4*	*4.0*	*4.1*	*4.2*	*4.1*	*4.5*
Serine	*6.5*	*6.4*	*6.3*	*6.3*	*6.1*	*5.7*	*5.4*	*5.3*	*5.4*	*4.8*
Glutamic acid	**18.1**	**19.0**	**18.8**	**18.8**	**18.6**	**21.9**	**22.3**	**22.4**	**22.1**	**23.2**
Proline	**9.1**	**8.9**	**9.5**	**9.7**	**9.8**	**10.6**	**11.0**	**10.9**	**11.1**	**9.6**
Glycine	3.1	3.0	3.1	3.0	3.0	2.6	2.3	2.3	2.2	1.8
Alanine	4.4	4.3	4.3	4.2	3.8	3.6	2.8	2.8	2.9	3.0
Cystine	*1.8*	*1.7*	*1.8*	*1.7*	*1.5*	*1.4*	*1.5*	*1.5*	*1.5*	*0.6*
Valine	*5.0*	*5.1*	*5.1*	*4.6*	*4.7*	*4.1*	*3.8*	*3.9*	*3.8*	*4.8*
Methionine	1.7	1.7	1.7	1.8	1.8	1.9	1.6	1.6	1.6	1.8
Isoleucine	2.4	2.5	2.4	2.6	3.2	3.0	2.9	2.9	2.9	4.2
Leucine	9.9	10.1	10.4	10.5	10.1	10.4	9.8	9.9	9.7	8.7
Tyrosine	4.0	4.1	3.8	4.1	4.1	3.9	3.9	3.9	3.8	4.5
Phenylalanine	4.4	4.4	4.4	4.2	3.8	3.6	3.8	3.8	3.7	4.8
Lysine	6.3	6.7	6.2	6.2	6.3	6.2	7.2	7.0	6.9	8.1
Histidine	2.1	2.1	2.2	2.2	2.3	2.0	2.3	2.3	2.2	3.0
Tryptophan	1.6	1.6	1.6	1.5	1.6	1.4	1.6	1.6	1.6	1.5
Arginine	6.1	5.7	5.6	5.6	5.9	5.4	5.6	5.5	5.6	3.3

[a] From our earlier investigations based on milk samples from 32 cows

Table 6

Biological value of colostrum protein and milk protein, calculated by MORUP *and* OLESEN *(1976), relative to time after parturition*

Time after parturition (hours)	Biological value	Time after parturition (days)	Biological value
0	118.0	5	118.8
8–14	122.9	10	108.6
20–28	127.6	20	107.6
44–52	129.5	45–60	106.4
68–76	122.5		

[a] One value calculated for each time after parturition based on the average concentration of amino acids (Table 5) and the method of MORUP and OLESEN (1976)

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References

BEREZVAI, F. & RÁKÓCZI, I. (1966): Újabb adatok a kocatej összetételének változásához a laktáció folyamán. (Further data on changes in sow's milk composition during lactation.) *Magy. Állatorv. Lap.*, *212*, 448–491.

BUTLER, J. E. (1971): Review of the bovine immunoglobulins. *J. Dairy Sci.*, *54*, 1315–1316.

CSAPÓ J. (1982): Gyors módszer élelmiszerek és takarmányok cisztintartalmának meghatározására ioncserés oszlopkromatográfiával. (Fast method for the determination of cystine content of food and feed by ion exchange column chromatography.) *Élelmiszerv. Közl.*, *4*, 163–172.

CSAPÓ J. (1984): *Colostrum and milk composition of several genotypes of cattle*. Ph. D. Thesis. PANNON Agricultural University Faculty of Animal Science, Kaposvár. p. 120.

CSAPÓ J. & CSAPÓ JNÉ (1986): Takarmányok és élelmiszerek triptofántartalmának meghatározása fotometriásan és ioncserés oszlopkromatográfiával. (Determination of tryptophan content of food and feed by ion exchange column chromatography and photometrically.) *Élelmiszerv. Közl.*, *3*, 150–161.

CSAPÓ J., CSAPÓ-KISS, ZS., MARTIN, T. G., SZENTPÉTERI, J. & WOLF, GY. (1994): Composition of colostrum from goats, ewes and cows producing twins. *Int. Dairy J.*, *4*, 445–458.

CSAPÓ-KISS, ZS., STEFLER, J., MARTIN, T. G., MAKRAY, S. & CSAPÓ, J. (1995): Composition of mare's colostrum and milk. Protein content, amino acid composition and biological value. *Int. Dairy J.*, *5*, 403–415.

DOURMAND, J. Y., ETIENNE, M. & NOBLET, J. (1991): A contribution to the study of amino acid requirements for lactation in sows. *Journées Rech. Porcine en France*, *23*, 61–68.

DUEE, P. H. & JUNG, J. (1973): Amino acid composition of sow's milk. *Ann. Zootech.*, *22*, 243–247.

ELLIOTT, R. F., VANDER, NOOT, G. W., GILBREATH, R. L. & FISHER, H. (1971): Effect of dietary protein level on composition changes in sow colostrum and milk. *J. Anim. Sci.*, *32*, 1128–1137.

ERLICH, P. (1892): Ober Immunität durch Vererbung und Säugung. *Z. Hyg. Infekt. Krankh.*, *12*, 183.

FRENYÓ, V. L., PETHES, GY., ANTAL, T. & SZABÓ, I. (1981): A sertés kolosztrum- és vérsavó-IgG koncentrációjának változása az idő függvényében. (Change in IgG concentration of colostrum and serum in sows.) *Magy. Állatorv. Lap.*, *36*, 315–317.

GURR, M. I. (1981): Review of the progress of dairy science: Human and artificial milks for infant feeding. *J. Dairy Res.*, *48*, 519–554.

HARDY, R. N. (1965): The breakdown of ^{131}I gamma-globulin in the digestive tract of the newborn pig intestine. *J. Physiol.*, *205*, 435–451.

HENNIG, A. & ANKE, M. (1966): Die Zusammensetzung der Sauenmilch verschiedener Laktationsstadien. (Rohnährstoffe, Mengenelemente, Spurenelemente.) *Arch. Tierz.*, *9*, 312–329.

INOUE, T. (1981): Possible factors influencing the immunoglobulin M concentration in swine colostrum. *Am. J. Vet. Res.*, *42*, 1429–1432.

JENSEN, P. T. (1978): Trypsin inhibitor and immunoglobulins in porcine colostrum. *Acta Vet. Scand.*, *19*, 475–477.

JENSEN, P. T. & PEDERSEN, K. B. (1979): Studied on immunoglobulins an trypsin inhibitor in colostrum and milk from sows and in serum of their piglets. *Acta Vet. Scand.*, *20*, 60–72.

KING, R. H., RAYNER, C. J. & KERR, M. (1993): A note on the amino acid composition of sow's milk. *Anim. Prod.*, *57*, 500–502.

KLOBASA, F., WERHAHN, E. & BUTLER, J. E. (1981): Regulation of humoral immunity in the piglet by immunoglobulins of maternal origin. *Res. Vet. Sci., 31*, 195–206.

KLOBASA, F., BUTLER, J. E., WERHAHN, E. & HABE, F. (1986): Maternal-neonatal immunoregulation in swine. II. Influence of multiparity on de novo synthesis by piglets. *Vet. Immun. Immunopath., 11*, 149–156.

KLOBASA, F., WERHAHN, E. & BUTLER, J. E. (1987): Composition of sow milk during lactation. *J. Anim. Sci., 64*, 1458–1466.

KLAVER, J., VAN KEMPEN, G. J. M., DE LANGE, P. G. B., VERSTEGEN, M. W. A. & BOER, H. J. (1981): Milk composition and daily yield of different milk components as affected by sow condition and lactation/feeding regiment. *J. Anim. Sci., 52*, 1091–1097.

KOVÁCS, J. (1961): *Fehérhússertés szoptatókocák takarmányhasznosítása.* (Food utilization of suckling large white sows.) Mezőgazd. Kiadó, Budapest, p. 21.

KOVÁCS, F., KALICH, J. & SALLAI, J. (1967): Egyes környezeti tényezők hatása a sertés termelésére és egészségére. III. A colostrum mint környezeti faktor, különös tekintettel a malackori betegségek megelőzésére. (The effect of certain environmental factors on the health and production of pigs. III. The colostrum as an environmental factor with special respect to the prevention of diseases of piglet age.) *Magy. Állatorv. Lap., 22*, 557–560.

LECCE, J. G., MATRONE, G. & MORGAN, D. O. (1962): Porcine neonatal nutrition, absorption of unaltered nonporcine proteins, and polyvinylpyrrolidine from gut of piglets and subsequent effects on maturation of serum profile. *J. Nutr., 73*, 159–171.

LUCAS, I. A. M. & LODGE, G. A. (1961): *The nutrition of the young pig.* A review. Technical Communication No. 22. Commonwealth Bureau of Animal Nutrition.

MORUP, K. & OLESEN, E. S. (1976): New method for prediction of protein value from essential amino acid patterns. *Nutr. Rep. Int., 13*, 355–365.

NEUHAUS, U. (1961): Die Milchleistung der Sau und die Zusammensetzung und Eigenschaften der Sauenmilch. *Z. Tierz. Züchtungsbiol., 75*, 160–167.

NOBLET, J. & ETIENNE, M. (1986): Effect of energy level in lactating sows on yield and composition of milk and nutrient balance of piglets. *J. Anim. Sci., 63*, 1888–1896.

ONDERSCHEKA, K. (1969): Untersuchungen über Menge und Zusammensetzung der Schweinemilch und den Einfluss dieser Faktoren auf das Wachstum Ferkel. Wien. *Tieraerztl. Monatsschr., 56*, 1–14.

PAYNE, L. C. & MARSH, C. L. (1962): Absorption of gamma-globulin by the small intestine. *Federation Proceedings, 103*, 751–756.

RERAT, A. & DNEE, P. H. (1975): Ernährung und Reproduction der Sau. *Obersichten Tierernaehr., 3*, 249–258.

SALMON-LEGAGNUER, E. (1964): Quelques aspects des relations nutritionnelles entre la gestation et la lactation chez la truie. *Ann. Zootech., 14*, 1–12.

SPEER, V. C., BROWN, H., QUINN, L. & CATRON, D. V. (1959): The cessation of antibody absorption in the young pig. *J. Immun., 83*, 632.

WERHAHN, E., KLOBASA, F. & BUTLER, J. E. (1981): Investigation of some factors which influence the absorption of IgG by neonatal piglet. *Vet. Immun. Immunopath., 2*, 35–51.

WHITE, C. E., HEAD, H. H., BACHMAN, K. C. & BAZER, F. W. (1984): Yield and composition of milk and weight gain of nursing pigs from sows fed diets containing fructose or dextrose. *J. Anim. Sci., 59*, 141–150.

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COMPOSITION OF SOW'S COLOSTRUM AND MILK
II. FATTY ACID COMPOSITION, AND CONTENTS OF FAT, VITAMINS AND MACRO- AND MICROELEMENTS

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Total solids and fat contents, fatty acid composition, fat soluble- (A, D_3, E, K_3) and vitamin C contents, macro- and microelement contents were determined in colostrum and milk samples from 10 Danish Large White, 10 Danish Duroc and 10 Norwegian Landrace sows. It was established that the total solids (24.03%) and fat content (5.32%) of first colostrum increased to 48–72 h of lactation (total solids: 27%, fat content: 13.1%), and afterwards decreased to 18.7% and 6.5% at the end of lactation. The fat of sow's milk contained saturated fatty acids with 4–12 C numbers only in very low concentrations (in fact just above the limit of identification). Sow's milk contained significantly more unsaturated fatty acids than cow's milk. Particularly great differences were found in the case of linolenic acid with sow's milk containing significantly more than cow's milk. Sow's milk contained more ash (0.843%), calcium (1965 mg kg^{-1}), phosphorus (1510 mg kg^{-1}), zinc (6.49 mg kg^{-1}), iron (2.44 mg kg^{-1}) and copper (1.34 mg kg^{-1}), and less potassium (748 mg kg^{-1}), sodium (387 mg kg^{-1}) and magnesium (111 mg kg^{-1}) than cow's milk, while there were no differences between the two species in manganese content. Potassium, sodium, iron and copper contents decreased, while calcium and phosphorus contents increased during lactation. Contents of vitamins A, D_3, E, K_3 and C of colostrum (1.61, 0.015, 3.69, 0.092 and 68.4 mg kg^{-1}) were found, with the exception of vitamin K_3, to be one and a half to twice as great as that of late lactation milk (0.92, 0.009, 2.53, 0.089 and 45.3 mg kg^{-1}).

Sow's milk contained vitamins at two to three times the concentrations in cow's milk. There were no significant differences among breeds or interaction between breeds and time of sampling with regard to composition of colostrum and milk.

Keywords: sow's colostrum and milk, fat content, fatty acid composition of milk fat, vitamin content, macro- and microelements.

Milk contains amino acids, proteins, fat and fatty acids, vitamins and macro- and microelements which are essential in the diet of man, and 1 l of milk can meet 20–60% of man's daily requirements for these nutrients. Cows, goats and ewes produce this essential food product from feed ingredients which are unsuitable for man's consumption, and of limited value for monogastric animals. The position is

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different in the case of sows. Sows consume foods, which are suitable for man's consumption, so the sow is a rival of man in the food chain. Knowledge of sow's milk composition could be used in formulating diets for early weaned or orphan piglets.

The objectives of this study were 1) to determine the composition of sow's colostrum and milk, 2) to describe compositional changes from farrowing to 60 days after farrowing and 3) to estimate differences between genotypes and variation among individuals within genotype with regard to milk composition.

The mammary gland develops in the last stage of pregnancy in non-milking sows. Milk production normally starts at parturition, but may begin one or two days before or after parturition. During the final hours of gestation, a highly pigmented and viscous colostral secretion is accumulated in the mammary gland. This secretion, called colostrum or precolostrum depending on time of collection, has as its predominant components globulin and albumin and smaller amounts of casein (KLOBASA et al., 1987). They reported that during the first 6 days of lactation, colostrum had more dry matter and protein content and less fat and lactose content than normal milk. After the colostral period, the fat and lactose content of milk increased, while that of dry matter remained constant. BEREZVAI and RÁKÓCZI (1966) reported that colostrum contained more dry matter, fat, ash, calcium, phosphorus and iron than normal sow's milk. HENNIG and ANKE (1966) stated that colostrum had more dry matter, sodium, potassium and copper than normal milk and that ash, calcium, magnesium, phosphorus and manganese contents increased during lactation. KLAVER and co-workers (1981), compared composition of milk from sows with poor and very good body conditions, and found that sows in good condition produced more milk which contained more energy than that from sows in poor condition. They reported that level of feeding did not influence milk composition in early lactation. NOBLET and ETIENNE (1986) examined composition of milk from sows consuming low and high energy diets and reported that energy content of diet did not influence lactose and ash contents, but that dry matter, fat and energy contents of milk were significantly higher in milk from sows consuming low energy food. In their opinion, higher dry matter content was largely due to higher fat content of milk. Higher fat content was attributed to the fact that sows consuming low energy food mobilised their body fat reserves better than did sows consuming high energy food. Feeding with fructose or dextrose did not influence fat content of milk, but the lactose content of milk was significantly higher in milk from sows consuming fructose than that from sows consuming dextrose (WHITE et al., 1984).

KLOBASA and FARRIES (1986) found that, in colostrum of the first day of lactation, there were no short chain fatty acids up to carbon number 10. Butyric acid was detected only at the end of lactation, and capronic acid after the 28th day of lactation. Oleic acid was the predominant fatty acid, followed by palmitic and linolic acids. The proportions of fatty acids changed continuously during lactation. They

stated that the fatty acid spectrum of sow's milk fat differed considerably from the milk fat of ruminants. DROCHNER and MARKUS (1987) analysed the composition of milk of sows whose diet contained 8% soybean oil, and reported that the fat content of sow's milk increased by 4–5% in the first two weeks, but only by 2% in the fourth and fifth weeks of lactation. The linoleic acid content of milk fat increased when soybean oil was fed. The content of lactose in the milk of the sows was not affected by soybean oil. ELLIOTT and co-workers (1971) reported that sow's milk fat contains far less 4–8 carbon saturated fatty acids than the milk fat of cows. Milk fat of sows did not contain butyric acid at all. Myristoleic acid was not found in sow's milk, but linolenic and linoleic acids, were in higher concentrations in sow's milk.

KIRCHGESSNER and co-workers (1981) established that colostrum from the Cu-depleted sows showed a greatly reduced Cu content relative to that of sows given an adequate Cu supply. The provision of adequate copper after parturition produced normal Cu levels within five days. According to GURR (1981) sow's milk contains less sodium and potassium and more calcium, magnesium, phosphorus, iron, zinc and manganese than cow's milk. The ratio of calcium:phosphorus is slightly greater in sow's milk than in cow's milk. Sow's milk contains two to three times as much vitamin A and four to five times as much vitamin C as cow's milk (ELLIOT et al., 1971). No references were found concerning contents of other vitamins in sow's milk.

1. Materials and methods

Milk samples from 10 Danish Large White, 10 Danish Duroc and 10 Norwegian Landrace sows were collected and analysed. Sows were milked on the following schedule: Colostrum and milk samples (50–100 cm^3) were taken by hand milking immediately after parturition, at the 12th, 24th and 48th h after parturition, on the 3rd, 5th, 10th and 20th days of lactation, and at between 45–60 days of lactation. The milkings were performed by two qualified pig keepers. During the experiments the pigs suckled their mothers. Only the first colostrum was taken before the pig could suckle. After the first colostrum, all of the mammary gland was milked completely, and the milk well mixed prior to sampling. The pigs were separated from the sow approximately 10 min prior to milking and milk let down was initiated by a warm water wash of the udder. Since time following last nursing by the pigs was not controlled, that factor could influence concentration of samples. However, the effect of such variability on data analysis would be to make the tests of significance conservative (i.e. to find fewer differences than actually exist).

Milk samples, stored at −25 °C, were thawed in water at +35 °C and blended. Dry matter of colostrum and milk samples was determined by HUNGARIAN STANDARD (1981) by drying to constant weight at 105 °C. Fat content was determined by the Gerber method according to HUNGARIAN STANDARD (1982).

The fatty acid contents of the milk fat were determined in the form of fatty acid methyl esters by a Packard 419 type gas chromatograph, a flame ionisation detector and a Hewlett-Packard 33900 type electronic integrator. In the quantitative evaluation, the relative percentage proportions of the methyl esters were regarded as equal to the proportions of the corresponding peaks in the chromatogram (CSAPÓ et al., 1986).

To determine vitamins A-, D_3- and E-contents of milk, samples (5 cm^3) were saponified by alcoholic pyrogallol solution and 2.5 cm^3 80% potassium hydroxide. The resulting material was extracted in an alcohol-n-hexane system. The extract was distilled and diluted in 200 μl methanol; 20 μl of the solution was injected on a 250 × 5 mm column packed with 10 μm granulation Partisil ODS, and the vitamin concentrations were determined on a Pye UNICAM LC–XP HPLC. Elution was carried out with an 85:15 solution of methanol:water at 1.4 cm^3/min drift speed. The basis for quantitative evaluation was vitamin standards made by MERCK. Vitamin K_3 was determined on a solution obtained by chloroform extraction of an alkalescent substrate. The extracted vitamin K was detected on 251 nm. Vitamin C content of milk samples was determined by the method of RADEFF (1938).

Ash content was determined by the HUNGARIAN STANDARD (1978). The macro- and microelements which were present in the ash as metallic oxides were converted to chlorides by hydrochloric acid and taken into solution. The metallic contents were determined by UNICAM SP–191 type atomic absorption spectrophotometer. Phosphorus content was determined by Spekol photometer by measuring the blue colour created by ammonium molybdenate.

The composition of cow's milk was determined on samples from 32 cows by the same methods as were used for sow's milk. The cows were under summer feeding conditions, based principally on grass. The sample consisted of 17 Holstein-Friesian sired crossbred cows (62.5% Holstein-Friesian, 25% Jersey and 12.5% Hungarian red spotted) which were in the second or third lactation and 15 Hungaro-Friesian cows in the first lactation (CSAPÓ, 1984; CSAPÓ et al., 1995).

The data were analysed by analyses of variance and regression analyses. Comparisons of individual pairs of means were used in establishing points of maxima and minima for the individual constituents.

2. Results and discussion

Data for each trait were analysed by analysis of variance with main effects of bread (2 d.f.) and time of collection (8 d.f.) plus the breed by time interaction effect in the model. Neither breed nor interaction were found to represent a significant ($P > 0.25$) source of variance for any of the traits. Therefore, the results reported here described only the time trends.

2.1. Dry matter and fat content of colostrum and milk

Dry matter and fat contents and changes associated with stage of lactation (colostrum to 45–60 days) for sows are shown in Table 1. Dry matter contents at different times after farrowing, with 95% confidence intervals of the means are shown in Fig. 1. The dry matter content of the colostrum immediately after parturition averaged 24.03%. Dry matter content remained unchanged to 24 h of lactation. Afterwards, it increased and reached a maximum between 48–72 h of lactation with 27.03–27.37%. The dry matter content of the milk decreased to 22% at 5 days, to 20% at 10 days and to 19% at 20 days of lactation, and this value remained unchanged to the end of lactation.

Table 1

Dry matter and fat contents of colostrum and milk of sows
(g/100 g)

Time after parturition (h/day)	Dry matter		Fat	
	Average	S.D.[a]	Average	S.D.[a]
0	24.03[c]	2.99	5.32[g]	2.05
8–14	23.01[c]	3.52	6.13[fg]	3.01
20–28	23.40[c]	2.82	9.99[cd]	3.56
44–52	27.37[b]	2.72	12.90[b]	3.11
68–76	27.03[b]	3.93	12.99[b]	2.47
5	22.32[c]	3.77	10.78[c]	2.33
10	19.74[b]	1.15	9.02[de]	1.92
20	18.60[e]	1.05	7.92[e]	1.54
45–60	18.69[e]	1.35	6.49[f]	1.42
Cow[h]	12.55	1.22	3.86	0.55

[a] Standard error of mean = 0.1826 S. D.; 95% confidence interval for mean is means ±0.3578 S.D.
[b, c, d, e, f, g] = Means in the same column having no superscripts in common differ ($P < 0.5$)
[h] From our earlier investigations based on milk samples from 32 cows

When the changes in the dry matter content relative to time after farrowing were compared with those of other farm animals which were studied earlier (CSAPÓ, 1984; CSAPÓ et al., 1994, 1995; CSAPÓ-KISS et al., 1995), the maximum dry matter content at 48–72 h of lactation was very atypical. In most other farm animals, we obtained a logarithmic decrease in the dry matter content after parturition. KLOBASA and co-workers (1987) reported somewhat similar results in the change in dry matter content. They measured higher dry matter content at 72 h and 5 days than at 12 and 24 h of lactation, but the differences were much smaller than those reported here.

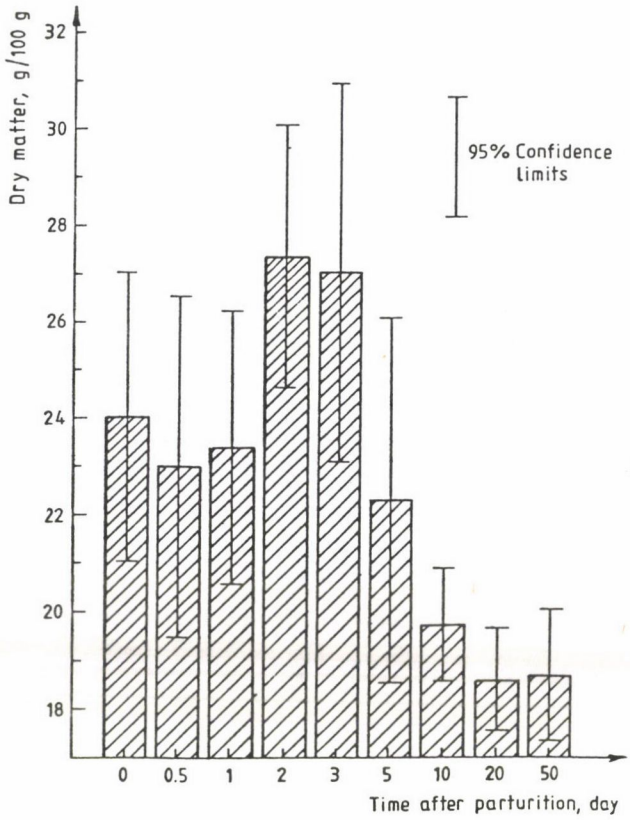

Fig. 1. Dry matter content of colostrum and milk of sows

The fat content of the colostrum (Fig. 2) was also the highest (12.90–12.99%) at 48–72 h of lactation, which was more than twice the level measured at the beginning or at 12 h of lactation (5–6%). It is probable that the increase in dry matter content was largely due to the increase in fat content. Fat content of sow's milk decreased more moderately than dry matter content during lactation, and at 5–10 days of lactation was 9–11%. Afterwards the fat content of the milk gradually decreased, and was 7.9% on the 20th day of lactation and 6.5% at the 45–60 day of lactation. Considering samples 0–72 h to represent colostrum, the dry matter and fat contents of colostrum were higher than those of milk.

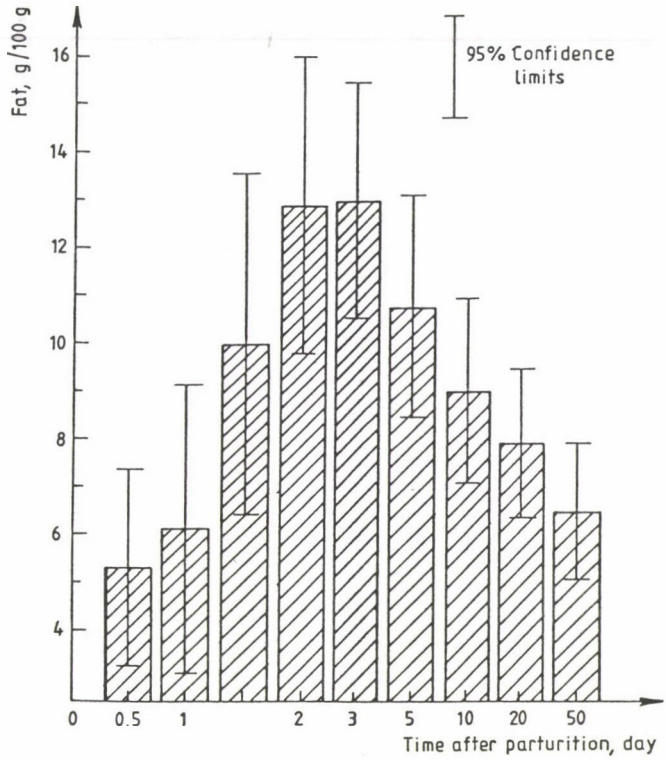

Fig. 2. Fat content of colostrum and milk of sows

Tendencies in the changes of fat content during lactation were very similar to those obtained by KLOBASA and co-workers (1987), but the levels observed were much higher in this study. The differences could be due to different breeds, feeding regimes or by the method of sampling of colostrum and milk. We tried to milk (except the first colostrum) all of the mammary glands totally, and it is well known that the last milk drawn is more concentrated in fat. In our experiment, the pigs suckled their mothers, and we determined the composition of milk comparable to that consumed by the pigs. In the sow, as in other farm animals, differences in milk sampling techniques can cause great differences in the dry matter and fat contents.

Dry matter and fat content of sow's milk and cow's milk were compared. Sow's milk (5–60 days) contained 58 and 120%, respectively, more dry matter and fat than did cow's milk.

2.2. Macro- and microelement content of colostrum and milk

Macro- and microelement contents and changes associated with stage of lactation (colostrum to 45–60 days) for sows are shown in Tables 2 and 3. Comparisons of macro- and microelements of sow's milk and cow's milk are also shown. Analytical methods were totally identical in the two species.

Table 2

Macro element contents of colostrum and milk of sows

Time after parturition (h/day)		Ash (g per 100 g)	Macro elements (mg per kg)				
			Potassium	Sodium	Calcium	Phosphorus	Magnesium
0							
	$\bar{x}$	0.662	1100	685	686	1017	78.5
	S.D.[a]	0.068	65	181	290	140	20.5
8–14							
	$\bar{x}$	0.660	997	802	820	1002	83.9
	S.D.	0.1240	88	203	296	169	15.7
20–28							
	$\bar{x}$	0.651	936	764	992	1092	90.8
	S.D.	0.166	180	228	333	132	17.4
44–52							
	$\bar{x}$	0.755	911	658	1157	1088	97.6
	S.D.	0.073	120	153	436	166	15.7
68–76							
	$\bar{x}$	0.817	841	709	1490	1104	107.3
	S.D.	0.076	124	282	278	153	18.0
5							
	$\bar{x}$	0.813	952	491	1632	1239	94.5
	S.D.	0.040	223	315	285	229	11.3
10							
	$\bar{x}$	0.796	836	470	1659	1202	93.1
	S.D.	0.037	63	179	153	90	7.1
20							
	$\bar{x}$	0.808	808	394	1746	1323	101.4
	S.D.	0.057	63	37	129	105	5.1
45–60							
	$\bar{x}$	0.843	748	387	1965	1510	110.7
	S.D.	0.027	109	64	150	44	7.7
Cow[b]							
	$\bar{x}$	0.753	1204	504	1287	996	139
	S.D.	0.031	68	33	143	11	12

[a] Standard error of mean = 0.1826 S.D.; 95% confidence interval for mean ±0.3578 S.D.

[b] From our earlier investigations based on milk samples from 32 cows

Table 3

Microelement contents of colostrum and milk of sows

Time after parturition (h/day)		Microelements (mg kg^{-1})			
		Zinc	Iron	Copper	Manganese
0					
	$\bar{x}$	15.70	1.70	3.77	0.062
	S.D.	4.98	0.24	1.24	0.031
8–14					
	$\bar{x}$	12.99	1.88	3.25	0.051
	S.D.	5.01	0.78	1.26	0.011
20–28					
	$\bar{x}$	12.44	2.39	3.32	0.108
	S.D.	4.34	1.35	0.96	0.069
44–52					
	$\bar{x}$	8.82	2.73	3.63	0.058
	S.D.	3.03	0.88	0.87	0.024
68–76					
	$\bar{x}$	5.75	2.92	3.77	0.061
	S.D.	2.53	1.09	1.39	0.015
5					
	$\bar{x}$	5.84	2.35	1.67	0.077
	S.D.	1.41	0.99	0.56	0.036
10					
	$\bar{x}$	5.64	2.21	1.24	0.073
	S.D.	1.11	0.61	0.16	0.029
20					
	$\bar{x}$	6.14	2.02	1.33	0.081
	S.D.	0.87	0.66	0.12	0.028
45–60					
	$\bar{x}$	6.49	2.44	1.34	0.100
	S.D.	0.06	1.15	0.20	0.049
Cow[b]					
	$\bar{x}$	5.63	1.07	0.302	0.093
	S.D.	0.19	0.32	0.055	0.013

[a] Standard error of mean = 0.1826 S. D.; 95% confidence interval for mean is mean ±0.3578 S.D.

[b] From our earlier investigations based on milk samples from 32 cows

Ash content of sow's colostrum was measured as 0.662%, and this level was constant during the first day of lactation. The ash content increased to 0.755 at 44–52 h of lactation, and to 0.817% at the end of the third day of lactation. From 3 days to the end of lactation, ash content ranged from 0.796 to 0.843%. After the colostral period the ash content of sow's milk was 10% higher than that of cow's milk. The early increase in ash content are not typical of patterns observed for other species studied in this laboratory.

The potassium content of colostrum was greatest immediately after parturition (1100 mg kg^{-1}). This value continuously decreased during lactation to 750–800 mg kg^{-1}, which was approximately 30–35% lower than that in cow's milk. The sodium content of sow's colostrum and milk was 658 to 802 mg kg^{-1} in the first three days of lactation and afterwards decreased to levels of 400–500 mg kg^{-1}, and remained unchanged during the remainder of the lactation. This value was approximately 20% less than that in cow's milk.

The calcium content of the colostrum increased from 686 mg kg^{-1} to 1157 mg kg^{-1} during the first two days of lactation, and reached a maximum of 1965 mg kg^{-1} at the end of lactation. Calcium content of sow's milk was 25% higher than that of cow's milk. Phosphorus content of colostrum changed only slightly during the first 48 h of lactation (1017–1088 mg kg^{-1}), and afterwards increased in a pattern similar to that of calcium and reached its maximum (1510 mg kg^{-1}) at the end of lactation. Comparing the magnesium content of first colostrum with the magnesium content of first colostrum from other farm animals, it was surprising that the first colostrum had such very low magnesium content (78.5 mg kg^{-1}) relative to the later stages of lactation. The magnesium content increased to 97.6 mg kg^{-1} by the second day of lactation and ranged from 93.1 to 110.9 mg kg^{-1} far the remained of the lactation. The magnesium content was approximately 33–40% lower than that of cow's milk.

The zinc content of the colostrum was highest at parturition (15.70 mg kg^{-1}), which continuously decreased to the 3rd day or lactation (5.95 mg kg^{-1}), and afterwards remained constant (5.69 to 6.49 mg kg^{-1}) to the end of lactation. The zinc contents of sow's- and cow's milk were virtually identical. The iron content of the colostrum was lowest at parturition (1.70 mg kg^{-1}), reached a maximum at 2–3 days of lactation (2.73–2.92 mg kg^{-1}), and afterwards remained high to the end of lactation (2.2–2.4 mg kg^{-1}). The iron content of sow's milk was more than twice as high as that of cow's milk.

The copper content of sow's colostrum did not show significant change during the first 3 days of lactation (3.25–3.77 mg kg^{-1}), but later decreased to 1.24–1.34 mg kg^{-1} between 10–60 days of lactation. The copper content of sow's milk was four times as high as that of cow's milk. Manganese content reached its maximum at 24 h after parturition (0.108 mg kg^{-1}), afterwards decreased to 2–3 days of lactation (0.058–0.061 mg kg^{-1}), and then continuously increased to the end of lactation (0.100 mg kg^{-1}). The manganese contents of sow's milk were almost identical.

In this study colostrum (0–72 h) was found to have lower ash, calcium, phosphorus, magnesium, iron and manganese contents than milk. However, contents of potassium, sodium, zinc and copper were higher in colostrum than in milk. BEREZVAI and RÁKÓCZI (1966) reported that sow's colostrum had lower levels of

dry matter, fat, ash, calcium and phosphorus than those reported here. HENNIG and ANKE (1966) also found that sodium, potassium an copper contents were greater than those of milk. Their work also reported time change patterns very similar those reported here for ash, calcium, magnesium, phosphorus and manganese contents.

The nature of the changes of mineral contents over time followed several patterns: 1) continuous decrease (potassium), 2) continuous increase (calcium, phosphorus), 3) increase to day 2 or 3 and than remain constant (ash, magnesium, iron), 4) decrease to day two or three and remain relatively constant (sodium, zinc) and 5) decrease after 3 days (copper).

Mineral contents of sow's milk and cow's milk were compared. Sow's milk (5–60 days) contained 10, 25, 50, 100 and 300%, respectively, more ash, calcium, phosphorus, iron and copper than did cow's milk. However, sow's milk contained 20, 30 and 35%, respectively, less sodium, potassium and magnesium than did cow's milk. Zinc and manganese contents were essentially equal. These findings were in good agreement with those of GURR (1981) who reported sow's milk to contain more calcium, phosphorus, iron an copper and less sodium and potassium than cow's milk.

2.3. Vitamin content of colostrum and milk

Vitamin content and changes associated with stage of lactation (colostrum to 46–60 days) for sows are shown in Table 4. Comparisons of vitamin contents of sow's milk and cow's milk are also shown in Table 4. There were no significant ($p > 0.10$) changes in vitamin contents during the colostral period (0 vs 3 days). The decline in vitamin C, however, approached significance. The vitamin contents of milk (45–60 days) were lower ($p < 0.05$) than those of colostrum for all except vitamin K_3.

Table 4

Vitamin contents of sow's colostrum and milk

Vitamin	Time after parturition			
(mg kg^{-1})	hours		days	Cow[a]
	0	68–76	45–60	
A	1.61	1.83	0.92	0.35
D_3	0.015	0.014	0.009	0.003
E	3.69	3.74	2.53	1.14
K_3	0.092	0.101	0.089	0.032
C	68.4	57.9	45.3	15.3

[a] From our earlier investigations based on milk samples from 32 cows

Vitamins A, D_3, E and K_3 are fat soluble and contained in the milk fat. Considering the changes in fat content reported previously (5.3% at parturition, 12.9% at 2–3 days and 6.5% at 45–60 days), expression of vitamins per unit of fat result in a much higher concentration (2.0 to 2.5 times) of vitamins in the milk fat drawn immediately after parturition than in milk fat drawn at 3 or 45–60 days of lactation. Because vitamin C is not bound to milk fat, its concentration should not be expressed as a proportion of fat.

No reports were found concerning the vitamin content of colostrum milked immediately after parturition, or about changes in the vitamin content during lactation. Values were reported for vitamins A and C contents of sow's milk by ELLIOTT and co-workers (1971). They reported 40–144 $\mu g/100\ cm^3$ vitamin A in milk, which agrees with our data on sow's milk, but are lower than our values for colostrum. They reported about 5600–11300 $\mu g/100\ cm^3$ vitamin C in sow's milk which seemed to be very high in comparison to our results, but were comparable to our data on first-drawn colostrum. With regard to the other vitamins there appear to be no comparable data in the literature.

Comparison of the vitamin contents of sow's milk with vitamin content of cows milk, revealed that concentrations of the five vitamins ranged from 2.2 to 3.0 times as great in sow's milk as in cow's milk.

2.4. Fatty acid composition of colostrum fat and milk fat

Table 5 shows fatty acid composition of milk fat and time changes, expressed as relative percentages of fatty acid methyl esters. Value needed to compare fatty acid compositions of sow's milk and cow's milk are also shown.

As the table was arranged, the most striking factor was that sow's colostrum did not contain even traces of butyric, capronic, caprylic and lauric acids, prior to 72 h of lactation and no capric acid prior to 5 day of lactation. In these investigations, the milk fat of sow's milk contained no nonadecanoic acid at all. After the 5th to 10th days of lactation, we could identify butyric, capronic, caprylic and lauric acid from the milk fat in traces, but the amounts of these fatty acids did not reach 0.1 relative percent. The same could be stated for myristoleic, pentadecanoic, pentadecylic and behenic acid contents of milk fat, while heptadecylic and eicosatric+erucic acid contents of milk fat were between 0.1–0.2%. (We could not separate the eicosatric and erucic acid by our gas chromatographic method.) The above fatty acids were only present in very low concentrations in the milk fat, and were only 1% of all fatty acids. The reason for discussing them is largely because there are so few data about fatty acid composition of sow's milk fat in the literature, and partly because we were able to identify fatty acids, in very low concentrations, which has not been discussed in the literature. For example, we identified myristoleic acid as well as four odd carbon

number pentadecanoic, pentadecylic, margaric and heptadecylic acids. However, it appeared that the milk fat of sow's milk did not contain nonadecanoic acid. Our investigations similarly confirmed that the milk fat of sow's milk contained behenic acid and, with great probability, eicozatric- and erucic acid. The amount of the 13 fatty acids was so low and the standard deviations so large that we could not make a statement about the changes during lactation in the case of these fatty acids. Similar to results of KLOBASA and FARRIES (1986), we found that, immediately after parturition, the first colostrum did not contain butyric acid, but contradictory to their results, we found that the low carbon number fatty acids (4–12) appeared in milk after 5–10 days of lactation. In contrast to results of ELLIOTT and co-workers (1971), we identified not only butyric acid but also caprylic and lauric acids from sow's milk fat in measurable concentrations.

Table 5

Fatty acid composition of the lipids in colostrum and milk of sows
(Relative percentages of the fatty acid methyl esters)

Fatty acid		Time after parturition									
		hours					days				
		0	8–14	20–28	44–52	68–76	5	10	20	45–60	Cow[a]
Butyric acid	(4:0)	0	0	0	0	0.05	0.06	0.05	0.08	0.09	0.52
Capronic acid	(6:0)	0	0	0	0	0.06	0.09	0.09	0.09	0.09	0.56
Caprylic acid	(8:0)	0	0	0	0	0	0.02	0.03	0.03	0.03	0.27
Capric acid	(10:0)	0	0	0	0	0.01	0.01	0.01	0.01	0.02	2.61
Lauric acid	(12:0)	0	0	0	0	0.02	0.01	0.01	0.02	0.02	4.35
Myristic acid	(14:0)	3.20	3.21	3.18	3.06	3.06	3.12	3.61	3.71	4.02	14.00
Myristoleic acid	(14:1)	0.01	0.02	0.03	0.01	0.02	0.03	0.02	0	0.01	1.41
Pentadecanoic acid	(15:0)	0.03	0.02	0.03	0.03	0.01	0.01	0.01	0.01	0.01	1.32
Pentadecylic acid	(15:1)	0.01	0	0.01	0	0.02	0.02	0.02	0	0.02	0.23
Palmitic acid	(14:0)	33.3	32.2	31.1	30.8	29.5	27.6	36.4	37.0	36.6	44.06
Palmitoleic acid	(16:1)	5.47	5.56	5.52	5.32	5.37	5.46	6.04	9.10	10.52	2.08
Margaric acid	(17:0)	0.08	0.09	0.05	0.06	0.07	0.08	0.06	0.09	0.08	0.60
Heptadecylic acid	(17:1)	0.13	0.14	0.14	0.17	0.13	0.16	0.12	0.14	0.15	0.46
Stearic acid	(18:0)	6.31	6.41	6.34	6.27	6.36	6.32	6.09	6.02	5.93	7.94
Oleic acid	(18:1)	37.5	38.4	39.5	38.8	39.4	40.3	32.9	33.0	32.3	17.25
Nonadecanoic acid	(19:0)	0	0	0	0	0	0	0	0	0	0.03
Linoleic acid	(18:2)	12.7	12.6	12.7	14.0	14.1	14.5	12.6	8.9	8.4	1.72
Arachidic acid	(20:4)	0.42	0.44	0.46	0.48	0.67	0.87	0.52	0.51	0.50	0.19
Linolenic acid	(18:3)	0.74	0.77	0.83	0.91	1.02	1.11	1.02	1.14	1.04	0.09
Behenic acid	(22:0)	0.01	0.02	0.01	0.01	0	0.01	0.02	0.01	0.01	0.15
Eicosatric acid Erucic acid	(20:3) (22:1)	0.11	0.12	0.09	0.11	0.12	0.15	0.12	0.13	0.14	0.20

[a] From our earlier investigations based on milk samples from 32 cows

The fatty acids not mentioned earlier – myristic, palmitic, palmitoleic, stearic, oleic and linoleic acids – amount to more than 97% of sow's milk fat. Of these fatty acids, palmitic and oleic acids have the greatest importance, because they constitute 68% of the total. The palmitic acid content of the fat of colostrum immediately after parturition was 33.3%, afterwards it decreased to 27.6% at 5 days of lactation, and then continuously increased to the end of lactation (36.6%). The palmitoleic acid did not change at the beginning of lactation, but, at 10 days, started to increase, and by the end of lactation had almost doubled. Myristic acid remained constant at 3.1 to 4.0%. Stearic acid also remained constant at 5.9 to 6.4%. Oleic acid was constant through 5 days (37.5 to 40.3%), but decreased in late lactation. Linoleic acid was slightly higher at 3–5 days of lactation (14.0 to 14.5%) than the levels before (12.7%) and after (8.4 to 12.6%). In contradiction of results reported by KLOBASA and FARRIES (1986), we found that palmitic and oleic acids were in nearly equal concentrations in the milk fat of sow's milk. In 3rd and 4th places were palmitoleic and linoleic acids, respectively. In the case of myristoleic acid our results differed from results reported by ELLIOTT and co-workers (1971), who could not identify myristoleic acid, in measurable concentration, from sow's milk.

The differences in fatty acid composition of sow's- and cow's milk are primarily in the areas of low carbon fatty acids and saturation. Sow's milk fat contains low carbon fatty acids only at barely detectable levels with only 0.2% of the total, while the amounts of these fatty acids in cow's milk fat represents 8% of the total. The milk fat of cow's milk contains more myristic, palmitic, and stearic acids, and far less palmitoleic, oleic, linoleic and linolenic acids than is found in sow's milk. From the middle to the end of lactation, 48% of the fatty acids are saturated, and 52% are unsaturated in sow's milk fat. Comparable data for cow's milk fat are 22 and 78%, respectively. The ratio of saturated to unsaturated fatty acids in sow's milk fat is 0.92, while the ratio for cow's milk is 0.28.

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References

BEREZVAI, F. & RÁKÓCZI, I. (1966): Újabb adatok a kocatej összetételének változásához a laktáció folyamán. (Further data on changes in sow's milk composition during lactation.) *Magy. Állatorv. Lap.*, *212*, 448–491.

CSAPÓ, J. (1984): *Colostrum and milk composition of several genotypes of cattle*. Ph. D. Thesis. PANNON Agricultural University Faculty of Animal Science, Kaposvár, p. 120.

CSAPÓ, J., SUGÁR, L., HORN, A. & CSAPÓ, JNÉ (1986): Chemical composition of milk from red deer, roe and fallow deer kept in captivity. *Acta agron. hung., 3 – 4*, 359 – 372.

CSAPÓ, J., CSAPÓ-KISS, ZS., MARTIN, T. G., SZENTPÉTERI, J. & WOLF, GY. (1994): Composition of colostrum from goats, ewes and cows producing twins. *Int. Dairy J., 4*, 445 – 458.

CSAPÓ, J., STEFLER, J., MARTIN, T. G., MAKRAY, S. & CSAPÓ-KISS, ZS. (1995): Composition of mare's colostrum and milk. Fat content and fatty acid composition and vitamin content. *Int. Dairy J., 5*, 393 – 402.

CSAPÓ-KISS, ZS., STEFLER, J., MARTIN, T. G., MAKRAY, S. & CSAPÓ, J. (1995): Composition of mare's colostrum and milk. Protein content, amino acid composition and biological value. *Int. Dairy. J., 5*, 403 – 415.

DROCHNER, W. & MARKUS, W. (1987): Einzatz hochaufgefetteter Rationen in der Ernährung hochgravider und laktierebder Sauen. *Dt. tierärztl. Wschr., 94*, 424 – 426.

ELLIOTT, R. F., VANDER NOOT, G. W., GILBREATH, R. L. & FISHER, H. (1971): Effect of dietary protein level on composition changes in sow colostrum and milk. *J. Anim. Sci., 32*, 1128 – 1137.

GURR, M. I. (1981): Review of the progress of dairy science: Human and artificial milks for infant feeding. *J. Dairy Res., 48*, 519 – 554.

HENNIG, A. & ANKE, M. (1966): Die Zusammensetzung der Sauenmilch verschiedener Laktationsstadien. (Rohnährstoffe, Mengenelemente, Spurenelemente). *Arch. Tierz., 9*, 312 – 329.

HUNGARIAN STANDARD (1978): *Nyers hamutartalom meghatározása.* (Determination of raw ash content.) MSz 6830.

HUNGARIAN STANDARD (1981): *A tej szárazanyag tartalmának meghatározása.* (Determination of total solids in milk.) MSz 3744.

HUNGARIAN STANDARD (1982): *Tej és ízesített tejitalok zsírtartalmának meghatározása.* (Determination of fat content of milk and flavoured beverages.) MSz 3703.

KIRCHGESSNER, M., GRASSMANN, E. & MADER, H. (1981): Zum Cu-Gehalt von Sauenmilch bei unterschiedlicher Cu-Versorgung. *Z. Tierphysiol., Tierernähr. Futtermittelk., 45*, 261 – 267.

KLOBASA, F. & FARRIES, E. (1986): Veränderungen des Fettsäurespectrums im Milchfett von Sauen im Verlauf der Laktation. *Milchwissenschaft, 41*, 146 – 148.

KLOBASA, F., WERHAHN, E. & BUTLER, J. E. (1987): Composition of sow milk during lactation. *J. Anim. Sci., 64*, 1458 – 1466.

KLAVER, J., VAN KEMPEN, G. J. M., DE LANGE, P. G. B., VERSTEGEN, M. W. A. & BOER, H. J. (1981): Milk composition and daily yield of different milk components as affected by sow condition and lactation/feeding regiment. *J. Anim. Sci., 52*, 1091 – 1097.

NOBLET, J. & ETIENNE, M. (1986): Effect of energy level in lactating sows on yield and composition of milk and nutrient balance of piglets. *J. Anim. Sci., 63*, 1888 – 1896.

RADEFF, T. (1938): Eine Methode zur Bestimmung des Vitamin-C in der Milch. *Milchwiss. Forsch., 19* 187 – 192.

WHITE, C. E., HEAD, H. H., BACHMAN, K. C. & BAZER, F. W. (1984): Yield and composition of milk and weight gain of nursing pigs from sows fed diets containing fructose or dextrose. *J. Anim. Sci., 59*, 141 – 150.

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MIGRATION ANALYSIS OF ELEMENTS FROM COMPOST AND CASING MATERIAL TO THE FRUIT BODIES IN CULTIVATED MUSHROOMS (*AGARICUS BISPORUS*)

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The macro and trace element uptake of cultivated mushroom was investigated from spiked (macro and trace elements) soil and casing materials. The soil samples and the mushroom samples were analysed after careful sampling, sample preparation and simultaneous multielement ICP–AES determination. In case of some element treatment positive effect, in other cases negative effect on the amount of crops was observed. Out of all elements used in treatments mainly the intake of Ni and Cd was measured, which can be important from the toxicological point of view.

Keywords: macro- and microelement treatment, trace element analysis, mushroom, toxicity

Four million tons of mushroom a year are grown all over the world, more than half of it is champignon (*Agaricus bisporus*). This kind of mushroom rules the market in Europe and America. The main producers are the USA, China, France, the Netherlands and United Kingdom. Between 1990 and 1993 Hungary doubled the production of cultivated champignon mushrooms and reached 20 thousand tons a year, and reached the European average (2 kg/person/year).

Examination of cultivated food in agriculture is important for several reasons. On the one hand, more and more toxic trace elements will get into the plant (and food) from the environment, like water, air, soil (CHANG & HAYES, 1978; PECK, 1977). On the other hand, the optimization of growing process can result in increasing yields – without decreasing the quality (VAN GRIENSEN, 1987).

In our experiments the development of cultivated mushrooms (in cellars) was examined changing the macro and trace element content of the environment during the growing period (so-called production waves). Macroelements (K, Na, Mg, Ca), essential elements (Mn, Zn, Cu, Co) and toxic trace elements (Cd, Cr, Hg, Ni, Pb)

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(BENTON JONES, 1984) were added to the compost and to the casing (covering) material.

1. Methods

The addition of elements was carried out with two methods described in Table 1. In method 1 the salt solution of certain elements one by one was mixed with the compost and with the casing material. The concentration of 13 elements was determined from the compost and in case of macro elements the concentration was increased by 10, 20, 50 and 100%, in case of trace elements it was increased by 100, 200, 500 and 1000%. The elements were added to the compost (25 kg per plastic bags) in the form of inorganic salt solutions (chlorides and nitrates), except for Ca and Mg, which were added in solid state. In all cases two parallel investigations were made.

Table 1

Trace and macro elements added to the compost and casing material (in the percent of the original quantity present in the soil)

	Concentration of elements added		
	separately to	both to	
	the compost (%)	the compost (%)	the casing material (%)
MICRO elements	1000	100	100
Cd, Co, Cr, +		200	200
Cu, Hg, Mn, +		500	500
Ni, Pb, Zn		1000	1000
MACRO elements	100	10	10
K, Na, Mg, Ca		20	20
		50	50
		100	100

The casing material – about five kg/bag – was sprayed with 200 cm^3 salt solution.

The harvest from the bags is periodical (production waves). Samples were taken in the first four waves. The time between production waves is about 1–1.5 weeks. The main crop of mushrooms appears between the first and fourth waves, after the fourth wave the product is much less.

The change in element concentration were investigated in the soil, compost and casing material during the above-mentioned production waves. Macro and trace element concentration changes of the compost and the casing material were

investigated at the beginning and end of the production. Because of the industrial conditions, there was no chance to investigate the element ballance during the experiment.

1.1. Sampling, sample preparation

The sampling was executed according to the methods of BLOCK (1979). Samples taken from the compost and the casing material, as well as the mushroom samples were dried at 105 °C and then powdered in an agate mill then the whole amount was transferred though a 60 μm plastic sieve. In the experiment 8.00 g compost, 8.00 g casing material and 25.00 g mushroom sample was investigated. The average dry matter content was: in mushroom 7%, in compost 37–42% and in casing material 55–65%.

At each experiment 4–4 mushroom samples were taken from the two parallel bags. They were cut into 4 to 8 pieces, depending on the size of the mushrooms with plastic knife and exactly 25 g was weighed in from the 8 mushrooms.

The compost was vertically pushed out from the bag using a plastic tube of 2.5 cm in diameter. Soil samples were taken at four different heights and collected on the drier plate. The sample amount was 8 g.

The casing material was sampled at 5 various places of the covering material of the production sack.

The sample preparation was carried out according to the method of FODOR & MOLNÁR (1993). At the digestion process 200–200 g were measured into closed PTFE beaker of 100 cm^3, and digested by 2 cm^3 cc. HNO_3 and 2 cm^3 H_2O_2 of 30%. The samples were digested in pressure cooker with the addition of water. The digestion time was 30 min (PECK, 1977). In case of mushroom samples the solution was crystal clear and in case of compost a colourless solution containing silicic acid was obtained. Each solution was filled up to 10–10 cm^3 with bidistilled water.

1.2. Instrumental conditions

At the calibration of inductively coupled plasma (ICP) spectrometer (Thermo-Jarrell-Ash, ICAP–9000) the matrix and the concentration of calibration solutions were matched. For the determination of elements a policromator was used. The main parameters of measurements were: output power 1.05 kW, nebulizator (Babington type) flow rate 0.4 dm^3 min^{-1}, plasma gas flow 14 dm^3 Ar min^{-1}. At both side of spectral lines background correction was used at 7 s integration time.

2. Results

The migration of macro and trace elements between the compost and the mushroom and also between the casing material and mushroom was measured. The results are summarized in figures.

With the increasing concentration of macroelements (K, Mg, Na) in the compost and casing material no concentration change was measured in mushroom samples. With the addition of Ca to the compost the Ca concentration of the mushroom increased three times compared to its original concentration in the first three production waves, but at the fourth wave just a small increase was measured.

The Cd (toxic element) gets into the mushroom both from the soil and casing material. Adding 2–10 times more Cd to the soil (compared to the original concentration, together with other microelements and separately) its content increased in the mushrooms according to the added quantity (Fig. 1).

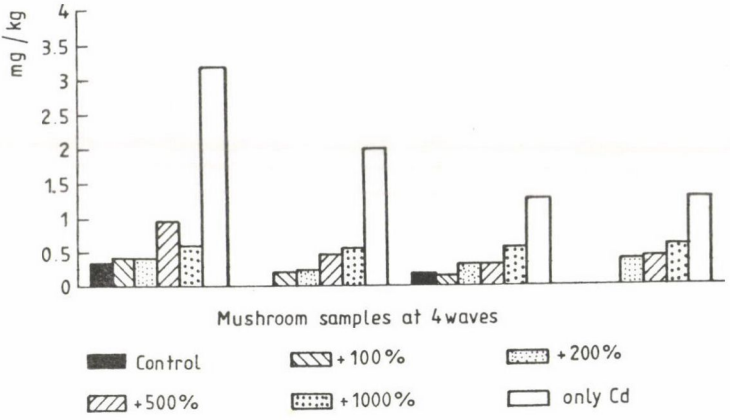

Fig. 1. The effect of combined microelement (and only 10 mg Cd/kg) mixture added to the compost on the average Cd content of mushroom

Adding increasing quantities of Cr, Co, Hg and Pb to the soil and to the casing material resulted in no change in the amount of these elements in any of the production waves of mushroom.

In case of Cu and Mn from the compost and in case of Zn from the casing material slight migration was found into the mushroom, but no real increasing effect (more than 5%) was measured.

The behaviour of Ni was surprising. The Ni concentration increased in the first production wave, when it was added to the compost. During the further waves cultivated mushrooms take 3–5 times more Ni from the compost and casing material than that of the control. The average of the measurements is shown in Figs 2 and 3.

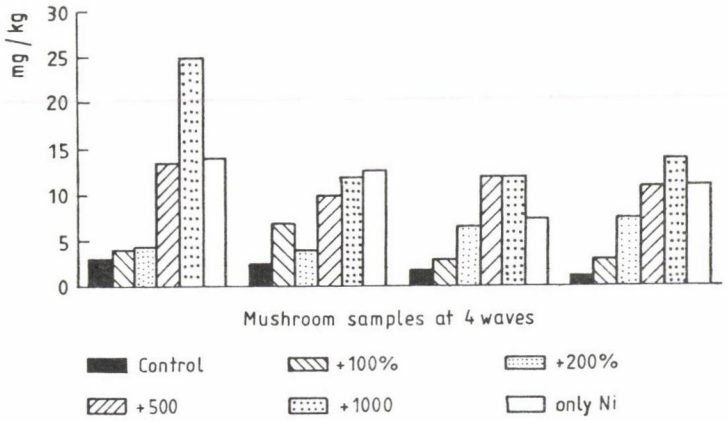

Fig. 2. The effect of combined microelement (and only 10 mgNi/kg) mixture added to the compost on the average Ni content of mushroom

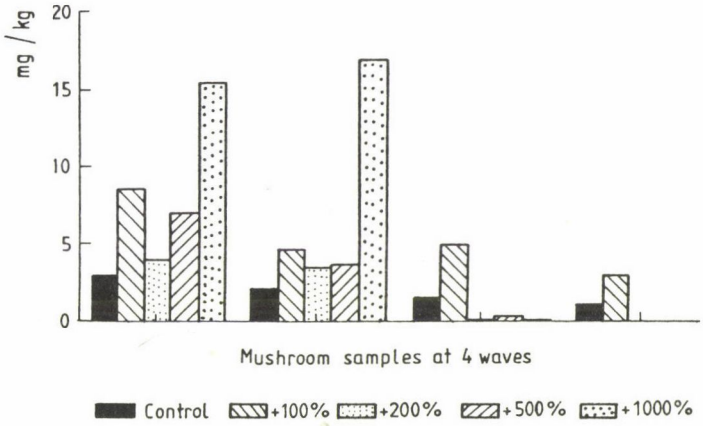

Fig. 3. The effect of combined microelement mixture added to the casing material on the average Ni content of mushroom

The effect of addition of the macro and trace elements to the quantity of the product was as follows:

The addition of the macro elements (K, Na, Mg, Ca) both to the compost and the casing material caused just a slight increase in the quantity of crop. The addition of trace elements to the soil either one by one, or in combination, did not cause change in the yield of mushrooms. In case of Hg treatment the fruit bodies appeared later and their quantity was below the average. One of the most important results of experiment is that trace elements added to the casing material caused real decrease in corps.

3. Conclusions

At the mushroom cultivation the knowledge of Cd and Ni content of compost and casing material is very important because champignon mushroom accumulates these two toxic elements in its fruit body. Determination of Hg content is also important since it causes drastic decrease in yield. The concentration of these elements has to be kept as low as possible. The optimal concentration of main elements – K, Na, Ca, Mg – increases yield, but only Ca increased the element concentration of the mushroom, mostly during the first three production waves.

In contrast to the previous results, certain elements of the casing material build in the fruit body.

Mushroom is part of the healthy human diet because of its essential amino-acid, low carbohydrate and high mineral content, unless it is contamined with toxic trace elements (Cd and Ni) during production.

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References

BENTON JONES, J. (1984): *Soil test and plant analysis*. Benton Press, Athens, Georgia, pp. 1–36.

BLOCK, R. A. (1979): *Handbook of decomposition methods in analytical chemistry*. J. Wiley, New York, pp. 50–134.

CHANG, S. T. & HAYES, W. A. (1978): *Biology and technology of cultivated mushrooms*. J. Thompson, London, pp. 1–48.

FODOR, P. & MOLNÁR, E. (1993): Honey as an environmental indicator. *Microchim Acta*, *112*, 113–118.

VAN GRIENSEN, L. J. L. D. (1987): *The cultivation of mushrooms*. Dekker, The Netherlands, pp. 1–87.

PECK, T. R. (1977): *Soil testing: Correlating and interpreting the analytical results*. ASA, Madison, Wisconsin, pp. 28–106.

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YEASTS WITH β-D-GLUCOSIDASE ACTIVITY: PROPERTIES AND POSSIBLE APPLICATION IN WINEMAKING PROCESSES

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For the better utilisation of the hidden aromapotential of white wines we selected 4 isolates from yeast strains which had β-D-glucosidase activity and tested for the enzyme inductibility on different carbon sources. Crude enzyme extracts were made and the β-D--glucosidase activity was measured after induction with salicin. The α-L-arabinosidase and α--L-rhamnosidase activities were tested too.

The β-D-glucosidase activity of the samples at pH 3.5 was 8–15% of the maximum activity measured in pH range 3–6, 5–15% etanol increased the β-D-glucosidase activity. K_M and $K_{glucose}$ values were 1.45 mmol and 44 mmol (from 46058, *Hansenula*) and 2.8 mmol and 165 mmol (from Tokaj 7, *Sacch. cerevisiae*).

Winery experiment showed, that in Traminer wines treated with enzyme extract or commercial enzymes 30–60% of the initial activity was preserved after one month contact time. By sensory analysis higher quality was established only in wines treated with a mixed yeast culture and with commercial enzymes.

Keywords: β-D-glucosidase, terpene glycosidé hydrolysis, wine, wine yeasts

It is well known that the primary aroma substances originated from grapes are responsible for the varietal flavour character of wines and significantly influence the quality.

A very important group of the characteristic aroma substances, especially in fragrant grape cultivars is the mono- and sesquiterpenol derivatives (MARAIS, 1983). Certain winetechnological operations are favourable for the better utilisation of the variety flavour.

Considerable part of the aroma substances is located in the skin of the berries, therefore the maceration promotes the extraction of aroma compounds. One part of the aroma compounds found in grapes are in free volatile and odorous form, others are glycosidically bound aroma precursors (CORDONNIER & BAYONOVE, 1974; WILLIAMS et al., 1982).

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These glycosides are not hydrolysed during the wine making process and represent a so-called "hidden aroma potential" in wine. In many grape cultivars the concentration of glycosidically bound monoterpenols is higher than that of free, volatile terpenols (GUNATA et al., 1985).

Several publications deal with the enhancement of the aromatic character of different white wine sorts by increasing the free aroma component content of wines by hydrolysing the aroma precursors with enzymes having β-D-glucosidase activity (GROSSMANN & RAPP, 1988; GUNATA et al., 1990). The bound terpenols are present in grapes mainly as disaccharide glycosides: as 6-0-α-L-arabinofuranosyl-β-D--glucopyranosides and 6-0-α-L-rhamnopyranosyl-β-D-glucopyranosides or as β-D--glucopyranosides of geraniol, nerol and linalool (WILLIAMS et al., 1982; GUNATA et al., 1985). Their enzymatic hydrolysis is a sequential process, which requires first α-L--arabinosidase and α-L-rhamnosidase enzymes to the cleavage of the disaccharide (1→6) linkage. Monoterpenols are then liberated by action of β-glucosidase (GUNATA et al., 1988).

In the last years some commercial pectolytic enzymes having, β-D-glucosidase activity have been developed which can hydrolize the glycosidic precursors during vinification (CANAL-LLAUBERAS, 1992; BAYONOVE et al., 1992).

Traditionally, wines have been produced by natural fermentation by yeasts originating from grapes and wineries. There are great variations in the quantity and distribution of the relevant yeast species which can be mainly related to differences in ecosystems and vintages. For this reason, during the last few years many winemakers have used pure cultures or mixed pured yeast cultures isolated from their own wine region. In the microvinification processes some mixed yeast cultures (*Hansenula*, *Candida*, *Saccharomyces*, *Saccharomyces cerevisiae*) have intracellular β-D--glucosidase activity (GROSSMANN et al., 1987; DUBOURDIEU et al., 1988). Using a genetically transformed yeast strain, which secrets fungal enzyme to the must, it is possible to detect an increase in the flavour of the wine (PÉREZ-GONZÁLEZ et al., 1993). Our further aim is to select such wine yeast cultures from the Hungarian endemic strains.

The better utilisation of the hidden aroma potential of white wines has been studied for 3 years in our institute. In connection with this, yeasts and some moulds from the Culture Collection of the Research Institute of Viticulture and Enology were tested after induction on selective media for their β-D-glucosidase activity. The induction of β-D-glucosidase production in yeast strains was possible by growing on salicin (as the only carbon source) containing medium (GROSSMANN et al., 1987). From the most promising yeast strains intracellular extracts were made and their β-D-glucosidase activity was measured. The activities were compared with some commercial pectolytic enzyme preparations which have considerable β-D-glucosidase activity, too (MIKLÓSY & PÖLÖS, 1992, 1993).

The glucoamylase synthesis in a yeast strain is regulated by induction and/or catabolite repression mechanisms (PARK & AZUMA, 1990). To overcome the effect of glucose on glucoamylase synthesis mutagenic experiments and selection of spontaneous mutant isolates were carried out to obtain glucose derepressed mutants. They were selected against 2-deoxy-D-glucose as used for isolation of derepressed mutants producing enzymes like glucoamylase, α-amylase and β-glucosidase and invertase (TUBB, 1986; GHOSH et al., 1991). These investigations led to the isolation of 5 mutant strains capable of producing high yield of glucoamylase in presence of glucose.

In this work the β-D-glucosidase and also the α-L-arabinosidase and α-L--rhamnosidase activity of two *Hansenula*, one *Candida*, and one *Saccharomyces cerevisiae* yeast strains were studied. We determined some enzyme kinetic parameters of the β-D-glucosidase originated from two yeast strains. The yeasts and yeast extracts and three commercial enzyme preparations were tested in the wine making processes, too.

1. Materials and methods

1.1. The yeast strains

Candida intermedia	'333'
Hansenula sp.	'342'
Saccharomyces cerevisiae	'Tokaj 7 (Hungarian E. Micr. C.), Badacsony 1
Hansenula anomala	'46058' (ATCC)
Hansenula anomala	'46131' (ATCC)

1.2. Commercial enzymes

Novoferm 12 L (Novo Nordisk Ferment Ag.)
Glucanex (Novo Nordisk Ferment Ag.)
Trenolin Bukett (Erbslöh)

1.3. Media and culture conditions

YEP agar medium containing (g dm^{-3}), 10 g glucose, 20 g yeast extract, 10 g peptone and 20 g agar (OXOID) in 1 dm^{-3} distilled water.

SZVM agar medium containing 10 g glucose, 5 g $(NH_4)_2SO_4$, 1 g KH_2PO_4, 0.5 g $MgSO_4 \times 7\ H_2O$ and 20 g agar (OXOID) in 1 dm^3 distilled water.

SZVM–NG agar medium containing 10 g glucose, 20 g yeast extract, 10 g peptone, 20 g agar (OXOID) and 1.0 g p-nitrophenyl-β-D-glucopyranoside in 1 dm^3 distilled water.

SZVM–D agar medium: the SZVM–NG agar medium was supplemented with 1 g dm^{-3} 2-deoxy-glucose.

K medium containing 2.0 g glucose, 5 g $(NH_4)_2SO_4$, 1 g KH_2PO_4 and 0.5 g $MgSO_4 \times 7\ H_2O$ in 1 dm^3 distilled water.

A medium containing 2.0 g arabinose (Aldrich), 5 g $(NH_4)_2SO_4$, 1 g KH_2PO_4 and 0.5 g $MgSO_4 \times 7\ H_2O$ in 1 dm^3 distilled water.

R medium containing 2.0 g rutin (Aldrich), 5 g $(NH_4)_2SO_4$, 1 g KH_2PO_4 and 0.5 g $MgSO_4 \times 7\ H_2O$ in 1 dm^3 distilled water.

SZ medium containing 2.0 g salicin (DIFCO), 5 g $(NH_4)_2SO_4$, 1 g KH_2PO_4 and 0.5 g $MgSO_4 \times 7\ H_2O$ in 1 dm^3 distilled water.

1.4. UV-mutagenic treatment

The '333', '342' and *Hansenula anomala* '46058' and '46131' strains were grown on YEP liquid medium until log phase. The cultures were centrifuged at 4000 × g for 10 min at 4 °C. The precipitated cells were resuspended in sterile deionised water and adjusted to 2×10^7 cell dm^{-3} of the water and spreaded on SZVM–D agar plates. These were UV treated (until 90–95% of cells were killed) and were kept in dark overnight, then they were incubated at 28 °C for 4 days.

1.5. Selection of mutant/spontaneously mutant strains

The *S. cerevisiae* Badacsony 1 and Tokaj 7 strains grown on SZVM–D liquid medium for 4 days until log phase, cell culture was washed three times in physiological NaCl solution. The $2 \times 10^6 - 2 \times 10^6$ cells were spread out on SZVM–NG agar plates. These were incubated at 28 °C for 2 days with subsequent incubation at 20 °C for 2 days. The yeast strains producing β-glucosidase exhibited colour zones around colonies.

1.6. Induction of β-D-glucosidase enzymes by additives

The cells were harvested from YEP medium at log phase and were collected after washing them twice in sterile deionized water.

The 1.50 g of wet weight of cell masses were suspended and harvested in 2.0 g dm^{-3} rutin or arabinose or salicin or glucose (as a control) as carbon source containing SZVM liquid medium on a reciprocal shaker at 2.5 Hz at 28 °C for 2 days.

1.7. Preparation of crude endoglucosidases

The cells were collected in 20 cm^3 0.01 mol phosphate buffer (pH = 6.5) and were ultrasonicated at +4 °C for 4 min, then mixed with 4.0 g $(NH_4)_2SO_4$ and were kept at 4 °C for 20 min. The pellets were centrifuged at 6000 × g for 10 min.

The supernatants were dialyzed overnight against 0.01 mol phosphate buffer (pH = 6.5).

1.8. Determination of enzyme activity

β-D-glucosidase, α-L-arabinosidase and α-L-rhamnosidase activities were determined according to BIRON and co-workers (1988) using the appropriate glycosides of p-nitrophenol as substrates. The assays were carried out at 24 °C or at 40 °C in sodium acetate buffer at pH 3.5 or 5. Measuring the effect of pH on β-D--glucosidase activity buffer solutions pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 were applied. The p-nitrophenol released by hydrolysis was determined spectrophotometrically at 405 nm. For inhibition studies 5, 10, 20, 50, 100 $g\,l^{-1}$ glucose or 5, 10, 12, 15% ethanol were added to the buffer solution. The accuracy of the determination is ±3%. Protein concentration of various samples was determined by the method of LOWRY and co-workers (1951) using bovine serum albumin (BSA) as standard.

1.9. Vinification experiments

In 1992 Cserszegi fűszeres (Irsai Oliver x Traminer) grape from Érsekhalma and Traminer grape from Mőcsény, in 1993 Traminer grape from Mőcsény were used in winemaking processes. Figure 1 shows the scheme of enzyme treatments. In 1992 the influence of enzyme preparations was combined with maceration. The musts were fermented either with Lalvin K1 wine yeast or with a mixed flora of yeasts having β-D-glucosidase activity. Enzyme extracts and commercial enzyme preparations were added to young wines.

The wines were judged by a trained panel of 12–14 members in 1992 after 3 months contact time, in 1993 after 7 months contact time. Pair tests were used to compare the treated wines with the control ones.

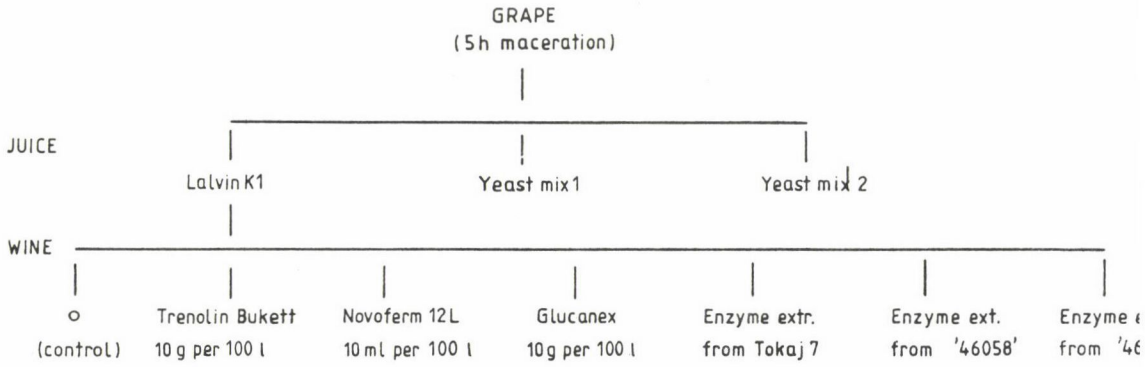

Fig. 1. The scheme of enzymic treatments in must and wine

2. Results and conclusions

2.1. Selection of yeast strains having β-D-glucosidase activity

The Hungarian endemic must fermenting yeast strains (*Saccharomyces cerevisiae*, *Hansenula* sp., *Candida intermedia*) have been tested for β-D-glucosidase activity. Only 4 strains were able to grow on SZ agar plates. The strains had low activity for the splitting of salicin. The use of these selected and induced strains might result in small increase in the free terpenol content during the winemaking process.

2.2. Reduction of glucose repressing effect

'Glucose derepressed' or 'partially derepressed' UV mutants were isolated from strains '333', '342', *H. anomala* '46058 and '46131', and spontaneous 'glucose derepressed' isolates were selected which appeared as well growing strains on SZ medium when treated with 2-deoxy-D-glucose (Table 1). Four strains were isolated, which were able to split salicin in the presence of 10 g l^{-1} glucose. These isolates were applied for microvinification experiments.

Table 1

Selection of mutant/spontaneous mutant strains

Isolates	On SZVM–SZ medium	Isolates	On SZVM–SZ medium	Isolates	On SZVM–SZ medium
	'333'		Tokaj 7		Badacsony 1
/1	(+)	/1	(+)	/1	(+)
/2	(+)	/2	–	/2	(+)
/3	–	/3	–	/3	–
/4	(+)	/4	–	/4	–
/5	–	/5	(+)	/5	–
/6	–	/7	+	/6	–
/7	(+)	/8	(+)	/7	–
/8	(+)	/9	–	/11	(+)
/9	(+)	/10	–	/12	–
/10	–	/16	–	/13	–
/11	–	/17	–	/14	–
/12	(+)	/18	(+)	/15	–
/13	–	/19	(+)	/16	–
/14	–	/20	(+)	/21	–
/15	(+)	/21	–	/22	(+)
/16	(+)	/22	–	/23	–
/17	+	/23	–	/24	–
/18	–	/24	(+)	/25	–
/19	–	/26	–	/26	–
/20	(+)	/27	–	/27	–
/21	–	/28	–	/28	–
/22	–	/29	(+)	/29	–
/23	(+)	/30	–	/30	–
/24	–				
/26	–				
/27	–				
/30	–				
	'342'		*Hansenula* '46058'		*Hansenula* '46131'
/3	–	/1	+ +	/2	–
/4	–	/2	–	/3	–
/7	(+)	/3	–	/4	(+)
/8	–	/4	–	/8	–
/9	–	/5	–	/9	–
/11	+	/7	(+)	/10	+ +
/12	–	/8	(+)	/11	–
/13	–	/9	(+)	/12	–
/14	–	/10	–	/13	(+)
/21	–	/11	–	/14	(+)
/22	–	/16	(+)	/16	–
/23	(+)	/17	–	/17	(+)
/24	–	/18	–	/18	–
/28	(+)	/19	(+)	/19	–
/30	(+)	/20	(+)	/21	–
				/24	(+)
				/25	–
				/26	–
				/27	–
				/31	–

+ + = well growing; + = growing; (+) = weak; – = not growing

2.3. Possibility of the induction of β-D-glucosidase enzyme synthesis

Out of the tested strains the '333' *Candida intermedia*, the 'Tokaj 7' *Saccharomyces cerevisiae* and the '46131' and '46058' *Hansenula* yeasts have been chosen and grown in selective nutrient solutions containing different β-D-glucosidase inductive carbon sources. Table 2 shows the β-D-glucosidase activities of the intracellular extracts of these strains at 24 °C, pH = 3.5 and at 15 g l^{-1} glucose content, these circumstances are close to winery application.

Table 2

The β-D-glucosidase activity of some yeast strains grown in nutrient solutions containing different β-glucosidase inductive carbon sources

Nutrient solutions	Yeast strains '333' U/ml[a]	'333' U/mg[b]	Tokaj 7 U/ml[a]	Tokaj 7 U/mg[b]	'46058' U/ml[a]	'46058' U/mg[b]	'46131' U/ml[a]	'46131' U/mg[b]
K	4×10^{-4}	1.1×10^{-3}	5×10^{-3}	3.4×10^{-3}	1.4×10^{-1}	3.7×10^{-2}	1.2×10^{-1}	3.2×10^{-2}
A	1.5×10^{-3}	6×10^{-1}	4.4×10^{-3}	3.4×10^{-3}	1.2×10^{-1}	3.2×10^{-2}	1.2×10^{-1}	2.8×10^{-2}
R	1.7×10^{-3}	1.3×10^{-3}	3.2×10^{-3}	3.9×10^{-3}	1×10^{-1}	4.6×10^{-2}	4×10^{-2}	3.5×10^{-2}
S	1.9×10^{-3}	2.7×10^{-3}	3.9×10^{-3}	4×10^{-3}	1.3×10^{-1}	1.7×10^{-1}	1×10^{-1}	7.3×10^{-2}

[a] U activity expressed as released p-nitrophenol μmol/min/ml enzyme solution at 24 °C, pH = 3.5 and 15 g l^{-1} glucose content.

[b] U activity expressed as released p-nitrophenol μmol/min/mg protein content at 24 °C, pH = 3.5 and 15 g l^{-1} glucose content.

[c] K: nutrient solution with glucose carbon source
A: nutrient solution with arabinose carbon source
R: nutrient solution with rutin carbon source
S: nutrient solution with salicin carbon source

The β-D-glucosidase enzyme production doubled in the '333' yeast cells in nutrient solution containing salicin. The increase of β-D-glucosidase enzyme activity in the 'Tokaj 7' yeast cells is approximately 10% in nutrient solutions containing rutin or salicin. The activity increase is relatively higher in case of '46058' and '46131' strains. It is more than fourfold in case of the '46058' strain.

2.4. The α-L-rhamnosidase and α-L-arabinofuranosidase side activities of the crude enzyme extracts

According to GUNATA and co-workers (1988) the hydrolysis of the grape monoterpenyl disaccharide-glycosides is a sequential reaction which involves the removal of the terminal unit by cleavage of the disaccharide (1→6) linkage by either

α-L-rhamnosidase or α-L-arabinofuranosidase with production of monoterpenyl glucosides first. Monoterpenol is then liberated by the action of β-D-glucosidase. Therefore over the β-D-glucosidase activity, the knowledge of the α-L-rhamnosidase and α-L-arabinofuranosidase activities of the enzyme extracts is also very important. These activities have been determined by the appropriate glycosides of p-nitrophenol and are shown in Table 3. The investigated yeast strains have 2–3 order of magnitude lower α-L-arabinofuranosidase activity and 3–5 order lower α-L--rhamnosidase activity than β-D-glucosidase activity. The α-L-rhamnosidase activity in the *Hansenula* '46058' and '46131' yeast strains is relatively higher than in the extracts from 'Tokaj 7' *Saccharomyces cerevisiae*.

Table 3

Side activities of different yeast strains

Yeast strains	α-L-rhamnosidase activity		α-L-arabinofuranosidase activity		β-D-glucosidase activity	
	U/ml[a]	U/mg[b]	U/ml[a]	U/mg[b]	U/ml[a]	U/mg[b]
46058	1.59×10^{-3}	9.8×10^{-4}	7.2×10^{-3}	4.45×10^{-2}	1.4	0.86
46131	6.52×10^{-4}	5.65×10^{-4}	4.89×10^{-3}	4.24×10^{-3}	1.12	0.97
Tokaj 7	3.4×10^{-6}	3.53×10^{-6}	1.4×10^{-4}	1.45×10^{-4}	3.96×10^{-2}	4.1×10^{-2}

[a] U activity expressed as released p-nitrophenol μmol min^{-1} ml^{-1} enzyme solution

[b] U activity expressed as released p-nitrophenol μmol min^{-1} ml^{-1} protein content

2.5. Influence of pH on the β-D-glucosidase activity

The β-D-glucosidase activity of different yeast extracts was measured in pH range from 3 to 6. Results are shown in Fig. 2. In case of 'Tokaj 7' yeast strain $10^2\times$ β-D-glucosidase activity was plotted. The enzyme activity of the intracellular enzyme extracts from '46058' and '46131' yeast strains is increasing throughout the whole examined pH range, that is the optimum pH of β-D-glucosidase enzymes of these yeast strains is higher than 6.

It is well known (DIXON & WEBB, 1979), that the effect of pH on the enzyme activity is ascribed to the combination of different factors. Among others, changes of the ionization state of the different components of the system, changes of the substrate concentration to saturate the enzyme and destruction of the enzyme play an important role. The differences in the effect of pH on β-D-glucosidase activity at the different yeast strains propound the hypothese that the protein structure of the β-D-glucosidase enzyme in 'Tokaj 7' yeast strain is different from that of the enzyme in the *Hansenula* yeast strains.

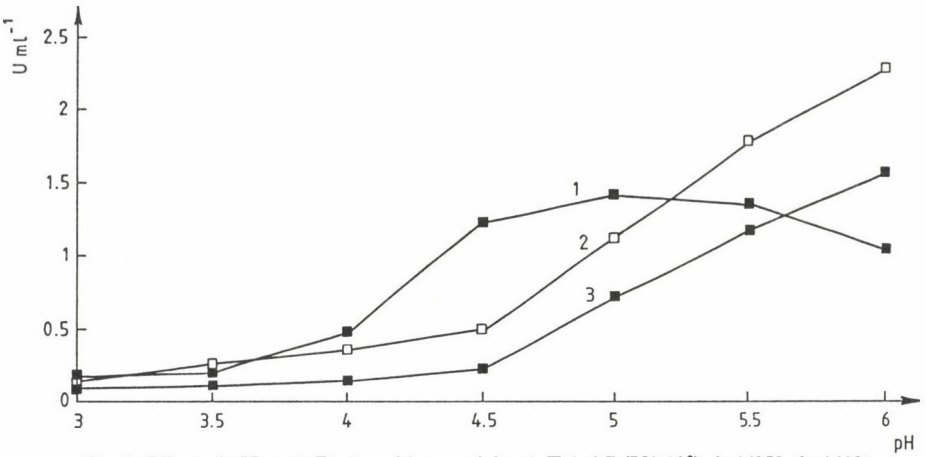

Fig. 2. Effect of pH on β-D-glucosidase activity. 1: Tokaj 7 (U* 10^2); 2: 46058; 3: 46131

At pH = 3.5, which means the approximate pH value of winery applications, 14% in 'Tokaj 7' strains, 8% in '46131' strains, 15% in '46058' strains of the β-D--glucosidase enzyme activity is preserved after 1 month as compared to the maximum activity value measured in the given pH range (Fig. 2). During several months contact time the remaining relatively low activity value may be sufficient to increase the free aroma content of wines by hydrolysing the bound monoterpenyl glycosides.

2.6. Determination of some enzyme kinetic parameters of β-D-glucosidase from 'Tokaj 7' and '46058' yeast strains

We investigated some kinetic details of the hydrolysis reaction by β-D-glucosidase enzymes from a *Saccharomyces cer.* (Tokaj 7) and *Hansenula* (46058) yeast strains. When the temperature was increased from 24 °C to 40 °C the β-D-glucosidase activity (both yeast strains) was doubled.

Five-fifteen % ethanol increased β-D-glucosidase activity by 30–35%, therefore during winemaking the alcohol will not have inhibition effect.

The competitive inhibition effect of glucose for β-D-glucosidase enzymes is known. $K_{glucose}$ values were determined by the simple graphical method of Dixon (DIXON & WEBB, 1979).

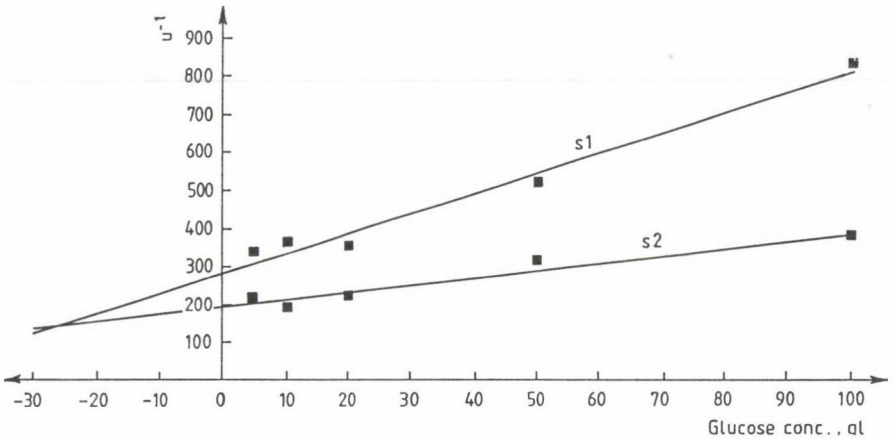

Fig. 3. Graphical determination of glucose inhibition constant of Tokaj 7 yeast's β-D-glucosidase. s1: y = 5.33x+284.11, r2 = 0.98; s2: y = 1.98x+194.2, r2 = 0.97

The method is illustrated in Fig. 3 on example of β-D-glucosidase from 'Tokaj 7'. If reciprocal velocity values are determined at different glucose concentrations, keeping substrate concentration constant, a straight line is obtained on plotting 1/U against glucose concentration. A second straight line is obtained with another substrate concentration and the point of intersection gives $-K_{glucose}$ directly.

K_M values were determined on the basis of well-known Lineweaver-Burk plots (DIXON & WEBB, 1979). The data are shown in Table 4. Determinations were carried out at 24 °C and pH = 3.5.

Table 4

Some enzyme kinetic parameters of β-D-glucosidase from two yeast strains

Kinetic parameters	Yeast strains	
	'46058	'Tokaj 7'
K_M	1.45 mmol	2.8 mmol
$K_{glucose}$	44 mmol	165 mmol

2.7. Results of winery applications

The first winery applications were carried out in 1992. Cserszegi fűszeres and Traminer juices and wines, which are expected to have high bound monoterpenol content, were treated with yeasts having β-D-glucosidase activity or intracellular

extracts of some yeasts or with commercial enzymes, which over the pectinase activity had significant β-D-glucosidase activity, too (see Fig. 1). According to the results of sensory analysis the influence of enzymes on the taste of wines was more explicit in samples having had increased skin contact, too (MIKLÓSY & PÖLÖS, 1993). It is well known, that after maceration part of the bound monoterpenols which are found in larger proportion in skin, become accessible, too. Therefore in 1993 the enzymes and enzyme extracts were given to Traminer musts and wines only after skin contact. The enzymic extracts from 'Tokaj 7', '46131' and '46058' yeast strains as well as commercial enzymes Glucanex, Trenolin Bukett and Novoferm 12 L were used.

The β-D-glucosidase activities of these preparations in wines were measured as a function of time and the results are shown in Fig. 4. The activities are plotted as per cents of the initial enzyme activities measured in wine. It can be seen, that after one month 30% of the initial activity could be measured in wines treated with enzyme extracts from 'Tokaj 7'. This value was 40–60% in wines treated with intracellular extracts from the other yeast strains.

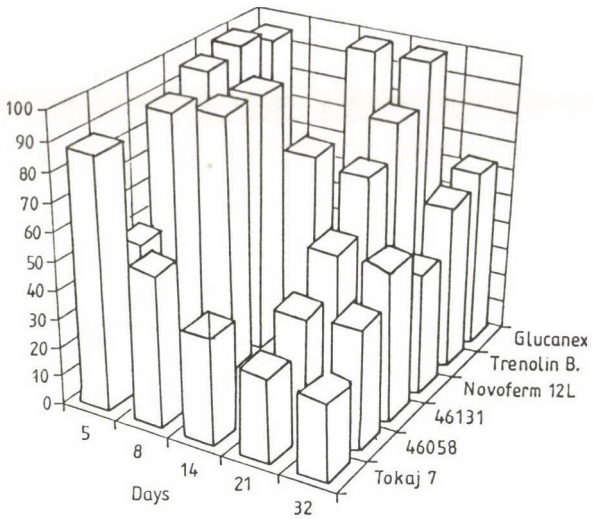

Fig. 4. Decrease of β-D-glucosidase activity in wine

The sensory analysis of wines took place after 6 months, the results are summarized in Table 5. The wine treated with Trenolin Bukett was considered by more than 85% of the wine tasters better in aroma, fruity taste and in overall quality, than the control one. The differences were not as strong by wines treated with the other commercial enzymes. More than 70% of the wine tasters found the overall quality and flavour better at the Novoferm 12L treated wines than the control one and in case of Glucanex the preference was only more than 60%.

Table 5

Results of the sensory analysis of different enzyme treated Traminer wines

Wines treated with	Odour	Strength of fruity flavour	Harmony
Tokaj 7 extr.		(+)	
46131 extr.	(+)	(+)	(+)
46058 extr.			
Yeast mix 1			
Yeast mix 2	+ +	+ +	+ +
Glucanex	+	+	+
Trenolin Bukett	+ + +	+ + +	+ + +
Novoferm 12L	+	+ +	+ +

+ + + preference > 85%
+ + preference > 70%
+ preference > 60%
(+) preference > 50%

From our preparations, a mixed yeast culture, which contained 50–50% 'Tokaj 7' and '46058' yeast cells, showed the best features. The wine fermented with this flora was found better by more than 70% of the wine tasters in aroma, taste and in general harmony, than the control wine in several repeated tastings. The wines treated with intracellular extracts of yeasts did not show significant improvement in aroma and taste compared with the control wine. Although the β-D-glucosidase activity has been measured for a longer time in wines treated with those extracts, this activity was not sufficient to get more free aroma compounds, than the recognition threshold or these preparations are not pure enough and might contain substances which cause off-flavour in wine.

Further on it would be necessary

- to improve our extraction method (purified enzymes) and to determine aroma compound contents of wines in order to follow analytically the effect of enzyme treatments on wine flavour,
- to examine the effectivity of these (induced) strains during the vinification,
- to improve the fermentation process for better utilisation of the hidden aromapotential of white grapes.

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PREPARATION OF TITANIUM ENRICHED *SACCHAROMYCES CEREVISIAE*

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Under adequate circumstances yeasts are capable of accumulating, and incorporating into organic compounds large amounts of trace elements.

Use of nutrient medium supplemented with 20 $\mu g\ cm^{-3}$ Ti-ascorbate resulted in a titanium-accumulation in the range of 1500–2000 $\mu g\ g^{-1}$ dried yeast. With the addition of Ti-ascorbate to the yeast culture in the exponential phase of growth high Ti-binding to the cell components can be attained, instead of adsorption on the surface.

Keywords: trace elements, titanium, yeast, accumulation

Titanium is found in rather large amount in the soil, however, because of the insolubility of titanium compounds plants can generally take it up in very small quantities.

Titanium, in form of water-soluble chelate can stimulate both plant and animal organisms, which can be explained with the increase of activities of enzyme systems. PAIS (1983) and ALCARAZ and co-workers (1994) noticed that in plant Ti increases the crop and affects the internal parameters beneficially. In animals Ti stimulates the growth and improves the utilization of fodder. Ti-ascorbate can play an important role in the strengthening of immune system therefore in the protection against diseases (PAIS et al., 1989).

There is no fear about any toxicity problems in plant cultivation, in feeding animals or in human nutrition.

Summarizing titanium cannot be considered as an essential trace element, on the other hand there is no doubt about its physiologically beneficial role (PAIS et al., 1989).

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1. Materials and methods

Baker's yeast (*Saccharomyces cerevisiae*) was used in the experiments.

As Ti source Ti-ascorbate was prepared from ascorbic acid with analytical purity and titanium-tetrachloride in ion-free aqueous medium, the strongly acidic solution was adjusted to 4.4 pH with potassium hydroxide solution.

In the course of preliminary experiments as well as in preparing the inoculum for cultivation in laboratory scale fermenters, yeasts were cultivated in shaking flasks where conditions and composition of the nutrient medium were identical. Compositions of media are given in Tables 1 and 2.

Table 1

Composition of nutrient medium for shaking flasks
($g\ dm^{-3}$)

Glucose	50.0 $g\ dm^{-3}$
$(NH_4)_2SO_4$	4.5 "
KH_2PO_4	1.5 "
$MgSO_4 \cdot 7H_2O$	1.2 "
Yeast extract	3.5 "

in tap water
pH = 4.5 (with 15% HCl)
sterilization: 1.2 bar, 15 min. in autoclave

Table 2

Composition of nutrient medium for lab fermenter
($g\ dm^{-3}$)

Glucose	70.0 $g\ dm^{-3}$
$(NH_4)_2SO_4$	6.5 "
KH_2PO_4	2.5 "
$MgSO_4 \cdot 7H_2O$	1.7 "
Yeast extract	5.0 "

in tap water
ph = 4.5 (with 15% HCl)
sterilization: 1.2 bar, 25 min. in autoclave

After inoculation from agar surface yeast was frown in shaking flasks (100 cm^3 broth in 750 cm^3 Erlenmeyer flask, 350 r.p.m., 30 °C, 20–24 h).

In our experiments we used 10 dm^3 laboratory scale fermenters with 5 dm^3 actual broth volume. Cultivation was carried out at 30 °C, 700 r.p.m., batch technique,

1 v/v min and/or 0.5 v/v min aeration. The amount of inoculum was 10% of the broth volume.

In the course of cultivation yeast growth was followed by cell density measurement. Samples were measured spectrophotometrically at 550 nm using the non-inoculated medium as blank, applying 10-fold dilution. For dilution 0.01 mol dm^{-3} HCl was used.

Glucose content of samples was determined by Schoorl's reduction method.

Separation of biomass from the nutrient media was carried out by centrifugation for 30 min at 6000 r.p.m. After washing with tap water 3-times and repeated centrifugation – the yeast biomass was supplemented with acetone in excess, then the biomass precipitated in the form of fine flocculants could be filtered with a water jet pump. After filtration yeast was dried at room temperature and stored in small bottles until analysis.

Ti-content of the samples was determined by Inductive Coupled Plasma Atomic Emission System (ICP–AES). Dried yeast samples were prepared for analysis as follows: yeast samples (0.5 g) were taken into an acid-resistant Teflon tank specially designed for digestion. Samples with 3 cm^3 cc HNO_3 + 3 cm^3 H_2O_2 each, were to stand overnight at room temperature, then boiled at 100 °C, 30 min. After cooling samples were filled up to 10 cm^3 with 2× distilled water.

Amino acid composition was measured by aminochrom II OE–914 type automatic analyzer. Sample preparation (except for determination of cysteine): to a sample corresponding to 60 mg protein, 10 cm^3 6 mol dm^{-3} HCl (containing 2.5% DMSO) was added, then after bubbling through with nitrogen gas (3 min), samples were hydrolysed at 110 °C for 22 h. When hydrolysis was completed, samples were partially neutralized with 10 cm^3 of 5 mol dm^{-3} NaOH solution and finally supplemented to a volume of 25 cm^3 with Na-citrate (pH = 2.2).

For determination of cysteine, to a sample (corresponding to 30 mg protein) 10 cm^3 of 3 mol dm^{-3} p-toluene sulfonic acid (containing 0.2% triptamine) was added, then hydrolysed as described above. After partial neutralization with NaOH sample was filled up to end volume with distilled water.

Characteristic analytical parameters:

ion-exchange resin DC 4A
buffer solutions (Pico buffer II: A, B, C)
flow rate: 2 cm^3 h^{-1}
ninhydrine flow rate: 10 cm^3 h^{-1}
column temp.: T_1 = 35 °C, T_2 = 65 °C, T_3 = 75 °C
time of analysis: 100 min
detection: at 640 and 440 nm

For the measurement of Ti incorporation into yeast cells the biomass was sonicated. The dried yeast powder was suspended in phosphate buffer (pH = 6.0) in 10-fold dilution, and the cells were disrupted in ULTRASONIC homogenizer equipment (Braun, 300 W) for 3×3 min. Suspension containing disrupted cells was then stored till utilization at −24 °C. Before the ICP measurement the thawed samples were centrifuged 30 000 r.p.m., 30 min. After separation of supernatant from cell fragments 0.2 cm^3 3% trichloro-acetic acid was added to the solution (10 cm^3) and the precipitated proteins were removed by membrane filtration. The filtrate and dried cell fragments were then analysed.

2. Results and discussion

As preliminary experiments, cultivation in shaking flasks was carried out in nutrient medium supplemented with Ti-ascorbate in different concentrations. We wanted to know, whether the above-described Ti introduction affects yeast growth and whether the yeast is capable of accumulating titanium. Our results presented in Fig. 1 clearly show that at the Ti concentrations applied, Ti-ascorbate does not hinder yeast growth significantly and Ti incorporation is proportional to the amount introduced.

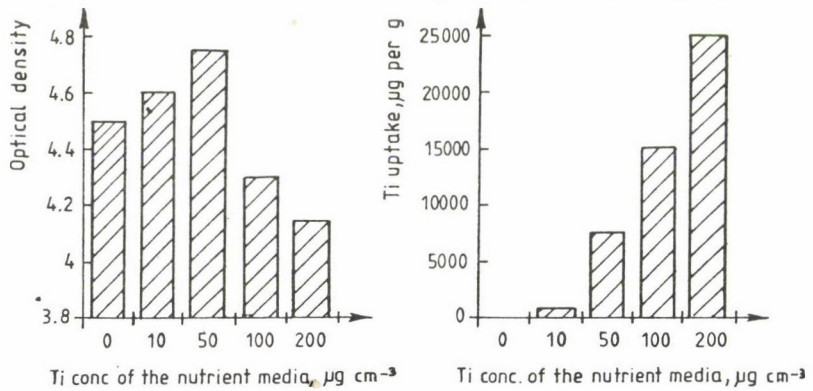

Fig. 1. *Saccharomyces cerevisiae* cultivated in shaking flasks with Ti-ascorbate supplementation

At 100 and 200 $\mu g\ cm^{-3}$ Ti concentrations a yellow precipitate was observed, therefore in our further experiments 10 and 20 $\mu g\ cm^{-3}$ Ti concentrations were applied. In further experiments yeast was cultivated in laboratory scale fermenter, where titanium was added in 10 and 20 $\mu g\ cm^{-3}$ concentrations. Our results have been summarized in Tables 3 and 4. The values of concentrations in Figure and Tables refer to titanium (not Ti-ascorbate).

Table 3

1st fermentation: Ti addition in the amount of 10 μg cm^{-3}

Time	OD	Ti in medium (μg cm^{-3})	Glucose in medium (g dm^{-3})	pH
0	0.45			4.4
2	0.65		64.7	4.0
5	1.35	→1.83		3.5
6	2.65	1.48	42.1	3.2
8	3.75	0.89		3.0
10	4.80	0.83	17.2	3.0
24	5.15	0.83	5.4	2.9

Ti uptake 820 μg g^{-1}
biomass 4.8 g dm^{-3}
calculated Ti uptake 1900 μg g^{-1}

Note: the arrows point to 10 μg cm^{-3} Ti addition

Table 4

2nd fermentation: Ti addition in the amount of 20 μg cm^{-3}

Time	OD	Ti in medium (μg cm^{-3})	Glucose in medium (g dm^{-3})	pH
0	0.47		64.7	4.4
2	0.70			4.1
5	1.10	→1.76		3.8
6	2.45	→2.36	39.0	3.5
8	2.95	1.50		3.2
10	4.15	1.32	17.6	3.0
24	4.85	1.32	4.8	2.8

Ti uptake 2074 μg g^{-1}
biomass 5.5 g dm^{-3}
calculated Ti uptake 2410 μg g^{-1}

Note: the arrows point to 10 μg cm^{-3} Ti addition

Theoretical Ti uptake was calculated on the basis of Ti addition and Ti content of the sample was measured at the end of fermentation by following formula:

$$Ti_{theoretical} = [(c_t - c_r)/m]1000 \ [\mu g \ g^{-1}]$$

c_t: total amount of Ti addition [μg cm^{-3}]
c_r: Ti residue measured in medium at the end of fermentation [μg cm^{-3}]
m: dry biomass formed [g dm^{-3}]

Titanium uptake means the amount of titanium measured in 1 g of dried yeast.

An interesting observation: Ti-ascorbate addition cannot be monitored by measurement data. Ti-ascorbate uptake seems to be an immediate reaction, which may be attributed to rapid assimilation of ascorbic acid.

The reason for the difference between calculated values and measured amounts of Ti uptake is that a part of Ti is not incorporated, only absorbed on the surface and it is removed by washing and by acetone precipitation.

It should be noted that investigations with ^{45}Ti isotope carried out on the basis of earlier experiments (KIMURA et al., 1988) showed that Ti-ascorbate passes through the cell membrane and links to protein (very likely to transferrin).

Experiments in lab scale fermenters were carried out by detailed examinations of 20 $\mu g\ cm^{-3}$ Ti addition. Changing the conditions of cultivation the effect of pH and aeration is presented. In Table 5 measurement data for pH = 4.0 are collected, in Table 6, those for pH = 5.0 are shown.

Table 5

3rd fermentation: Ti addition in the amount of 20 $\mu g\ cm^{-3}$ (pH = 4.0)

Time	OD	Ti in medium ($\mu g\ cm^{-3}$)	Glucose in medium ($g\ dm^{-3}$)
0	0.30		65.1
2	0.40		
4	1.05	→2.75	27.6
5	1.45	→4.90	
7	2.85	3.85	
9	4.15	3.75	11.6
24	4.85	5.45	1.3

Ti uptake 1565 $\mu g\ g^{-1}$
biomass 6.1 $g\ dm^{-3}$
calculated Ti uptake 2380 $\mu g\ g^{-1}$

Note: the arrows point to 10 $\mu g\ cm^{-3}$ Ti addition

Table 6

4th fermentation: Ti addition in the amount of 20 μg cm^{-3}
(pH = 5.0)

Time	OD	Ti in medium (μg cm^{-3})	Glucose in medium (g dm^{-3})
0	0.45		66.2
2	0.75		
4	1.15	→2.93	29.9
5	1.40	→5.57	
7	3.05	4.83	
9	3.90	4.50	4.2
24	5.00	5.19	1.3

Ti uptake 1580 μg g^{-1}
biomass 6.3 g dm^{-3}
calculated Ti uptake 2350 μg g^{-1}

Note: the arrows point to 10 μg cm^{-3} Ti addition

With the increase (and simultaneous control) of the pH value, Ti uptake decreased in comparison with productions without pH adjustment. No significant changes can be observed in Ti accumulation and yeast growth between the experiments at pH = 4.0 and pH = 5.0.

Table 7

5th fermentation: Ti addition in the amount of 20 μg cm^{-3}
(aeration: 0.5 v/v min)

Time	OD	Ti in medium (μg cm^{-3})	Glucose in medium (g dm^{-3})	pH
0	0.30		67.5	4.5
3	0.55			4.2
4	0.85	→1.94		3.7
5	1.30	→2.87	32.4	3.5
7	2.65	1.32		3.2
9	3.15	0.86	18.5	2.9
24	4.60	4.80	3.5	2.8

Ti uptake 1800 μg g^{-1}
biomass 5.4 g dm^{-3}
calculated Ti uptake 2810 μg g^{-1}

Note: the arrows point to 10 μg cm^{-3} Ti addition

The results in Table 7 were obtained by cultivation with reduced aeration. In comparison with data given in Table 4 the only difference is in rate of aeration, which was 0.5 v/v min instead of 1 v/v min. Under conditions of reduced aeration Ti absorption in yeast was somewhat lower than observed with 1 v/v min aeration, however the difference was not significant.

For measuring Ti incorporation cells were disrupted in ultrasonic wave equipment. After separation of the solid moieties and the filtrate Ti contents of the fractions were measured by ICP. Results are given in Table 8.

Table 8

Study of Ti incorporation

	Numbering of fermenter experiments			
	2 pH = chang.	3 pH = 4.0	4 pH = 5	5 aer. = 0.5 v/v min
Ti uptake [$\mu g\ g^{-1}$]	2074	1565	1580	1800
Ti content of dried cell fragments after disruption [$\mu g\ g^{-1}$]	2064	1755	2051	1994
Ti content of filtrate after disruption [$\mu g\ g^{-1}$]	40.2	19.7	11.9	66.2

Results obtained verify that most of accumulated titanium is incorporated into the water insoluble organic compounds since there was hardly any Ti detectable in the filtrate after cell disruption.

Amino acid analysis has also been performed for titanium containing yeasts obtained by cultivation at pH = 5.0 and without pH adjustment. These data are shown in Table 9 compared to amino acid composition of the control yeast.

Ti uptake caused simultaneous increase in amino acid concentration. It may be expected that Ti-ascorbate also increases amino acid synthesis in other eucariotic cells.

Ti enriched yeast may be utilized in paramedical products as well.

Table 9

Amino acid content of Ti enriched and control baker's yeast

Amino acid	Amino acid content (mg per g) Fermentation 2nd	4th	Control
	Ti uptake (μg per g) 2074	1580	
Asp	57.23	60.48	42.8
Tre	29.95	31.12	24.6
Ser	28.33	29.57	18.5
Glu	53.68	60.01	63.4
Pro	16.53	17.65	15.4
Gly	20.67	22.88	16.5
Ala	31.30	34.68	27.7
Cys	3.65	4.43	3.0
Val	26.96	35.45	18.7
Met	3.86	8.28	5.3
Ile	16.97	22.01	18.3
Leu	47.33	43.88	26.5
Tyr	17.86	19.70	17.5
Phe	14.28	16.34	18.2
Lys	52.85	59.90	38.4
His	14.76	11.79	11.5
Trp	5.52	12.19	4.8
Arg	51.08	42.17	29.3
Total	492.81	532.53	400.4

3. Conclusion

Baker's yeast (*Saccharomyces cerevisiae*) is able to assimilate Ti-ascorbate and accumulate titanium in its cells. The addition of 20 μg cm^{-3} Ti in the exponential phase of yeast growth resulted in 1500–2000 μg Ti/g dried yeast enrichment. Ti incorporation into the cells led to approximately 20–30% protein increase compared to normal baker's yeast. In the cells accumulated Ti is very likely present in organic bond.

References

ALCARAZ, C. F., CARVAJAL, M., FRUTOS, M. J., GIMÉNEZ, J. L., MARTÍNEZ-SÁNCHEZ, F. & PASTOR, J. J. (1994): The physiological role of titanium in *Capsicum annuum* L. plants. A review of the Spanish research during the 1989–1994 period. *Proceeding 6th International Trace Element Symposium, Budapest* (PAIS, I. et al., Eds), pp. 75–111.

JANZSÓ, B., SUHAJDA, Á. & SZABÓ, G. (1989): *Production of selenium yeasts.* Paper presented at IV. Trilateral Conference on Yeasts, Sárospatak, July 24 – 28.

JANZSÓ, B., SUHAJDA, Á., HOLLÓ, J., SZABÓ, G., EXTERDE, S., MAROSSY, K. & HADADY, K. (1990): *Accumulation of different elements in yeasts.* Paper presented at 5th European Congress of Biotechnology, Copenhagen, July 8 – 13.

KIMURA, S., KAWAMURA, M., SHUDO, N. & PAIS, I. (1985): Nutritional studies on titanium-ascorbate. *Proceedings I. International Trace Element Symposium*, Budapest, pp. 239 – 249.

KIMURA, S., SAITOH, K., MORIMATSU, F., IDO, T. & ISHIWATA, K. (1988): Metabolic investigation of ^{45}Ti-compounds in rats. *Cyric Ann. Rep.*, 193 – 196.

PAIS, I. (1983): The biological importance of titanium. *J. Plant Nutr., 6*, 3 – 131.

PAIS, I. (1994): Trace elements need trace analysis. *Env. Sampl. for Trace Anal., 30*, 73 – 91.

PAIS, I., BOKORI, B., NAGY, B., SZILÁGYI, M., FEKETE, S. & KOLONICS, F. (1989): The role of titanium-ascorbate in animal nutrition and reproduction. *Proceedings VI. Internat. Trace Element Symp., Jena* (ANKE, M. et al., Eds), pp. 1356 – 1360.

PAIS, I. & FEHÉR, M. (1975): *Mikroelemet tartalmazó permetezőszerek kertészeti és mezőgazdasági növények termésében a beltartalmi értékek, elsősorban a cukortartalom növelésére.* (Trace element included artificial fertilizer for increase of internal parameters – mainly sugar content – in crop.) Hungarian Patent 170.693.

PAIS, I., JANZSÓ, B. & SUHAJDA, Á. (1992): *Titánnal dúsított élesztő előállítása.* (Production of Ti-enriched Yeast.) *Proceedings Lippay János Scientific Session, Food Industry Section*, pp. 263 – 265.

PAIS, I., NAGY, B., FEHÉR, D., PÁLFALVI, A., REIBLING, J., KIMURA, S., SZABÓ, Z. & MÉREI, L. (1990): *Eljárás élőlények, pl. haszonállatok, növények szaporodásbiológiai tulajdonságainak és kórokozókkal szemben való ellenállóképességének növelésére, a fiziológiai folyamatok kedvező befolyásolására.* (Process for increase of multiplication features and resistance against pathogens and beneficial influence of physiological processes of living organisms – e.g. domestic animals and plants). Hungarian Patent 200.095.

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COMPARISON OF SOME COMPONENTS FROM PIGS OF DIFFERENT BODY MASS KEPT IN NATURAL CONDITIONS

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A new genotype of Hungarian Large White (75%) x Mangalica (pig with curly bristles, 25%) pig was used in the experiment. The animals were kept in a farming system under strictly natural circumstances, neither antibiotics nor yield-increasing hormones were given to them. The experimental group was slaughtered after 12 – 13 moths at 180 kg of body mass while the control group had the traditional lifetime of 8 months at 120 kg of body mass.

As a result of the fattening crude protein content decreased and fat level considerably elevated in the various cuts of the group of older pigs.

In the aspect of cholesterol there was no difference between the groups. Linoleic acid showed a significantly lower level in the more fatty tissues of older pigs.

Thiamin and riboflavin contents markedly elevated nearly all cuts (ham, loin, neck-end, shoulder-blade) of the group of 180 kg, probably owing to the vitamin-rich diet performed. Iron, zinc, copper, potassium, sodium and calcium levels remained unchanged.

In the older pig group higher level of thiobarbituric acid reactive substances (TBARS) was observed probably due to the favourable possibilities for lipid peroxidation.

Keywords: pig genotype, natural conditions, nutrient composition, lipid peroxidation, high body-mass

Few data are available on the nutritive value of the so called "natural", "bio" or "organic" foods as compared to the traditionally produced ones (LUNDSTRÖM et al., 1992; OLIVER et al., 1992; VAN DER WAL et al., 1993).

The circumstances of natural keeping of pigs, i.e. the rigid environmental conditions of the isolated farming system, the deprivation of growth stimulating hormones and antibiotics made necessary to breed a new pig genotype more resistant than so far available. The University of Agricultural Science (Gödöllő, Hungary) developed a new cross-bred (Hungarian Large White /75%/ x mangalica (pig with curly bristles, 25%) for these purposes (HUNGAMANG STANDARD, 1990).

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The chemical composition in some major cuts of the new pig genotype kept in natural circumstances was already compared with those held traditionally under large-scale farming conditions. Two important characteristics of lipid peroxidation SOD and TBA reactive compounds were also measured (DWORSCHÁK et al., 1995).

For the production of the so-called "Bio-Herz" winter-salami the pork from pigs grown to a high body mass was necessary because of its delicious sensoric characteristics.

The aim of the second experiment was to compare the nutritients and other components in the major cuts of the new pig genotype grown to high body mass (160–200 kg) with those slaughtered at the usual body mass (120 kg). Both groups were kept in natural conditions.

1. Materials and methods

The pigs of the new genotype (75% Hungarian Large White x 25% mangalica) were kept on a "free range" farming system, in natural circumstances, with free moving possibilities. The feeding of animals was performed according to the prescription of Central Soya Co. and the University of Agricultural Science, Gödöllő, as laid down in a HUNGAMANG STANDARD (1990). The composition of the feed was the following (components in increasing order): peat with humic acid, bread grist, mineral and vitamin supplements, soy flour, soy flakes, bran, barley flakes, maize flakes. The feed and supplements were free of antibiotics, sulfonamides and yield increasing hormones. Green forage beetroots and turnips were grown on bio-farm (free from chemicals).

The control group (n = 5) of animals were slaughtered at about 120 kg of body mass, at the age of 8 months in a slaughterhouse of Budapest. The experimental group (n = 5) was treated the same way at about 180 kg of body mass at the age of 12–13 months. The carcasses were jointed and some major cuts and the liver were separated according to the HUNGARIAN STANDARD (1976). Representative samples (about 1000 g) were taken according to the relavant HUNGARIAN STANDARD (1983) for determination of the chemical composition.

Moisture, crude fat and nitrogen contents were measured according to the analytical manual of the A.O.A.C. (1984); crude protein content was calculated ($N \times 6.25$).

Cholesterol content was determined as follows: after digeration with HCl, the fat was extracted and then saponified. The non-saponified part was cleaned on thin-layer and the sterols were separated on GLC. The stationary phase was Chromosorb Q with 3% liquid phase of JXR, using nitrogen as carrier gas at a temperature of 270 °C and employing a flame ionization detector. Stigmasterol was adapted as internal standard.

Determination of fatty acid composition: fat was extracted with a mixture of chloroform-methanol 2:1. After evaporation, the residue was dissolved in hexane and then transesterified with 2 mol sodium methylate. After neutralization with acetic acid, water was removed by $CaCl_2$, and the solution was injected into the gas chromatograph CARLO ERBA Fractovap 2400, with nitrogen as carrier gas. Chromosorb Q a stationary phase and a liquid phase 5 CP and 10 C was used. Separation was carried out for both phases using a thermal program between 180 – 220 °C, and a flame ionization detector.

Thiamin and riboflavin were assayed by HPLC technique (BARNA & DWORSCHÁK, 1994).

Thiobarbituric acid reactive substances (TBARS) were measured after ascorbic acid induction and the results were expressed as malon-dialdehyde (MDA) according to OHKAWA and co-workers (1979).

The activity of superoxide dismutase (SOD) was measured according to SUN and ZIGMAN (1978) on the basis of inhibition of adrenaline oxidations.

Sodium, potassium and calcium were determined by a FLAMOM B flame-photometer after wet-ashing adapted for food (LINDNER & DWORSCHÁK, 1966).

Iron, zinc and copper were assayed by AAS technique after dry-ashing (ANON, 1971).

For statistical evaluation, two tailed Student's *t*-test was used.

2. Results and discussion

Table 1 shows the crude protein and crude fat content of the samples. All cuts of the pig group with high body mass have lower protein level than the group of usual weight. As for crude fat content the tendency is opposite: the older pigs have higher fat content especially in the originally thin cuts (ham, loin). These changes result in a generally higher dry weight in the cuts of older pigs and practically the same water--protein ratio for both groups. The results are in accordance with the general experiences so far gained in the practice.

Cholesterol levels did not show any definite trends between the two experimental groups, although in loin samples of the older pigs a significantly higher value were found (Table 2).

In most cuts of the pigs fattened to 180 kg lower levels for linoleic acid were found than in the control groups. These results are in accordance with the experiences found in the Research Institute for Animal Production "Schoonoord" (VAN DER WAL, 1994): an inversed relationship can be observed between fat content and linoleic acid level in pig tissues.

Table 1

Means ($\bar{x}$) and standard deviations (s) of crude protein and crude fat contents of naturally kept experimentation pigs (180 kg) and control animals (120 kg)

(n = 5)

	Crude protein (g per 100 g)			
	180 kg		120 kg	
	$\bar{x}$	s	$\bar{x}$	s
Ham	20.9	2.0	22.3	0.8
Loin	21.3	1.0	22.5	1.3
Neck-end*	18.3	2.1	20.3	0.3
Shoulder-blade*	17.6	1.4	19.4	1.2
Liver	20.7	1.5	20.9	1.4

	Crude fat (g per 100 g)			
	180 kg		120 kg	
	$\bar{x}$	s	$\bar{x}$	s
Ham**	12.6	4.2	6.1	1.0
Loin**	15.1	3.1	7.1	3.2
Neck-end	19.1	9.0	16.9	7.7
Shoulder-blade	16.2	5.2	12.2	4.2
Liver	3.2	1.1	4.0	0.4

* $p < 0.05$ ** $p < 0.01$

TBARS increased in the older animals, and in ham and loins samples these changes were significant. These increases can be attributed to the elevated fat content in the older pigs compared to that in the average age group (Table 3). The representative component of the enzymic defence system, the superoxide dismutase (SOD) activity increased only in the fatty cuts of older pigs. An opposite trend ruled in the thin cuts in correspondence with the observation, that the SOD activity decreases with the age (PUCSOK, 1994).

Table 4 introduces the thiamin and riboflavin levels of the various cuts. Figures are related to 100 g protein because the members of vitamin B-group is associated to proteins like co-enzymes in meats. Thiamin contents are significantly higher in all cuts of older pig group. Similar but smaller differences can be observed in the respect of riboflavin levels. No differences were found in the liver samples.

Table 2

Means ($\bar{x}$) and standard deviation (s) of cholesterol and linoleic acid contents of naturally kept experimental pigs (180 kg) and control animals (120 kg)

(n = 5)

	Cholesteros (g per 100 g)			
	180 kg		120 kg	
	$\bar{x}$	s	$\bar{x}$	s
Ham	17.4	5.3	20.2	3.9
Loin**	54.9	17.4	29.2	5.2
Neck-end	43.4	6.4	54.0	11.7
Shoulder-blade	42.0	8.6	32.3	16.9
Liver	250.7	97.2	281.5	26

	Linoleic acid ($C_{18:2}$) g per 100 g fatty acid			
	180 kg		120 kg	
	$\bar{x}$	s	$\bar{x}$	s
Ham*	8.8	1.6	12.1	3.1
Loin*	7.7	1.2	9.6	1.7
Neck-end	9.1	1.2	11.0	3.6
Shoulder-blade	8.4	1.1	7.8	3.2
Liver	13.0	1.3	13.0	0.6

* $p < 0.05$ ** $p < 0.01$

No reference was found to the comparison of vitamin levels in pigs of different ages. In case of cattle the trend is opposite: veal has higher thiamin content than beef (PEARSON & TAUBER, 1985). The higher thiamin levels in the cuts of older pigs might be attributed to the soy flour component in the feed rich in thiamin. CROSS and OVERBY (1988) supposed that the high thiamin level of pork is connected to the soy flour fed to the animals.

Iron, zinc, copper, potassium, sodium and calcium levels practically remained unchanged after ageing (Tables 5, 6, 7).

Table 3

Means ($\bar{x}$) and standard deviations (s) of TBA-reactive substances (given in MDA) and superoxide-dismutase (SOD) activity of naturally kept experimental pigs (180 kg) and control animals (120 kg)

(n = 5)

	MDA (nM per 100 g)			
	180 kg		120 kg	
	$\bar{x}$	s	$\bar{x}$	s
Ham**	11.3	1.8	8.2	1.4
Loin*	15.4	2.0	12.2	1.6
Neck-end	25.6	8.0	21.8	5.8
Shoulder-blade	18.2	2.4	17.2	5.1
Liver	50.9	7.0	53.5	5.8

	SOD (U mg^{-1})			
	180 kg		120 kg	
	$\bar{x}$	s	$\bar{x}$	s
Ham	4.4	0.3	5.5	1.3
Loin	7.2	0.7	7.5	0.3
Neck-end**	9.5	0.7	8.2	0.7
Shoulder-blade*	8.1	1.0	6.8	0.3
Liver	14.3	1.6	16.1	1.0

* $p < 0.05$ ** $p < 0.01$

Table 4

Means ($\bar{x}$) and standard deviations (s) of thiamin and riboflavin contents of naturally kept experimental pigs (180 kg) and control animals (120 kg)

(n = 5)

	Thiamin (μg per 100 g protein)			
	180 kg		120 kg	
	$\bar{x}$	s	$\bar{x}$	s
Ham**	3055	681	1460	404
Loin***	2544	444	1052	210
Neck-end*	1366	296	953	132
Shoulder-blade*	2329	684	1975	599
Liver	919	126	939	468

	Riboflavin (μg per 100 g protein)			
	180 kg		120 kg	
	$\bar{x}$	s	$\bar{x}$	s
Ham	604	58	519	105
Loin	765	74	670	98
Neck-end*	750	190	518	48
Shoulder-blade**	991	144	683	80
Liver	14175	1714	13424	1232

* $p < 0.025$ ** $p < 0.005$ ***$p < 0.001$

Table 5

Means ($\bar{x}$) and standard deviations (s) of sodium and potassium contents of naturally kept experimental pigs (180 kg) and control animals (120 kg)

(n = 5)

	Sodium (mg per 100 g)			
	180 kg		120 kg	
	$\bar{x}$	s	$\bar{x}$	s
Ham	60	7.1	59	4.2
Loin	121	18.5	112	14.8
Neck-end	63	5.7	63	2.7
Shoulder-blade	111	5.5	106	5.5
Liver	101	9.6	96	5.5

	Potassium (mg per 100 g)			
	180 kg		120 kg	
	$\bar{x}$	s	$\bar{x}$	s
Ham	276	17.1	300	23.7
Loin	475	61.3	443	97.3
Neck-end	206	28.8	227	25.9
Shoulder-blade	173	9.7	179	12.4
Liver	272	39.5	253	27.7

Table 6

Means ($\bar{x}$) and standard deviations (s) of calcium and iron contents of naturally kept experimental pigs (180 kg) and control animals (120 kg)

(n = 5)

	Calcium (mg per 100 g)			
	180 kg		120 kg	
	$\bar{x}$	s	$\bar{x}$	s
Ham	5.5	0.4	6.2	1.0
Loin	13.3	1.3	13.5	3.0
Neck-end	19.4	6.2	26.4	16.0
Shoulder-blade	7.7	2.1	7.1	1.2
Liver**	9.7	1.2	12.0	0.8

	Iron (μg per 100 g)			
	180 kg		120 kg	
	$\bar{x}$	s	$\bar{x}$	s
Ham	10.28	2.11	11.16	3.09
Loin*	9.44	2.75	14.68	4.77
Neck-end	19.04	4.31	21.36	5.66
Shoulder-blade	20.00	1.41	19.04	5.75
Liver	278.00	41.5	336.00	1167.00

* $p < 0.05$ ** $p < 0.01$

Table 7

Means ($\bar{x}$) and standard deviations () of zinc and copper contents of naturally kept experimental pigs (180 kg) and control animals (120 kg)

(n = 5)

	Zinc (mg per 100 g)			
	180 kg		120 kg	
	$\bar{x}$	s	$\bar{x}$	s
Ham	34.8	9.4	47.6	23.8
Loin	36.0	3.5	36.4	9.9
Neck-end	63.2	6.6	73.6	16.9
Shoulder-blade	47.6	8.6	44.8	8.4
Liver	58.0	10.9	65.8	12.7

	Copper (μg per 100 g)			
	180 kg		120 kg	
	$\bar{x}$	s	$\bar{x}$	s
Ham	0.60	0.05	0.53	0.10
Loin	0.45	0.29	0.45	0.13
Neck-end	0.51	0.31	0.55	0.23
Shoulder-blade	0.70	0.14	0.55	0.20
Liver	2.28	0.6	2.70	1.10

3. Conclusion

A new genotype of pig (Hungarian Large White /75%/ x Mangalica /25%/) was used in these experiment. The pigs were kept under natural circumstances.

Significant and often considerable differences were found between the chemical composition and other characteristics in the various cuts of pigs slaughtered at 120 kg and 180 kg body mass respectively. A part of these differences (thiamin, riboflavin) seems to be favourable, but others (protein, fat, linoleic acid) are detrimental from a nutritional viewpoint for the pigs grown to a high body mass.

The higher thiamin level in older pigs might be attributed to the constant intake of soy flour containing feed rich in thiamin.

In older animals the thiobarbituric acid (TBA) reactive substances increased probably as a result of higher fat concentration, causing a more intensive lipid peroxidation.

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Literature

ANON (1971): Perkin-Elmer analytical methods for atomic absorption spectrometry. Norwalk, CT, USA.

A.O.A.C. (1984): Official methods of analysis. (24.003, 24005, 24027) 14th ed. Arlington, VA, USA.

BARNA, É. & DWORSCHÁK, E. (1994): Determination of thiamine (vitamin B_1) and riboflavin (vitamin B_2) in meat and liver by high-performance liquid chromatography. *J. Chromat. A., 668*, 359–363.

CROSS, H. R. & OVERBY, A. J. (1988): Nutritive value aspects. – in: *World animal science, B. 3. Meat science, milk science and technology.* Elsevier, pp. 127–135.

DWORSCHÁK, E., BARNA, É., GERGELY, A., CZUCZY, P., HÓVÁRI, J., KONTRASZTI, M., GAÁL, Ö., RADNÓTI, L., BÍRÓ, GY. & KALTENECKER, J. (1995): Comparison of some components of pigs kept in natural (free-range) and large-scale conditions. *Meat Sci., 39*, 79–86.

HUNGAMANG STANDARD (1990): Quidelines for the breeding, keeping, feeding and hygienic conditions of NATUR–1 and NATUR–2 pigs. No. 272 (unpublished).

HUNGARIAN STANDARD (1976): *Sertés húsának hússzéki darabolása.* (Butcher's cutting of pork.) MSz. 6936.

HUNGARIAN STANDARD (1983): *Hús és húskészítmények mintavétele.* (Sampling of meats and meat products.) MSz 5871.

LINDNER, K. & DWORSCHÁK, E. (1966): Für Serienuntersuchungen geeignete flammenphotometrische Methode zur Bestimmung von Kalium, Natrium, Calcium und Magnesium in Lebensmitteln. *Z. Lebensmittelunters. u. -Forsch., 151*, 207–215.

LUNDSTRÖM, K., HAKANSSON, J., HANSSON, I., JOHANSSON, M. & ANDERSSON, K. (1992): Carcass and meat quality of F_2-crosses between European wild pig and domestic pig. – in: *Proceedings of 38th International Congress of Meat Science and Technology, Clermont-Ferrand, France*, Vol. 2. pp. 81–84.

OHKAWA, H., OHISHI, N. & YAGI, K. (1979): Assay for lipid peroxides in animal tissues by thiorbarbituric acid reaction. *Analyt. Biochem., 95*, 351–358.

OLIVER, M. A., GISPERT, M., GOU, P., BLASCO, A. & DIESTRE, A. (1992): Pig meat quality in crossbred experiments in the mediterranean. – in: *Proceedings of 38th International Congress of Meat Science and Technology, Clermont-Ferrand, France*, Vol. 2. pp. 109–112.

PEARSON, A. M. & TAUBER, F. W. (1985): Composition and nutritive value of raw materials and processed meats. – in: *Processed meats.* AV Publ. Comp. pp. 18–24.

PUCSOK, J. (1994): *Role of antioxidants in the nutrition.* paper presented at the 2nd Conference of the National Association of Hungarian Dieticians, Pécs, Oct. 14–16.

SUN, M. & ZIGMAN, S. (1978): An improved spectrophotometric assay for superoxide dismutase based on epinephrine autooxidation. *Analyt. Biochem., 90*, 81–83.

VAN DER WAL, P. G., MATEMAN, G., DE VRIES, A. W., VONDER, G. M. A., SMULDERS, F. J. M., GEESINK, G. H. & ENGEL, B. (1993): "Scharrel" (free range) pigs: carcass composition, meat quality and taste-panel studies. *Meat Sci., 34*, 27–37.

VAN DER WAL, P. G. (1994): Personal communication.

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THE OCCURRENCE OF TRANS-RESVERATROL IN SLOVENIAN RED AND WHITE WINES

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Some very recent studies suggested that the phytoalexin trans-resveratrol (3,5,4'--trihydroxystilbene) might be one of the compounds responsible for the prevention of heart diseases as a result of drinking wine. Recently new methods for its determination have been developed. In this work trans-resveratrol was quantitatively determined by HPLC in 45 red and 21 white wines from Slovenia. Samples were purified and enriched by passing them through a preconditioned C_{18} cartridge and eluting the trans-resveratrol with ethyl acetate. HPLC separation of trans-resveratrol was performed using a RP – C_{18} column. Detection was carried out with a UV detector at 310 nm. A processing variation coefficient of 3.7% was calculated. Ninety-four % of the error was caused by the sample preparation and 6% by the HPLC analysis itself. The detection limit was 0.25 mg l^{-1} for red wines and 0.01 mg l^{-1} for white ones, with a recovery rate of 99%. The time needed for 8 analyses was approximately 4 h. The content of trans-resveratrol in red wines ranged from 0.9 to 8.7 mg l^{-1} while in the white wines it was much lower – between n.d. and 0.6 mg l^{-1}.

Keywords: HPLC-analysis, stilbens, trans-resveratrol, wine

The phenolic compounds of wine have a large contribution to the colour, flavour and other sensorical properties of wine (OSZMIANSKI et al., 1988). At the moment phenolic substances in red wine are receiving considerable attention for their role in the prevention of coronary heart diseases (FRANKEL et al., 1993a, b; RENAUD & DE LOREGIL, 1992; WATERHOUSE & FRANKEL, 1993; WATERHOUSE, 1994; KANNER et al., 1994) because they inhibit the copper-catalysed oxidation of human LDL in vitro. SEGURA (1994) and SEIGNEUR and co-workers (1990) found that the consumption of red wine, but not that of white wine or ethanol, induced platelet hypoaggregation and an increase of HDL cholesterol both of which have cardioprotective activities in humans. Therefore, it has been suggested that the antioxidative activity of phenolic compounds may explain the "French paradox", a phenomenon that the inhabitants of Toulouse, whose alcohol intake is largely in the

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form of red wine, have a very low rate of cardiac diseases, despite their fat consumption rate being similar to that of the USA (RENAULD & DE LOREGIL, 1992). KINSELLA and co-workers (1993) described that phenolic compounds which are present in wine could provide significant antioxidative protection by several related mechanisms.

One of the compounds that may play an important role in cardioprotective action is the stilbenic phytoalexin trans-resveratrol (FRANKEL et al., 1993b). Resveratrol has attracted interest because its glucoside polydatin is traditionally used in the Asian herbal folks medicine "kojo-kon" for treatment of some cardiac diseases including atherosclerosis and inflammation (KIMURA et al., 1985). This traditional medicine is composed of the dried, powdered root of the Japanese Knotweed *Polygonum cuspidatum* (ARCHI et al., 1982). KIMURA and co-workers (1985) reported that resveratrol isolated from roots of *Polygonum cuspidatum* induced platelet hypoaggregation in an in vitro system using rat cells.

In the genus *Vitis vinifera* resveratrol was first noted in leaves in 1976 (LANDCAKE & PRYCE, 1976), and one year later in berries (LANDCAKE & PRYCE, 1977a). Due to the interest in its antifugal properties in grape vines resveratrol has been the subject of many studies (LANDCAKE & PRYCE, 1976; LANDCAKE & PRYCE, 1977b; JEANDET et al., 1991; BLAICH & BACHMANN, 1980; STEIN & HOOS, 1984). Nowadays different isomeric, glycosilated and polymeric forms of resveratrol like pterostilben and viniferin have been isolated and identified (MATTIVI & RENEIRO, 1992; LANDCAKE & PRYCE, 1976; 1977b). All stilbens in grapes are synthesized after stress, for example as a consequence of pathogenic infection or exposure to UV lights (JEANDET et al., 1992). Therefore the ability of stilben production has been considered as a grape disease resistance index (BARLASS et al., 1987; CREASY & COFFEE, 1988; PRYCE & LANDCAKE, 1977). As resveratrol is a phytoalexin its accumulation in plant cells is positively correlated with the resistance of grapevine varieties to grey mould (JEANDET et al., 1992; LANDCAKE & PRYCE, 1976; STEIN & BLAICH, 1985). Therefore it was reported to be a good marker for grape disease resistance (LANDCAKE & PRYCE, 1976; POOL et al., 1981).

Since resveratrol is supposed to be one of the compounds responsible for the reduced heart disease rate of wine drinkers, the determination of resveratrol in wine has been the object of some recent investigations (SIEMANN & CREASY, 1992; JEANDET et al., 1993; LAMUELA-RAVENTOS & WATERHOUSE, 1993; MATTIVI, 1993a, b; MATTIVI & NICOLINI, 1993; ROGGERO & ARCHIER, 1994; PEZET et al., 1994). As a result it was found that many different factors influenced the content of resveratrol in wines. Resveratrol synthesis is strongly related to the variety, and the content of resveratrol in different varieties was reported by several authors (SIEMANN & CREASY, 1992). It is known that there are large differences between vintage years which indicates the influence of environmental factors like temperature,

humidity etc. in the year of production. Furthermore MATTIVI and NICOLINI (1993) have demonstrated that the wine making technology makes an important contribution to the resveratrol content of wine.

For better evaluation, estimation and screening of all these factors many different analytical methods for extraction and determination of resveratrol in wines have been developed recently (JEANDET et al., 1993; SIEMANN & CREASY, 1992). Different methods of sample preparation like liquid-liquid extraction (LAMUELA-RAVENTOS & WATERHOUSE, 1993), solid-phase extraction (MATTIVI, 1993a, b; MATTIVI & NICOLINI, 1993), direct injection (PEZET et al., 1994) as well as different chromatographic methods e.g. HPLC and GS (ROGGERO & ARCHIER, 1994) were elaborated. Different detection techniques like UV, MS, fluorometric detector (PEZET et al., 1994) were also described.

1. Materials and methods

1.1. Materials

1.1.1. Wine samples. Forty-five red wines (11 Merlot samples, 13 Cabernet Sauvignon samples, 10 Refosk samples, 7 Blaufränkisch samples, 4 Pinot noir samples) and 21 white wines (11 Chardonnay samples and 10 Pinot gris samples) from Slovenia of different production areas, vintage years and technological processes which are currently available at the market were investigated.

1.1.2. Chemicals. Ethylacetate p.a. (Merck, Nr. 9623), Acetonitrile (Chromasolv, Riedel-de Haën Nr. 34852), Orthophosphoric acid (Riedel-de Haën Nr. 30417), HCl 37% (Riedel-de Haën Nr. 30721), Ethanol 96% (Riedel-de Haën Nr. 32294), Methanol (Chromosolv, Riedel-de Haën Nr. 34860), Potassium dihydrogen phosphate p.a. (Merck No. 4873), di-Sodium hydrogen phosphate dihydrate p.a. (Merck No. 6580), NaOH 1 mol l^{-1} (Titrisol, Merck No. 1.09956), Resveratrol (trans-3,4',5-trihydroxystilbene, Sigma R – 5010)), trans-4-Hydroxystilbene 98% (Janssen Chimica 12.193.68)

1.1.3. Solutions. pH 7.00 buffer – 3.52 g l^{-1} KH_2PO_4 and 7.26 g l^{-1} $Na_2HPO_4 \times H_2O$, Internal standard – 200 mg l^{-1} ethanolic solution of trans-4-hydroxystilbene

1.1.4. RP C_{18} cartridge. Bond Elut (Varian Part. Number 1211 – 3027)

1.2. Methods

1.2.1. Sample preparation. As numerous phenolic compounds present a maximum of absorption in the range of 280 – 320 nm preliminary fractionation is necessary for a good HPLC resolution (OSZMIANSKI et al., 1988). A simple and rapid

method for the isolation of stilbens was developed by MATTIVI (1993a) using the solid-phase extraction with C_{18} cartridges. Fifty ml wine and 0.5 ml internal standard were poured into a beaker, neutralized to pH 7.0 with 1N NaOH and made up to 100 ml with distilled water. The extraction of resveratrol was carried out on preconditioned RP – C_{18} cartridge. The cartridge was conditioned with 5 ml methanol, followed by 10 ml buffer solution, pH 7.0. Then 10 ml diluted and neutralised red wine were loaded onto the cartridge, for white wine samples 100 ml were used. The cartridge was washed with 20 ml buffer solution, pH 7.0. The washing solution was removed from the cartridge by using light nitrogen flux. The stilbens were then eluted with 5 ml ethyl acetate and collected in a conical centrifuge tube. The fraction thus obtained was evaporated to dryness on a rotary vacuum evaporator at 25 °C in order to remove the ethyl acetate without destroying the phenolic compounds. After evaporation the residue was completely redisolved in 5 ml methanol. The sample was put into a deep-freezer until the water remained was frozen (30 min) and then the ice crystals were removed by filtering. After elution the cartridge was re-equilibrated with 5 ml acid methanol (0.1% HCl) for immediate use. All samples were prepared in duplicate.

1.2.2. HPLC analysis. The Waters HPLC system consisted of two Waters – 501 pumps (Waters Associates Inc., Miliford, MA, USA), autosampler Waters WISP 712, multiwavelength UV detector Waters 490 E and Waters 810 Baseline Data Acquisition. Separation was carried out using a LiChrospher RP – C_{18} column (250 × 4 mm, 5 μm, Fa. Merck, Darmstadt, Germany) and a RP – C_{18} pre-column (4 × 4 mm, 5 μm) at 30 °C. Twenty-five μl of each sample was injected at least in two parallels. As mobile phase solvent A, 1 mmol H_3PO_4 and solvent B, CH_3CN were used. The gradient used is listed in Table 1. The flow rate was maintained at 2.5 ml min^{-1}. For the analysis the detector was set at a wavelength of 310 nm. Trans-resveratrol was identified by spiking with the standard (3,5,4'-trihydroxystilbene) under the same conditions and by comparison of the retention times. Quantification was performed from the peak-areas using a calibration curve of standard resveratrol (trans 3,4',5-trihydroxistilben) in concentrations ranging between 0.24 mg l^{-1} and 8 mg l^{-1}. Trans-4-hydroxystilbene was used as an internal standard.

1.3. Statistical analysis

Analysis of variance was carried out with the Statgraphics statistical program.

Table 1

Solvent gradient used in HPLC analysis

T (min)	A (%)	B (%)
0	87.5	12.5
25	73.7	26.3
30	37.5	62.5
40	37.5	62.5
41	0	100
50	0	100
51	87.5	12.5
60	87.5	12.5

2. Results and discussion

The HPLC analysis of a standard mixture on a C_{18} reversed-phase column gave two well-separated peaks, trans-resveratrol had an R_t of 24.61 min and the internal standard (trans-4-hydroxystilbene) an R_t of 37.98 min. A typical chromatogram of a prefractionated wine sample is shown in Fig. 1. The results obtained from the analysis of 21 white and 45 red commercially available Slovenian wines are presented in Tables 2 and 3. In red wines the trans-resveratrol

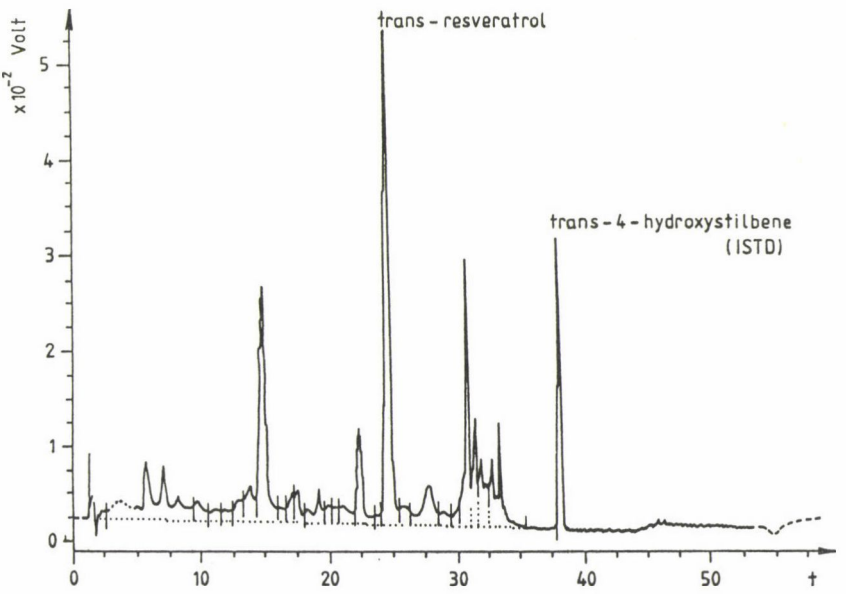

Fig. 1. HPLC determination of trans-resveratrol in Blaufränkisch (8.7 mg l^{-1})

Table 2

Concentration of trans-resveratrol (mg l^{-1}) in some Slovenian white wines

Variety	No. of samples (vintage)	Min	Max	$\bar{x}$	s
Chardonnay	4 (1993)	0.05	0.3	0.14	
	6 (1992)	0.12	0.6	0.22	
	1 (1991)	–	–	0.2	
Total	11	0.05	0.6	0.18	0.16
Pinot gris	4 (1993)	0.08	0.2	0.12	
	4 (1992)	n.d.	0.15	0.08	
	2 (1991)	0.1	0.2	0.15	
Total	10	n.d.	0.2	0.11	0.06
Subtotal per year	8 (1993)	0.05	0.3	0.13	0.08
	10 (1992)	n.d.	0.6	0.17	0.16
	3 (1991)	0.1	0.2	0.17	0.05
Total	21	n.d.	0.6	0.15	0.12

n.d = not detectable

concentration varied from 0.9 to 8.7 mg l^{-1} with an average value of 2.6 mg l^{-1} and a standard deviation (s.d.) of 1.3 mg l^{-1} (Table 2). In white wines, however, the values were much lower – between not detectable (n.d.) and 0.6 mg l^{-1} with an average value of 0.15 mg l^{-1} and a s.d. of 0.12 mg l^{-1} (Table 2). The values we found are similar to those published by MATTIVI, 1993a, b; MATTIVI & NICOLINI, 1993; PEZET et al., 1994, however, they are up to ten times higher in comparison to some previous studies (JEANDET et al., 1993; LAMUELA-RAVENTOS & WATERHOUSE, 1993; SIEMANN & CREASY, 1992). In accordance with previous studies red wines showed much higher trans-resveratrol contents than white wines (JEANDET et al., 1993; LAMUELA-RAVENTOS & WATERHOUSE, 1993; MATTIVI, 1993a, b; MATTIVI & NICOLINI, 1993; PEZET et al., 1994; ROGGERO & ARCHIER, 1994; SIEMANN & CREASY, 1992). In grape berries trans-resveratrol is mainly located in grape skins while its concentration is very low in the fruit flesh (JEANDET et al., 1991; SIEMANN & CREASY, 1992). Trans-resveratrol requires a relatively long contact time to be extracted. In the vinification process of white wines the grapes are pressed immediately or after only a short maceration time while the red wine is fermented initially on the skins and therefore can accumulate resveratrol.

Table 3

Concentration of trans-resveratrol (mg l^{-1}) in some Slovenian red wines

Variety	No. of samples (vintage)	Min	Max	$\bar{x}$	s
Merlot	3 (1993)	1.1	3.3	1.9	
	3 (1992)	2.2	2.8	2.4	
	2 (1991)	2.4	2.6	2.5	
	2 (1990)	1.2	5.4	3.3	
	1 (1989)	–	–	3.6	
Total	11	1.1	5.4	2.55	1.25
C. Sauvignon	4 (1993)	1.0	3.5	2.1	
	3 (1992)	1.6	2.2	1.9	
	2 (1991)	1.7	2.6	2.2	
	2 (1990)	1.5	2.2	1.9	
	1 (1989)	–	–	3.9	
	1 (1987)	–	–	3.1	
Total	13	1.0	3.9	2.23	0.84
Refosk	3 (1993)	2.4	3.5	2.9	
	2 (1992)	2.2	2.6	2.4	
	2 (1991)	2.5	4.7	3.6	
	1 (1990)	–	–	3.1	
	1 (1989)	–	–	2.6	
	1 (1988)	–	–	2.9	
Total	10	2.4	4.7	2.93	0.72
Blaufränkisch	3 (1993)	1.7	2.7	2.2	
	3 (1992)	1.8	8.7	4.1	
	1 (1990)	–	–	4.4	
Total	7	1.7	8.7	3.33	2.36
Pinot noir	1 (1993)	–	–	1.7	
	2 (1992)	3.3	4.5	3.9	
	1 (1991)	–	–	0.9	
Total	4	0.9	4.5	2.6	1.61
Subtotal per year	14 (1993)	1.0	3.5	2.2	0.86
	13 (1992)	1.6	8.7	2.9	1.90
	6 (1991)	0.9	4.7	2.4	1.09
	7 (1990)	1.2	5.4	2.8	1.60
	3 (1989)	2.6	3.9	3.4	0.70
	1 (1988)	–	–	2.9	–
	1 (1987)	–	–	3.1	–
Total	45	0.9	8.7	2.6	1.3

Only one of the white wine samples was significantly different from the others. It was a sample of late harvest Chardonnay wine in which the resveratrol content was much higher than expected (0.6 mg l^{-1}) since grapes are known to loose their ability to produce resveratrol during the ripening process (JEANDET et al., 1991). It is possible that such a late harvest date could mean some water evaporation and therefore a concentration of all the compounds, including trans-resveratrol.

Differences in the production of trans-resveratrol between varieties were reported by several authors, therefore the variety seems to be of prime importance (SIEMANN & CREASY, 1992). In our investigations the highest content of trans-resveratrol was found in a sample of Blaufränkisch wine with 8.7 mg l^{-1} and also the average value of all the samples within one variety was the highest in Blaufränkisch wines with 3.3 mg l^{-1}. One possible explanation might be found in the fact that the Blaufränkisch variety possesses a very high resistance to grey mould, which is supposed to be related to the resveratrol production. In the group of white wines the differences between varieties were less apparent.

Some remarkable differences in trans-resveratrol contents could also be found between samples belonging to the same varieties, but originating from different producers. Some of the explanations for these deviations could be geographical differences, differences in growing practices as well as in wine technology.

Additionally, the results for the same variety from the same winery were also very variable from one year to another. Taking the fact into account that wine making techniques in the wineries are more or less constant these findings could indicate that environmental conditions during the wine growing season also contribute to the differences in trans-resveratrol concentrations in wine (MATTIVI, 1993a, b; MATTIVI & NICOLINI, 1993; BAVARESCO, 1993).

3. Conclusion

The content of trans-resveratrol in wines seems to depend on many factors like the grape variety, vinification techniques, geographical origin, growing methods and ecological conditions. The choice of variety seems to be of prime importance for the concentration of trans-resveratrol in wine. Besides that, the resveratrol content seems to depend very strongly on the production technology.

In a recent study WATERHOUSE (1994) has established that a β-glucoside of trans-resveratrol is also present in significant amounts in grape berry skins. Moreover, ROGGERO and ARCHIER (1994) reported about significant amounts of β-glucoside of trans-resveratrol also in wines. Further studies to determine whether and to which degree the β-glucoside of trans-resveratrol contributes to the physiologically-available pool of resveratrol in wine should be carried out.

Preliminary studies on wine showed that when fermenting musts were treated with glucosidase containing enzyme mixtures, increased levels of trans-resveratrol could be detected in the resulting wines (WATERHOUSE, 1994). This suggested the presence of the β-glucoside of trans-resveratrol in the must.

For the better understanding of all these influences further detailed investigations in the fields of viticulture and oenology seem to be necessary.

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References

ARCHI, H., KIMURA, Y., OKUDA, H., BABA, K., KOZAWA, M. & ARICHI S. (1982): Effect of stilbene components of the roots of Polygonum cuspidatum Sieb. et Zucc. on lipid metabolism. *Chem. pharm. Bull.*, *30*, 1766 – 70.

BARLASS, M., MILLER, R. M. & DOUGLAS, T. J. (1987): Development of methods for screening grapevines for resistance to infection by Downy mildew. II. Resveratrol production. *Am. J. Enol. Vitic.*, *38*, 65 – 68.

BAVARESCO, L. (1993): Effect of potassium fertilizer on induce stilbene synthesis in different grapevine varieties. *Bull. O.I.V.*, 675 – 689, 751 – 752.

BLAICH, R. & BACHMANN, O. (1980): Die Resveratrolsynthese bei Vitaceen Induction und zytologische Beobachtungen. *Vitis*, *19*, 230 – 240.

CREASY, L. L. & COFFEE, M. (1988): Phytoalexin production potential in grape berries. *J. Am. Soc. Hort. Sci.*, 113 – 2, 230 – 234.

FRANKEL, E. N., KANNER, J., GERMAN, J. B., PARKS, E. & KINSELLA, J. E. (1993a): Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet*, *341*, 454 – 457.

FRANKEL, E. N., WATERHOUSE, A. L. & KINSELLA, J. E. (1993b): Inhibition of human LDL oxidation by resveratrol. *Lancet*, *341*, 1103 – 1104.

JEANDET, P., BESSIS, R. & GAUTHERON, B. (1991): The production of resveratrol (3,5,4'-trihydroxystilbene) by grape berries in different developmental stages. *Am. J. Enol. Vitic.*, *42*, 41 – 46.

JEANDET, P., BESSIS, R. & GAUTHERON, B. (1992): The production of resveratrol (3,5,4'-trihydroxystilbene) by grapevine in vitro cultures, and its application to screening for grey mould resistance. *J. Wine Res.*, *3*, 47 – 57.

JEANDET, P., BESSIS, R., MAUME, B. F. & SBAGHI, M. (1993): Analysis of resveratrol in Burgundy wines. *J. Wine Res.*, *4*, 79 – 85.

KANNER, J., FRANKEL, E. N., GRANIT, R., GERMAN, J. B. & KINSELLA, J. E. (1994): Natural antioxidants in grapes and wines. *J. agric. Fd Chem.*, *42*, 64 – 69.

KIMURA, Y., OKUDA, H. & ARICHI, S. (1985): Effect of stilbenes on arachidonate metabolism in leucocyts. *Biochim. Biophys. Acta*, *834*, 275 – 278.

KINSELLA, J. E., FRANKEL, E. N., GERMAN, B. & KANNER, J. (1993): Possible mechanisms for the protective role of antioxidants in wine and plant foods. *Fd Technology*, 85 – 89.

LAMUELA-RAVENTOS, R. M. & WATERHOUSE, A. L. (1993): Occurrence of resveratrol in selected California wines by a new HPLC method. *J. agric Fd Chem.*, *41*, 521 – 523.

LANDCAKE, P. & PRYCE, R. J. (1976): The production of resveratrol by Vitis vinifera and other members of the Vitacee as a response to infection or injury. *Physiol. Pl. Path.*, *9*, 77 – 86.

LANDCAKE, P. & PRYCE, R. J. (1977a): A new class of phytoalexins from grapevines. *Experientia, 33*, 151 – 152.

LANDCAKE, P. & PRYCE, R. J. (1977b): The production of resveratrol and the viniferins by grapevines in response to ultraviolet irradiation. *Phytochemistry, 16*, 1193 – 1196.

LANDCAKE, P. & MCCARTY, W. V. (1979): The relationship of resveratrol production to infection of grapevine leaves by Botrytis cinerea. *Vitis, 18*, 244 – 253.

MATTIVI, F. (1993a): Solid phase extraction of trans-resveratrol from wines for HPLC analysis. *Z. Lebensmittelunters. u. -Forsch., 196*, 522 – 525.

MATTIVI, F. (1993b): Il contenuto di resveratrolo nei vini rossi e rosati trentini del commercio. *Riv. Vitic. Enol.*, 37 – 45.

MATTIVI, F. & RENEIRO, F. (1992): Oligostilbens from the roots of genus Vitis. *Bull. Liais. Groupe Polyphenols, 16-I*, 116 – 119.

MATTIVI, F. & NICOLINI, G. (1993): Influenca della technica di vinificazione sul contenuto di resveratrolo dei vini. *L'enotechnico, 29*, 81 – 88.

OSZMIANSKI, J., RAMOS, T. & BOURZEIX, M. (1988): Fraction of phenolic compounds in red wine. *Am. J. Enol. Vitic., 39*, 259 – 262.

PEZET, R., PONT, V. & CUENAT, P. (1994): Method to determine resveratrol and pterostilbene in grape berries and wines using high-performance liquid chromatography and highly sensitive fluorimetric detection. *J. Chromatogr. A., 663*, 191 – 197.

POOL, R. M., CREASY, L. L. & FRACKELTON, A. S. (1981): Resveratrol and viniferins, their application to screening for disease resistance in grape breeding programs. *Vitis, 20*, 136 – 145.

PRYCE, R. J. & LANDCAKE, P. (1977): α-viniferin: an antifungal resveratrol trimer from grapevines. *Phytochemistry, 16*, 1452 – 1454.

RENAUD, S. & DE LOREGIL, M. (1992): Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet, 339*, 1523 – 1526.

ROGGERO, J. P. & ARCHIER, P. (1994): Dosage du resvératrol et de l'un de ses glycosides dans les vins. *Sci. Aliments., 14*, 99 – 107.

SEGURA, R. (1994): The protecting capacity of polyphenolic compounds against the peroxidative degradation of the essencial lipids of the human body. – in: *Bulletin of General assembly O.I.V., Paris*.

SEIGNEUR, M., BONNET, J., DORIAN, B., BENCHIMOL, D., DROUILLET, F., GOUVERNEUR, G., LARRUE, J., CROCKETT, R., BOISSEAU, M.-R., RIBEREAU-GAYON, P. & BRICAUD, H. (1990): Effect of the consumption of alcohol, white wine, and red wine on platelet function and serum lipids. *J. appl. Cardiology*, 215 – 222.

SIEMANN, E. H. & CREASY, L. L. (1992): Concentration of the phytoalexin resveratrol in wine. *Am. J. Enol. Vitic.*, 49 – 52.

STEIN, U. & HOOS, G. (1984): Induktions- und Nachweismethoden für Stilbene bei Vitaceen. *Vitis, 23*, 179 – 194.

STEIN, U. & BLAICH, R. (1985): Untersuchungen über Stilbenproduktion und Botrytisfälligkeit bei Vitis-Arten. *Vitis, 24*, 75 – 87.

WATERHOUSE, A. L. & FRANKEL, E. N. (1993): Wine antioxidants may reduce heart disease and cancer. – in: *Bulletin of General assembly O.I.V., San Francisco*.

WATERHOUSE, A. L. (1994): The non-polyphenolic compounds of grapes and wine which affect nutrition and health. – in: *Bulletin of General assembly O.I.V., Paris*.

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BOOK REVIEWS

Structure and development of meat animals and poultry

H. J. SWATLAND

Technomic Publishing Company, Inc., Lancaster, Basel, 1994, 606 pages

This book is a revised and updated version of Structure and development of meat animals, published in 1984.

There are many books that deal with animal growth and meat science from biochemical and endocrinological viewpoints but this book would help us to maintain and expand our knowledge of the subject from a structural and/or anatomical viewpoints as well.

Chapter 1 presents the basic information required to understand animal structure and slaughtering. Apart from this part the remainder of the book is concerned with the structure and development of muscle fat and bone in the commercial carcasses of meat animals.

Chapter 2 explains how the connective tissues of the carcass – bones, gristle and fat – shape the skeleton yet threaten meat quality. Chapter 3 gives a synopsis of US and Canadian carcass grading and meat cutting including more detail for the UK and Japan. (The section shows the trends that have emerged and how grading for meat yield and quality may evolve in the future.)

Chapter 4, a hands-on dissection guide for identifying the muscles of the carcass.

Chapter 5 shows how the commercial properties of meat are determined by the basic structure and biochemistry of striated muscle.

Chapters 6 and 7 describe the cellular basis of pre- and postnatal muscle growth and development. Chapter 6 gives an introduction to the histochemical fiber typing. Chapter 7 deals in detail with the postnatal cellular growth and development of meat.

Chapter 8 is devoted to animal growth and development.

Chapter 9 attempts to unite the information presented in earlier chapters to explain the origins of meat quality. This chapter concerns the conversion of muscles to meat.

A number of studies have been published in the field of meat science up to now therefore it is necessary from time to time to review the present state of knowledge of certain subjects. Each of the 600 pages of this book provides very important concise information on the above-mentioned topics with many figures. At the end of each chapter valuable, updated references are given for extensive literature survey. An index provides help for the reader in looking for different topics.

This book is easily understandable by everyone with basic knowledge in science and by those who are not familiar with the recent results in this field. It might be recommended for university students graduate and postgraduate ones for those who are devoted to science, food technologists, and everybody interested in meat science.

D. BÁNÁTI

Magnetic resonance imaging in foods

M. J. McCARTHY

Chapman and Hall, London, 1994, 110 pages

The book consists of six chapters in which the author gives not only an excellent introduction to the Nuclear Magnetic Resonance (NMR) and to the Magnetic Resonance Imaging (MRI) techniques with respect to their application in the field of the food industry but describes also specific applications like studying food structure, component distributions, phase changes, and transport properties. The book touches upon the potential applications for MRI in process control and quality assurance, too.

The first chapter introduces magnetic resonance phenomena, NMR in general, and MRI in detail. Applications and impact of MRI on food research as well as capabilities and limitations of this technique are discussed in this chapter.

The fundamental principles of MRI are described in the second chapter. An introduction to the quantum mechanical basic theory helps the researchers interested in more details to understand and interpret the MRI data. This chapter can be skipped by those individuals no interested in theoretical details.

Strategies for measuring food structure using MRI and procedures for analysing this information are presented in the third chapter. Some examples to measure certain structural features of foams, emulsions and suspensions, that cannot be measured by other experimental techniques are described here.

Possibilities of non-invasive measurements to determine moisture and lipid saturation distribution in foods are introduced in the fourth chapter. Correct analysis of the transport of a particular component in a food product is possible only when there is correct information on saturation distribution, material structure, material properties and thermodynamic data for the entire system. In this chapter it is shown that MRI can provide information on all of these items. As examples for moisture saturation measurements experiments with apples, potatoes, corn and gels are presented.

The fifth chapter deals with phase transitions. MRI can be used to quantify the kinetics of phase transitions during processing. The evaporation, freezing, melting, crystallisation, heat transfer etc. can be studied by MRI.

The future trends and conclusions are presented in the sixth chapter. It is shown that the applications of MRI in the study of food will continue to expand. The expansion of the application will most likely occur first in analytical applications and eventually in process control situations. The MRI will permit the examination of large molecules in an imaging mode.

All chapters are followed by a rich list of relevant literature to magnetic resonance imaging.

Despite much research on quantifying and predicting the changes in foods during processing and storage, there is still a considerable lack of fundamental information concerning the physicochemical changes and transport phenomena occurring in foods. One of the major difficulties to acquiring this knowledge has been the inability to measure food systems without altering the phenomena being measured. MRI is one solution of this problem; this is shown by M. J. McCarthy in his book.

K. KAFFKA

Analytical chemistry of foods

C. S. JAMES

Blackie Academic & Professional, London, Glasgow, Weinheim, New York, Tokyo, Melbourne, Madras, 1995, 178 pages

This book gives a comprehensive overview about the whole field known as analytical chemistry of foods. The author not only present both fundamental and applied aspects and practices of the chemical, biochemical and physical methods of foods but conforms to requirements set out in many of food laws. Moreover the book provides methods for qualitative and quantitative determination of illegal components in foods such as certain colouring materials, additives etc. The book consists of 7 chapters, representing two main parts: a theoretical and an experimental one. The first part, after a brief introduction (Chapter 1) deals with the traditional and modern analytical methods (Chapters 2–4). Chapter 2 is entitled "Assessment of analytical methods and data". This chapter provides an overview about requirements and choise of analytical methods, presentation of data, quality of data, statistical assessment of quality of data. Chapter 3 deals with "Principles of techniques used in food analysis". Due to the importance of this chapter it discusses all aspects of classical methods (such as titrimetric analysis, gravimetric procedures, solvent extraction methods, refractometry, polarimetry), the instrumental methods (spectroscopic methods, chromatography, electrophoresis) and the modern methods such as immunochemical assays. The latter topic is the most important of this chapter. Immunochemical methods may be used for detecting specific ingredients and contaminants in foods by ELISA tests. This topic deals with the problem how this test may be user for the detection of raw meat species such as beef, pork, horse, sheep, rabbit, poultry and furthermore, for the differentiation e.g. between chicken and turkey, sheep and goat. This chapter reviews the detection of different milk and milk products, identification of food proteins, specific proteins such as gluten or other proteins such as casein, whey proteins, soya and papain, detection of aflatoxin, veterinary and pesticide residues etc. Chapter 4 deals with the "Theory of analytical methods for specific food constituents". Topics discuss the different methods to determine carbohydrates, micronutrients (including mineral elements, ash and vitamins), to measure food energy (with bomb calorimetry, or by calculation of the constituents of the calorific value) and to determine the range of additives used in foods) such as salt, sulphur dioxide, benzoic acid, sorbic acid, nitrates, antioxidants: BHA, BHT, TBHQ). The second main part (Chapters 5 and 6) the "Experimental procedures" and "General food studies" present the methods described in the first "Theoretical part" used on the different foods according to standards (ISO, AOAC, BS, FAO/WHO). This part involves the use of described methods containing important practical informations for experts dealing with examination of foods. The last chapter (7) contains the additional reading material, relevant to the primary literature of the last years. This book is highly recommended to food controllers, to researchers in food science and to technologists, to high school and university students in relevant field as well as to all who are engaged in analytical chemistry.

M. SZENTGYÖRGYI

PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda, Budapest

Goods Analysis and Product Strategy in Marketing

by P. Tomcsányi

A blank spot of marketing management is revealed by the author of this book and highlighted by offering a methodology for the calculation of product utility. To have a better understanding of supporting decisions on product strategy in marketing P. Tomcsányi suggests a tool in goods analysis. The knowledge of product competitiveness calculated on the basis of utility contributes to strategic planning for product development. This methodology can be well applied in any section of industry, in agriculture, in trading and in profit and nonprofit services. Decision analytical tools can help to have a more realistic modeling of value judgment in the market.

About the author: corresponding member of the Hungarian Academy of Sciences, director for Gardening and Forestry of the National Institute for Agricultural Quality Control, professor of the University of Gardening and Food Industry, chairman of the Marketing Committee of the Hungarian Academy of Sciences, member of the Editorial Board of the Journal of International Food and Agribusiness Marketing (USA)

Readership: professionals in product development (in plant breeding, quality management, etc.) concerned with decision analysis for strategic marketing decisions and technical development (including postgraduate students).

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Concentrations of thiamin and riboflavin an some vegetable food
GARCÍA, F. P., DÍAZ, C. & HARDISSON, A.

Interpretation of the thermoluminescence phenomenon in milk protein concentrate powder
KISS, L. I. & KISPÉTER, J.

The influence of packaging and storage time on the colour of dehydrated raspberry
GVOZDENOVIC, J. & CURAKOVIC, M.

Effect of UV irradiation on lipid peroxidation in edible fats
LUGASI, A., HÓVÁRI, J., DWORSCHÁK, E., NESZLÉNYI, K., LEBOVICS, V. & ZSINKA, Á. J. N.

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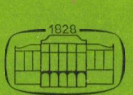

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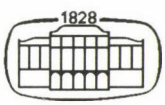

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CONCENTRATIONS OF THIAMIN AND RIBOFLAVIN IN SOME VEGETABLE FOOD

F. P. GARCÍA, C. DÍAZ and A. HARDISSON

Department of Analytical Chemistry, Food Science and Toxicology University of La Laguna, 38074-La Laguna. Spain

(Received: 31 May 1994; revision received: 25 May 1995; accepted: 8 June 1995)

Free and total fractions of thiamin and riboflavin were fluorimetrically determined in several vegetable foods: peas, cabbage, lettuce, cauliflower, and garlic. Concentrations of both fractions of riboflavin were higher than the corresponding concentrations of thiamin for all vegetable foods studied. Total riboflavin concentrations were higher than the free riboflavin fraction in the studied vegetable foods. No significant differences ($P > 0.05$), however, were found for peas, lettuce, and cabbage between both fractions of thiamin. Total thiamin and riboflavin concentrations were higher and lower, respectively, than others reported in the literature. Both fractions of each thiamin and riboflavin were correlated and the results indicate that there may be connection between them.

Keywords: thiamin, riboflavin, fluorimetric determinations, vegetable foods

Thiamin and riboflavin are water-soluble vitamins whose low intake can produce deficiency diseases in human beings with the corresponding risk for human health (COULTATE, 1984; BELITZ & GROSCH, 1985). Concentrations of vitamins in vegetable foods can vary considerably depending on several factors such as: differences in vegetable and soil types, cultivar, fertility, maturity and harvest, and post-harvest handling methods and conditions could contribute to that variability (HAGEN et al., 1979).

Determination of vitamins in foods can mainly be carried out for establishing food composition tables or for legal purposes concerning nutritional labelling. Although there are many available methods for the determination of thiamin and riboflavin in foods, most authors have preferred to use two: fluorimetric (ARQUÉS et al., 1988; A.O.A.C., 1980; BATISTA & BAETA, 1979; RYAN & INGLE, 1980; SOLIMAN, 1981; GARCÍA et al., 1993) and HPLC (AUGUSTIN, 1984; FELLMAN et al., 1982; FERNANDO & MURPHY, 1990; HAN et al., 1991; WATADA & TRAN, 1987; WILAMASIRI & WILLS, 1985). The main problems with the analysis of vitamins are their low stability with respect to several physicochemical factors and the presence of vitamers. The studied water-soluble vitamins are very sensitive to physicochemical factors such as: pH, temperature or light (GARCÍA et al., 1994). On the other hand,

vitamin activity may be due to a group of different chemical compounds, the vitamers. These compounds have a different vitamin activity and behavior with respect to the analytical signals (BRUBACHER et al., 1986).

In the present paper, the fluorimetric determination of thiamin and riboflavin with and without enzyme addition have been carried out with several vegetable foods in order to provide information about the concentration levels of these micronutrients in these types of food. Moreover, the different vitamin concentrations have been correlated to discover the relationship amongst them.

1. Materials and methods

1.1 Apparatus

- Fluorescence spectrophotometer Perkin-Elmer model MFP-44A with automatic recorder, arc of Xenon Osram XBO, differential corrected spectra unit DCSU-1, and heating water bath Selecta Frigitherm S 382.
- pH meter Digital radiometer PHM 84.
- Balance Sartorius-Werke GMBH.
- Centrifuge Dr. N. Gerber.
- Chromatography columns, approx. 30 cm × 10 mm i.d.

1.2 Reagents and solutions

- Thiamin hydrochloride standard solution (100 $\mu g\ cm^{-3}$). Accurately weighed 50 mg of thiamin chloride previously dried over P_2O_5 in dessicator and filled up to 500 cm^3 with acidified ethanol (pH = 3.5-4.5).
- Riboflavin standard solution (50 $\mu g\ cm^{-3}$). Fifty mg accurately weighed and dried over P_2O_5 in dessicator, then dissolved in 0.05 N acetic acid and filled up to pH 11.

Both standard solutions were stored in darkness, under toluene at 4 °C.

- Enzyme solution of alpha-Amylase (*Aspergillus oryzae*, activity 4.8 U mg^{-1}). It was prepared daily in 50% aqueous solution.
- Florisil (60-100 mesh).
- Other additional reagents required for the experimental procedures were of analytical quality.

1.3 Experimental procedures

In a recent paper (GARCÍA et al., 1993) methods for determination of both vitamins in vegetable foods have been optimized, based on methods described by A.O.A.C. (1980) with some modifications.

– Sample treatment: This is a common step for determination of both vitamins. Add a volume of 0.1 N of HCl equal to ~10 times dry weight of sample previously homogenized. Then digest at 100 °C for 30 min. After thawing they were vacuum filtered through Whatman n° 42 paper. If the filtrate was cloudy they were centrifuged for 20–30 min. at 2,000 r.p.m. The clean filtrate is filled up to 250 cm^3 with latter acid and pH adjusted to 4–4.5. This volume is divided into two equal parts for determination of free and combined fractions. The combined fraction is obtained after enzymatic treatment (α-amylase) and digestion (45 °C h^{-1}).

– Determination of thiamin: 3 cm^3 of the obtained solutions or the standard are vigorously mixed with an oxidant mixture formed by 1 cm^3 of methanol, 1 cm^3 of sodium hydroxide (30%) and 0.8 cm^3 potassium ferricyanide (1%). The thiocrome obtained is shaken and extracted with 13 cm^3 of isobutanol. The fluorescence signal in isobutanolic extract is measured at λ_{ex} = 374 nm and λ_{em} = 430 nm.

In order to eliminate interferences a blank analysis with every sample is carried out. This is obtained by means of equal treatment but without addition of oxidant agent (ferricyanide). Also, volumes of ferricyanide between 0.5–1.5 cm^3 were added to each analyzed sample to know the maximum fluorescence signal.

– Determination of riboflavin: Hundred cm^3 of the extract obtained in sample treatment is passed through a chromatographic column (10 mm i.d. × 35 cm) and then the riboflavin is eluted with pyridin 20% in acetic acid of 0.02 mol. Finally, the fluorescence of the first 50 cm^3 of eluate is measured at λ_{ex} = 470 nm and λ_{em} = 525 nm.

1.4 Samples

Sixty samples of five different fresh vegetables: 13 *Pisum sativum* L. (Peas), 14 *Brassica oleracea* L. *var. capitata* (Cabbage), 13 *Lactuca sativa* L. (Lettuce), 10 *Brassica oleracea* L. (Cauliflower), and *Allium porrum* L. (Garlic) have been analyzed. Samples were purchased in the main supermarkets in Santa Cruz and La Laguna (Tenerife) during 1990 and were frozen (–20 °C) and stored until analysis.

Statistical analysis of the data were carried out with SPSS programs found in the software of the Digital Computer VAX–VMS 11/780 V4–2 at the University of La Laguna.

2. Results and discussion

Table 1 shows the results (mg per 100 g) of the analysis of both vitamins differentiating two analyzed fractions: Total fraction (with added enzyme) and free fraction (without added enzyme). In all analyzed vegetables total riboflavin concentrations were sensitively higher (1.5–3 fold) than those of the free fraction

which indicates that most of this vitamin is combined. This is in accordance with other authors (MCBRIDGE & WYATT, 1983) who suggest that FAD and FMN are bound forms of riboflavin and occur more commonly in foods than the free form but have lower fluorescence. The ratios between total and free riboflavin were different in the five studied vegetables, arranged in the following sequence:

Cabbage (3.0) > Cauliflower (2.9) > Garlic (2.4) > Peas (2.1) > Lettuce (1.6)

Table 1

Main statistical parameters of free and total thiamin and riboflavin content (mg per 100 g) *in the studied vegetable foods*

Type of vegetable (n[a])		Thiamin		Riboflavin	
		Free	Total	Free	Total
Peas (13)	X ± SD[b]	0.109 ± 0.017	0.116 ± 0.019	0.114 ± 0.010	0.242 ± 0.027
	(M – m)[c]	(0.154 – 0.091)	(0.136 – 0.092)	(0.131 – 0.103)	(0.294 – 0210)
	C.V.[d]	15.6	16.4	8.4	11.0
Cabbage (14)	X ± SD	0.012 ± 0.005	0.013 ± 0.007	0.069 ± 0.015	0.207 ± 0.048
	(M – m)	(0.019 – 0.004)	(0.026 – 0.004)	(0.111 – 0.058)	(0.322 ± 0.141)
	C.V.	40.8	48.9	21.5	23.2
Lettuce (13)	X ± SD	0.014 ± 0.004	0.016 ± 0.004	0.084 ± 0.021	0.130 ± 0.020
	(M – m)	(0.020 – 0.005)	(0.024 – 0.012)	(0.135 – 0.040)	(0.159 – 0.107)
	C.V.	30.7	24.8	24.9	15.6
Cauliflower (10)	X ± SD	0.018 ± 0.007	0.028 ± 0.006	0.087 ± 0.016	0.206 ± 0.020
	(M – m)	(0.026 – 0.010)	(0.038 – 0.020)	(0.116 – 0.060)	(0.246 – 0.176)
	C.V.	37.6	21.4	18.4	9.8
Garlic (10)	X ± SD	0.021 ± 0.009	0.032 ± 0.013	0.099 ± 0.026	0.238 ± 0.044
	(M – m)	(0.032 – 0.010)	(0.047 – 0.016)	(0.133 – 0.065)	(0.302 – 0.186)
	C.V.	40.9	39.9	26.7	18.4
Overall (60)	X ± SD	0.037 ± 0.043	0.039 ± 0.037	0.089 ± 0.023	0.204 ± 0.051
	(M – m)	(0.154 – 0.004)	(0.136 – 0.004)	(0.135 – 0.040)	(0.322 – 0.107)
	C.V.	116.2	94.9	25.8	25.0

[a] n: number of samples
[b] X ± SD: Mean ± standard deviation
[c] M – m: Maximum – minimum
[d] C.V.: Coefficient of variation

Cabbages have lower ($P < 0.05$) free riboflavin concentration than lettuces and cauliflowers which, in turn, have lower concentrations ($P < 0.05$) than peas and garlic. One can conclude that the vegetable foods analyzed have different composition of vitamers of riboflavin.

Concentrations of two fractions of thiamin were lower than the corresponding fractions of riboflavin in all vegetable foods studied. However, the relative variation of the fractions of thiamin was always higher than in case of riboflavin.

Cauliflowers and garlic presented significantly higher ($P < 0.05$) levels of total thiamin than free thiamin, the ratios between both fractions being 1.56 and 1.52, respectively. However, for peas, lettuces and cabbages, no significant differences ($P > 0.05$) were found between the thiamin fractions (total and free). It suggests two things: the thiamin may exist predominantly in free form, or acidic (HCl) treatment (digestion), is enough to hydrolyze the combined fractions in those vegetable foods. Pea samples have shown mean values of the two thiamin fractions significantly ($P < 0.001$) higher than the other vegetables. Also, cauliflower and garlic samples show total thiamin levels higher ($P < 0.05$) than lettuces and cabbages.

Although fluorimetric method for the determination of the two vitamin fractions have been described (BATISTA & BAETA, 1979); no data are available in the literature for the free fraction of both vitamins. There is a large variation in the concentrations of these vitamins in the different vegetable foods (FOX et al., 1982; WILLS et al., 1984; WILLS et al., 1986). Thus, our results have been compared with the results reported in the papers that include data on the species of vegetable analyzed here. Also, a large variation can be observed depending on the region or country considered. Climatic conditions, type of soils, collection and storage, or added fertilizers among other factors can influence the vitamin concentrations of the vegetables. The levels of the total thiamin and riboflavin reported for: lettuce, cabbage and cauliflower from Australia (WILLS et al., 1984; WILLS et al., 1986), peas from Valencia (Spain) (ARQUÉS et al., 1988) or Moscow (Russia) (AUGUSTIN et al., 1983), and lettuce from Madrid (Spain) (CARBALLIDO & NAVARRO, 1983) were significantly higher for thiamin and lower for riboflavin than ours. WILLS and co-workers (1985) have compared the determination of both vitamins by fluorimetric and HPLC methods, finding no differences between them. They found mean values for thiamin in peas, cabbage and cauliflower approximately twice higher than ours. However, the sequence of the concentrations was equal in both cases: peas > cauliflower > cabbage. The concentrations of riboflavin in the studied vegetable were lower than the values reported in this paper. Also, in other paper, the same authors (SOLIMAN, 1981) have found concentrations of thiamin similar and riboflavin slightly lower than ours in cabbages from China.

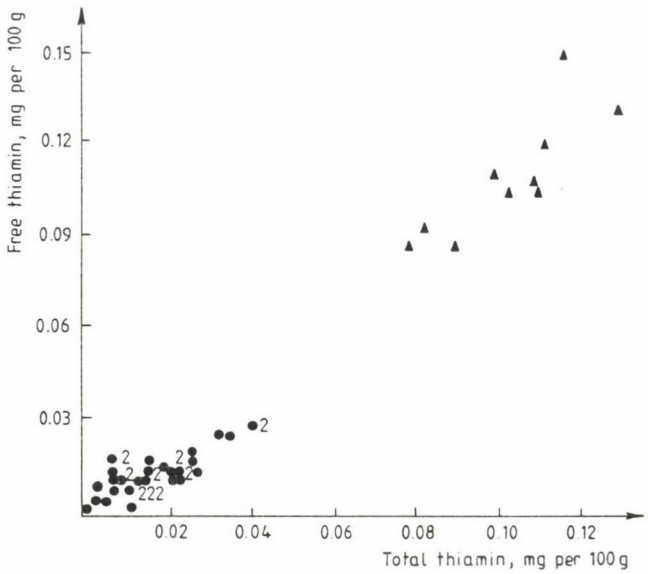

Fig. 1. Correlation between the two analyzed fractions of thiamin. ▲ = Samples of peas; ● = Rest of vegetables; 2 = When 2 samples are represented in the same point. Free thiamin (mg per 100 g) = = 1.09 Total riboflavin − 0.007 ($r = 0.979$; $p < 0.0001$)

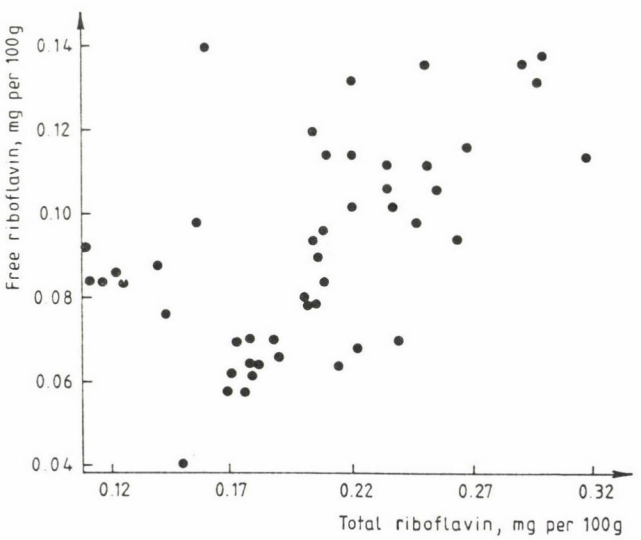

Fig. 2. Correlation between the two analyzed fractions of riboflavin. Free riboflavin (mg per 100 g) = = 0.24 Total riboflavin + 0.039 ($r = 0.523$; $p < 0.0001$)

Direct and logarithmic variables of the studied parameters have been correlated within and among the different vegetables. The correlation study within each vegetable was not significant because of the small number of data considered. In Fig. 1 the direct correlation between total and free thiamin fraction is shown, observing a positive slope near the unity and a very low intercept which confirms that almost all the thiamin found is in free fraction. Also, one can observe that the samples of peas have significantly higher contents of both fractions than the other vegetables. When the two analyzed fractions of riboflavin are directly correlated (Fig. 2), a correlation coefficient lower than in the latter correlation is detected. The slope was also positive, and much lower than in the aforementionated correlation, which indicates the important contribution of the bound fraction (vitamin phosphate esters) of riboflavin in the total fraction.

*

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References

ARQUÉS, M. J., FONT, G. & FARRÉ, R. (1988): Determinación de tiamina, riboflavina y B-caroteno en alimentos vegetales frescos y congelados. *Anal. Bromatol., 50*, 181 – 186.

A.O.A.C. (1980): *Official methods of Analysis*, 13th. Association of Official Analytical Chemists. Washington, DC, pp. 740 – 743.

AUGUSTIN, J. (1984): Simultaneous determination of thiamine and riboflavin in foods by liquid chromatography. *J. Assoc. Off. anal. Chem., 67*, 1012 – 1015.

AUGUSTIN, J., COLE, C. L., FELLMAN, J. K., MATTHEWS, R. H., TASSINARI, P. D. & WOO, H. (1983): Nutrient content of sprouted wheat and selected legumes. *Cereal Foods World, 28*, 358 – 361.

BATISTA, M. T. P. M. & BAETA, M. L. M. A. (1979): Dosagem da tiamina na levadura de cerveja. *Bol. Fac. Farm. Coimbra, 3*, 141 – 143.

BELITZ, H. D. & GROSCH, W. (1985): *Química de los alimentos.* Acribia, Zaragoza.

BRUBACHER, G., MÜLLER-MULOT, W. & SOUTHGATE, D. A. T. (1986): *Methods for determination of vitamins in food.* Elsevier Applied Science Publishers, London, pp. 3 – 20.

CARBALLIDO, A. & NAVARRO, M. A. (1983): Micronutrientes en lechuga, escarola y endibia. – Part I. Contenido vitamínico. *Anal. Bromatol., 35*, 123 – 134.

COULTATE, T. P. (1984): *Alimentos: Química de sus componentes.* Acribia, Zaragoza, pp. 146 – 177.

FELLMAN, J. K., ARTZ, W. E., TASSINARI, P. D., COLE, C. L. & AUGUSTIN, J. (1982): Simultaneous determination of thiamin and riboflavin in selected foods by high-performance liquid chromatography. *J. Fd Sci., 47*, 2048 – 2050.

FERNANDO, S. M. & MURPHY, P. A. (1990): HPLC determination of thiamin and riboflavin in soybeans and tofu. *J. agric. Fd Chem., 38*, 163 – 167.

FOX, M., LONCIN, M. & WEISS, M. (1982): Investigations into the influence of water activity, pH and heat treatment on the breakdown on thiamine in foods. *J. Fd Qual., 5*, 161 – 182.

GARCÍA, F. P., DÍAZ, C. & HARDISSON, A. (1993): Spectrofluorimetric determination of thiamin and riboflavin in vegetable foods. *Anal. Lett., 26*, 1977 – 1996.

GARCÍA, F. P., DÍAZ, C. & HARDISSON, A. (1994): Estabilidad de tiamina y riboflavina frente a algunos factores fisicoquímicos. *Afinidad*, *451*, 237–240.

HAGEN, R. E., ELKINS, E. R. & FARROW, R. P. (1979): Nutrient variations in canned fruits and vegetables. *HortScience*, *14*, 251–256.

HAN, Y., SONG, J., ZHAO, J. & DAI, Y. (1991): Simultaneous determination of vitamin B complex (thiamine, riboflavin, niacin, pyridoxine and folic acid) in foods by HPLC. *Chin. Chem. Lett.*, *2*, 645–648.

MCBRIDGE, D. E. & WYATT, C. J. (1983): Evaluation of a modified A.O.A.C. determination for thiamin and riboflavin in foods. *J. Fd Sci.*, *48*, 748–750.

RYAN, M. A. & INGLE, J. D. (1980): Fluorometric reaction rate method for the determination of thiamine. *Anal. Chem.*, *52*, 2177–2184.

SOLIMAN, A. G. M. (1981): Comparison of manual and benzenesulfonyl chloride-semiautomated thiochrome methods for determination of thiamine in foods. *J. Assoc. anal. Chem.*, *64*, 616–622.

WATADA, A. E. & TRAN, T. T. (1987): Vitamins C, B_1, and B_2 contents of stored fruits and vegetables as determined by high performance liquid chromatography. *J. Amer. Soc. Hort. Sci.*, *112*, 794–797.

WILAMASIRI, P. & WILLS, R. H. (1985): Simultaneous analysis of thiamin and riboflavin in foods by high-performance liquid chromatography. *J. Chromatogr.*, *318*, 412–416.

WILLS, R. B. H., LIM, J. S. K. & GREENFIELD, H. (1984): Composition of Australian foods. 23. Brassica vegetables. *Fd Technol. Australia*, *36*, 176–177.

WILLS, R. B. J., LIM, J. S. K. & GREENFIELD, J. (1986): Composition of Australian foods. 32. Leafy, stem and other vegetables. *Fd Technol. Australia*, *38*, 416–421.

WILLS, R. B. H., WIMALASIRI, P. & GREENFIELD, H. (1985): Comparative determination of thiamin and riboflavin in foods by high-performance chromatography and fluorimetric methods. *J. Micronutr. Anal.*, *1*, 23–29.

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CHOCOLATE PRE-CRYSTALLIZATION: A REVIEW

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The theoretical basis of cocoa butter pre-crystallization process during industrial production of chocolate is presented. Different physico-chemical transformation occurring during this process are reviewed. The polymorphic transformations of cocoa butter triglyceride, affecting the pre-crystallization, are emphasized. The paper presents a possibility for better technological control of the process as well as the advantages and disadvantages of some temperature-time regime of tempering which are being applied for the pre-crystallization of chocolate mass in the industrial scale production.

Keywords: chocolate, cocoa butter, polymorphism, pre-crystallization

Chocolate is made up of 28 to 42% cocoa butter, particles of saccharose (or some other sugar) and cocoa solids. Besides, chocolate contains 0.3 to 0.5% lecithin a natural emulsifier, and vaniline. The milk chocolate contains also all the components of milk solids (milk fat, proteins etc.) (FINCKE, 1965).

The melted chocolate mass is a complex rheological system (coarse suspension) the characteristics of which are influenced by the following factors:

- Temperature
- Amount of cocoa butter or other added fats
- Particle size distribution of cocoa solids
- Emulsifier content
- Moisture content.

During chocolate processing, by proper performation of technological processes the mentioned parameters should be brought into harmony, with the aim of obtaining a system, which will have a good defined physico-crystallographic and rheological behaviour after the solidification of chocolate mass. According to KLEINERT (1976) the crystallization of cocoa butter starts during pre-crystallization of the chocolate mass. The proper performing of this phase is essential to obtain a product of optimum quality characteristics. The quality characteristics of the

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chocolate may be reviewed from the standpoints of both producers and consumers (ANON, 1990).

From the standpoint of the producers the chocolate mass i.e. chocolate is good if:

- the viscous characteristics of the mass are optimal enabling normal handling during processing, transport, moulding or enrobing,
- the solidification time is short,
- the contraction is satisfactory enabling easy products demoulding.

From the consumers standpoint the chocolate should have the following characteristics:

- attractive visual look, gloss,
- appropriate hardness at room temperature,
- good snap,
- stability to temperature oscillations without change of characteristics (hardness, gloss),
- narrow melting range,
- good taste,
- good storage stability (fat bloom stability).

According to the findings of GAVRILOVIĆ and co-workers (1976–1977), as well as TIKVICKI (1991) a special attention has to be paid to the particle size distribution of cocoa solids, sugar and other solids present to achieve the demanded quality. The adequate particle size distribution can be obtained applying appropriate mills. On the other hand, the regulation of polymorphic transformations of cocoa butter in the following processing stages is a more complex problem. Namely, these transformations during pre-crystallization, and later during solidification of chocolate are of great significance for the quality of chocolate (SZÁNTÓ & MOHOS, 1973).

A series of details relevant to the cocoa butter crystallization are still unknown, but, on the bases of known facts, a lot of phenomena during pre-crystallization, chocolate solidification, as well as during storage of chocolate products can be explained. The topic of this work is the polymorphic tranformations occurring during chocolate pre-crystallization. These transformations are the bases for the explanation of the essence of some new technological procedures for improving this phase of chocolate processing. The aim of these innovations is to shorten the pre-crystallization process and to enable the regular flow of cocoa butter polymorphic transformations.

Polymorphism of cocoa butter

Polymorphism is defined as the ability of a substance to exist in more than one crystalline form under thermodynamically different conditions (GARTI & SATO, 1988).

The idea of polymorphic crystalline forms of cocoa butter, as well as of other fats, is not referring to the outside microscopic and macroscopic geometrical appearance of the fat crystals (HABITUS) but to the internal structure of the crystals on molecular level i.e. the packing of triglycerides in the molecular crystal lattice which can be defined in the arranged structure by X-ray diffractions spectrophotometry (CHAPMAN, 1965; SZÁNTÓ & MOHOS, 1973). The definition of certain polymorphic forms by this method will be explained later.

Two types of polymorphism exist: enanthiotropy and monotropy. The enanthiotropic polymorphism is characterized by a greater number of stable crystal forms in the given temperature range i.e. the transformations of crystal forms are reversible.

In monotropic polymorphism only one stable crystal form exists while the transformation of other crystal forms to the only stable form is irreversible.

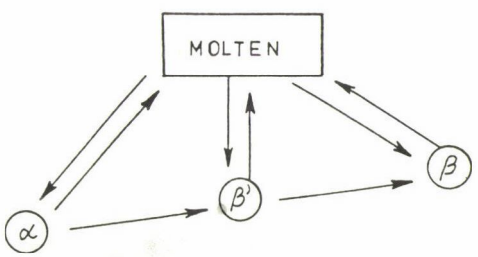

Fig. 1. Polymorphic transformations in fats

In vegetable oils and fats three polymorphic forms predominate (α, β', β) the formation of which depends on crystallization conditions i.e. the super cooling temperature (Fig. 1). The melted-liquid fat can directly transform into any polymorphic form but the transformation rate is different. Every polymorphic form may transform into a liquid state, i.e. can be melted. The formed polymorphic form may transform into another form only following the pathway $\alpha \rightarrow \beta' \rightarrow \beta$ i.e. the process is irreversible and this means that the polymorphism in fats is monotropic. As the β form is the most stable (the melting point is also the highest), fats stored for a longer period of time consist of only this crystalline form.

The polymorphic behaviour of cocoa butter compared to other vegetable oils and fats is quite a specific one. Namely, according to WILLE and LUTTON (1966) cocoa butter can exist in six polymorphic forms. The polymorphic forms are marked

Table 1

Nomenclature and melting points of polymorphic forms of cocoa butter (DIMICK & DAVIS, 1986)

Vaeck (1951)	Vaeck (1960)	Duck (1964)	Wille & Lutton (1966)	Chapman et al. (1971)	Lovegren et al. (1976)	Davis & Dimick (1986)
γ 18.0	γ 17	γ 18.0	I 17.3	I	VI 13.0	I 13.1
α 23.5	α 21–24	α 23.5	II 23.3	II	V 20.0	II 17.7
			III 25.5	III	IV 23.0	III 22.4
β"28.0	β 28	β"28.0	IV 27.3	IV 25.6	III 25.0	IV 26.4
β 34.5	β 34–35	β' 33.0	V 33.8	V 30.8	II 30.0	V 30.7
		β 34.4	VI 36.3	VI 32.2	I 35.5	VI 33.8

with Roman letters, but Greek letters is used as well. DIMICK and DAVIS (1986) had given a more detailed review of nomenclatures used for polymorphic forms of cocoa butter (Table 1).

The melting points of the polymorphic forms are also the indication of stability of certain crystalline form. As the nomenclature according to WILLE and LUTTON (1966) is more and more accepted, it will be utilized in this presentation.

Table 2

Triglyceride composition of cocoa butter (%)

Triglyceride	Composition (%)	Standard abbreviations for fatty acids	
POst	16.5 – 41.2	Ad – Arachidic	C20:0
StOSt	22.6 – 28.8	O – Oleic	C18:1
POP	12.0 – 18.4	St – Stearic	C18:0
StOO	2.7 – 8.9	L – Linoleic	C18:2
StLP	2.4 – 6.0	P – Palmitic	C16:0
PLSt	2.4 – 4.3		
POO	1.7 – 6.7		
StOAd	0.9 – 2.9		
PLP	1.1 – 2.7		
StLSt	0.9 – 2.7		
OOAd	0.8 – 1.8		
PPSt	0.4 – 1.2		
PStSt	0.2 – 1.5		
POL	0.2 – 1.1		
OOO	0.2 – 1.0		
StStSt	to 0.5		

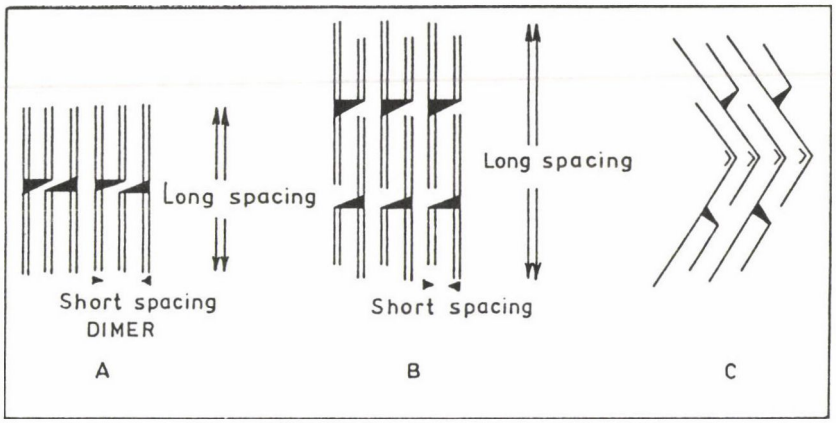

Fig. 2. Formation of dimer molecular triglyceride association A and B – trisaturated triglycerides – SSS type C – symmetrical monounsaturated triglycerides – SUS type

The complex polymorphism of cocoa butter is the result of its triglyceride composition (Table 2). 2-oleopalmitostearate, 2-oleodistearate and 2-oleodipalmitate are the most prevalent triglycerides in the cocoa butter and make up approximately 80 percent of the total. All these triglycerides contain an unsaturated fatty acid – the oleic acid, in number two position of the glycerol molecule, and due to that, the formation of an arranged structure is inhibited, as presented in the following review.

This work presents a simplified scheme of formation of the arranged structure of certain polymorphic triglyceride forms.

The results of previous investigations have shown that two associate triglyceride molecules – dimers – form the building blocks in the elementary cells of crystalline structure of fats. The triglycerides are believed to exist in tuning fork configuration in such a molecular association. The molecules in the association are antiparallel, as presented in Fig. 2 according to MANING and DIMICK (1985), CHALSERI and DIMICK (1987), CRAVEN (1988) and DIMICK (1991).

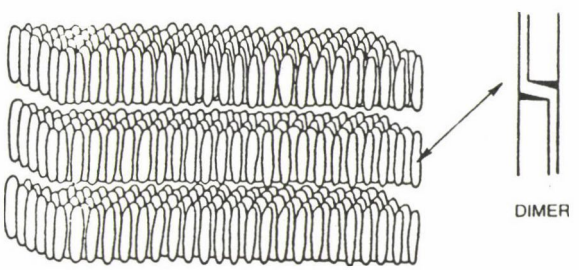

Fig. 3. Formation of arranged lamelar structure during triglyceride crystallization

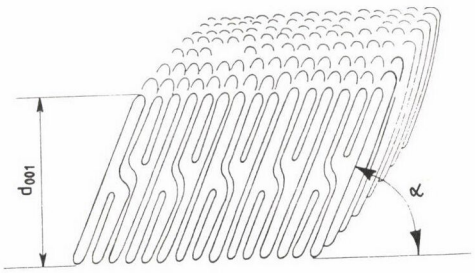

Fig. 4. Tilted layer of triglycerides in the crystalline structure

X-ray diffraction patterns can be employed to define the molecular arrangement of the dimers. X-ray analysis show that the spacings can be the short and the long ones (Fig. 2). According to CHAPMAN (1985) the short spacings are associated with the manner of packing of the hydrocarbon chains, whilst the long spacing is related to integral multiples of the chain length. With paraffinic hydrocarbons the long chains pack so as to obtain the highest possible van der Waals interaction and the chains pack parallel to each other. The molecular end groups associate with each other to form planes. As the result of polymorphic transformations, due to the change of arrangement of molecular associates in the lamelar arrangement, the long spacings change significantly (in less stable forms of the triglyceride dimers – presented as cigars – are vertically arranged) (Fig. 3).

CHALSERI and DIMICK (1987) have shown by the X-ray structure analysis data that the stable polymorphic forms of triglyceride molecular associations are tilted about 30° i.e. the angle between the horizontal layer and triglyceride chains is about 60° (Fig. 4).

Also these investigations have proved that this arrangement of triglyceride is energetically more convenient (the inter-molecular repulsive forces are smaller).

During crystallization the triglyceride molecules are approaching the arranged crystal structure. The formation of these structures is analogous to putting a puzzle together or to experiments with models performed by an architect. However, a principle in the nature during formation of these structures is to establish the maximum packing of the molecules. During the formation of arranged structure, the building blocks (triglyceride molecules) tend to occupy the energetically most favorable space arrangement and, in the same time, the smallest volume in the crystal. They tend to produce a more dense packing at the given temperature, so the intermolecular spacings are the smallest. This trend is the consequence of aggregate forces between the molecules (van der Waals forces). Sometimes these transformations are very rapid, sometimes they are slow, depending on the triglyceride composition i.e. on the ability of certain fatty acids in the triglycerides to arrange the structure of crystal lattice.

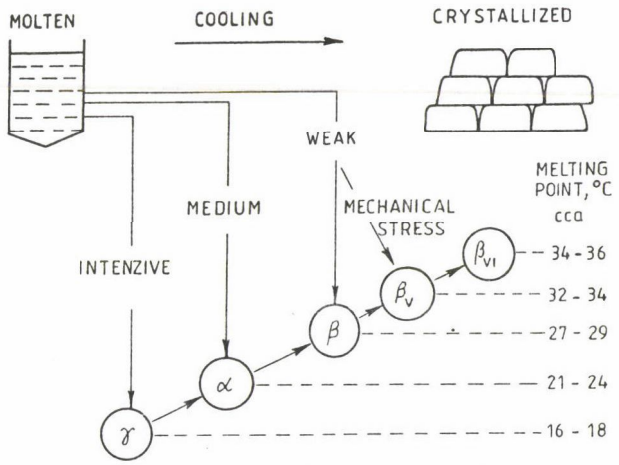

Fig. 5. Influence of cooling rate and shear force on the formation of polymorphic forms in cocoa butter

It is important to mention that the triglyceride crystallization in vegetable fats is very complex. The vegetable oils and fats consist of a large number of different triglycerides but during crystallization under certain conditions they form a unique crystal lattice. This means that triglycerides having different composition and melting point exist in the same crystal lattice. Such mixed crystals exhibit a number of anomalies which can be investigated through phase diagrams (GARTI & SATO, 1988). In case the crystallization mechanism of cocoa butter triglycerides on molecular level is known, it is possible to approach the aim that is to plan the physical and functional characteristics of the chocolate. In CRAVEN's opinion (1988) the formation of polymorphic forms of cocoa butter is influenced both by cooling rate and shear forces in the chocolate mass (Fig. 5).

Pre-crystallization of chocolate mass

The former discussion on crystallization and polymorphism of cocoa butter and experience gained through practical work clearly indicate that the optimum characteristics of the chocolate can be formed only when cocoa butter is in V-form. This can be achieved by proper performing of pre-crystallization and chocolate solidification, provided all the conditions necessary for the proper phase transformation of liquid chocolate mass to crystallographically solid chocolate are defined. This can be fulfilled through complex physico-chemical molecular transformations of cocoa butter triglycerides through several stages like nucleation, the growth of nuclei and production of three-dimensional crystal lattice. Due to the three-dimensional order, the system is firm and, on the other hand, all non-fat components are encapsulated. Several factors may influence the characteristics of the

crystalline lattice. The amount of crystallized triglycerides at the end of the process i.e. the size of individual crystals in the three-dimensional lattice is of special importance. These factors determine the density of the contact points in the structure finally determining the texture of the product. On the bases of the presented facts, chocolate is a solid the appearance of which is influenced by the structure.

During pre-crystallization (tempering) in the process of chocolate production, cocoa butter should crystallize in Form V i.e. a certain amount of these crystals has to be formed. In industrial conditions this can be achieved in two ways:

– by tempering the chocolate mass, which is achieved by a complex temperature-time process of super cooling and heating of the mass.

– by intensive mixing, which enables the formation of crystal nuclei and influences the growth and agglomeration of crystals applying the seeding technique i.e. introduction of a certain amount of powdered cocoa butter and chocolate or some triglyceride-based additive to the molten chocolate mass.

Due to these processes, the fractional crystallization of cocoa butter during pre-crystallization can be led in the desired direction.

One of the basic problems connected with the projecting of pre-crystallizers is the optimization of heat transfer during pre-crystallization. This means that the optimum ratio of heat exchange surface and the chocolate mass flow has to be chosen. The continuous removing of crystallized mass from the cooling surface is necessary for the effective heat transfer.

KLEINERT (1976) presented two different equipments (the vertical batch and horizontal continuous device, Fig. 6) as the example for the influence of constructive characteristics on the flow of pre-crystallization.

In the batch vertical device (Fig. 6, A) the heat transfer is performed through water-tempered walls, while the centrally installed mixer with scraping elements is used for the removal of the crystallized chocolate mass. In case of such construction the ratio of heat exchange surface and crystallization heat is unfavorable. As the cooling surface is not scrapped clean, the heat transfer is reduced, and these conditions favour the growth of crystals of undesirable size.

The continuous horizontal pre-crystallizer (Fig. 6, B) is a cylindrical device with intensive mixing, while the cooling is performed on the inside surface of the double cylinder. The coaxial mixer with specially designed scraper blades enables the continuous cleaning of the cooling surface. Usually two linked crystallizers are used for the pre-crystallization. The heat transfer in these devices is uniform. The special construction of the scrapes which moving in the narrow annular space causes turbulence in the chocolate mass. This results in an effective mixing of the mass, and it prevents the undesirable growth of the formed crystals. Namely, during mixing, the formed crystals are disintegrated, and the small crystal fragments are uniformly distributed through the whole mass.

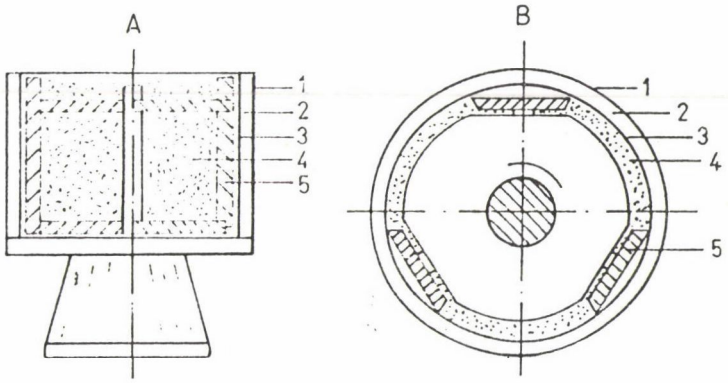

Fig. 6. Schematic view of the construction of batch (A) and horizontal continuous (B) device for chocolate mass pre-crystallization. 1: Outer skim; 2: cooling medium; 3: inner wall; 4: melted chocolate; 5: mixing device

The optimum pre-crystallization level of chocolate mass can be achieved in the described device, enabling obtaining of product having fine crystalline homogeneous structure.

Present-day different devices (batch, continuous and semi-continuous) are used in chocolate production for the chocolate mass tempering. The capacity of the devices may vary from 100 to 6000 kg h^{-1}. The length of pre-crystallization time depends on the capacity of the device and the quality of cocoa butter, i.e. the chocolate formulation. For instance, the total processing time for a 3000 kg tank is 5 to 7 h, while the actual formation of the seeds takes only 10 to 30 min (Buhler GmbH, Switzerland). Based on our discussion it is apparent that the efficiency of a pre-crystallizer depends on the given temperature-time regime of cooling/heating and mixing intensity i.e. construction of the device. The pre-crystallization level defines the concentration of the seeds in the pre-crystallized chocolate mass, and later also the number of crystals in V-polymorphic form in end product.

A few points are characteristic for the applied temperature-time regime of chocolate mass cooling/heating as presented in Fig. 7, using the Linetronik Thermofat computerized analyzer (Linetronik SA, Switzerland).

The first standpoint for the correct pre-crystallization is that the chocolate mass must be completely melted as to free it from crystal seeds. This can be achieved by heating the mass to 50 °C. After this, the mass is cooled to 32–33 °C (cooling stage I) to reach the saturation point of cocoa butter triglycerides with higher melting point. No formation of crystal seeds is to be expected in this phase. In the second cooling phase (cooling stage II), the mass is cooled to 27–29 °C. In this step, the mass is supercooled giving rise to a thermodynamic inbalance whose free energy enhances the formation of stable V and unstable II, III and IV polymorphic forms.

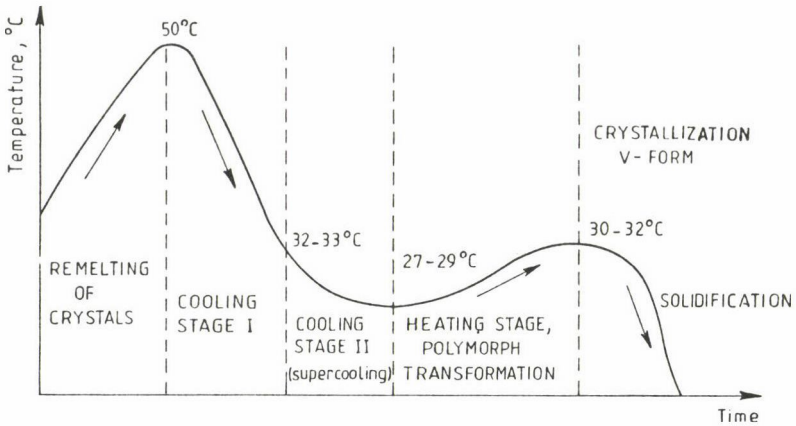

Fig. 7. Temperature-time regime of heating and cooling during chocolate mass pre-crystallization

The formation of nuclei is the result of two physico-chemical processes which take place during the formation of arranged structure i.e. nuclei. This is presented in Fig. 8.

The formation of new crystallization surfaces and volume takes place under different change of free energy. The energetic profile of nuclei formation as the three-dimensional structure is the resultant of these two processes. The positive free energy of a growing particle has a maximum value at a particle radius r*. This maximum means that the formation of arranged structure requires energy. According to AHRENS (1980) this energy is the activation energy for seed formation. Particles whose radius is smaller than r* have the tendency to be destroyed and dissolved again. Particles with large radius grow further and have the tendency to move towards the equilibrium of the solid phase under simultaneous reduction of the free energy. Crystallization nuclei whose radius is larger than r* are stable aggregates and consist of 500 to 1000 triglyceride molecules (BERGER, 1976). The speed with which the crystal nuclei are formed is defined as the number of formed nuclei in a definite period. The second cooling stage is ended when a sufficient number of crystal seeds per volume unit has been formed in the chocolate mass. As a significant heat is liberated due to crystal growth, further cooling is of no use. On the contrary, the mass is heated to 30–32 °C to enable the polymorphic transformation (transition of nonstable to V-form) of cocoa butter triglycerides during the phase transformations (crystallization).

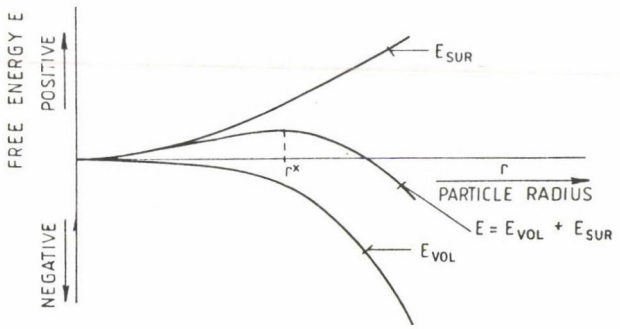

Fig. 8. Change of free energy of triglyceride molecules during seed formation.
E_{SUR}: $E_{surface}$; E_{VOL}: E_{volume}; r: particle radius

The pre-crystallization of chocolate mass in industrial scale takes place under the following temperature-time tempering regime:

- three-stage conventional process,
- four-stage cyclo-thermic process (STM-Temper),
- Kreuter interval tempering process.

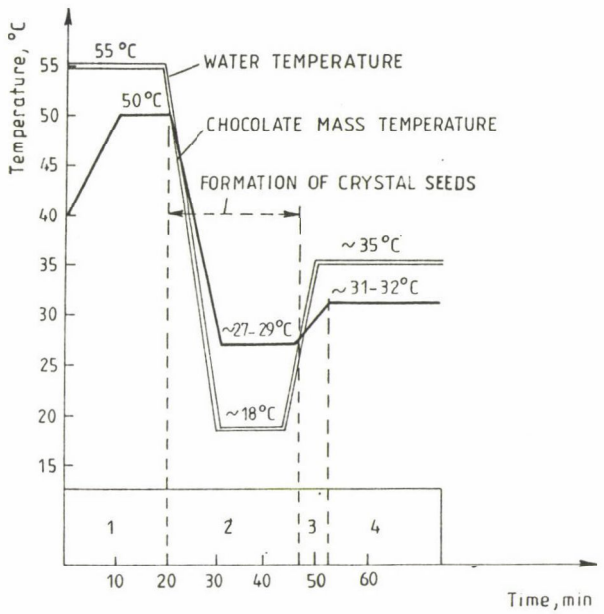

Fig. 9. Temperature-time programme of heating and cooling during three-stage conventional tempering of chocolate mass. 1: Remelting stage; 2: cooling stage (super-cooling); 3: heating stage; 4: processing

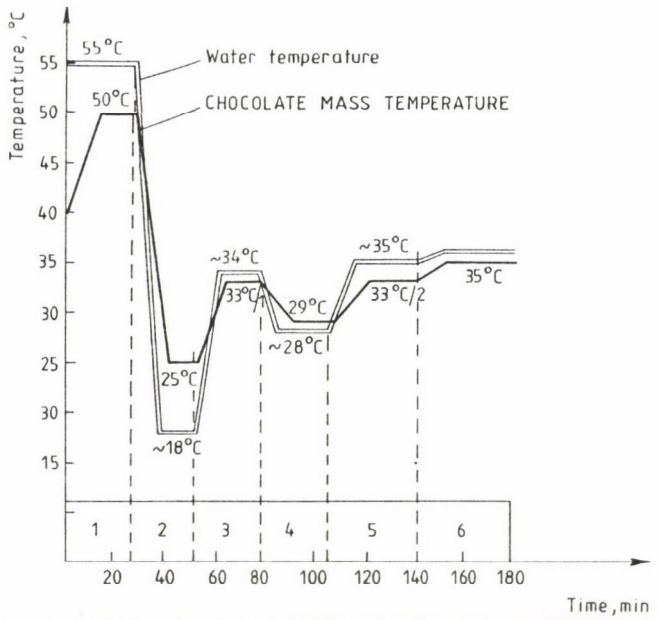

Fig. 10. Cyclo-thermic tempering process of chocolate mass. 1: Melting stage; 2: cooling stage I; 3: heating stage I; 4: cooling stage II; 5: heating stage II; 6: processing

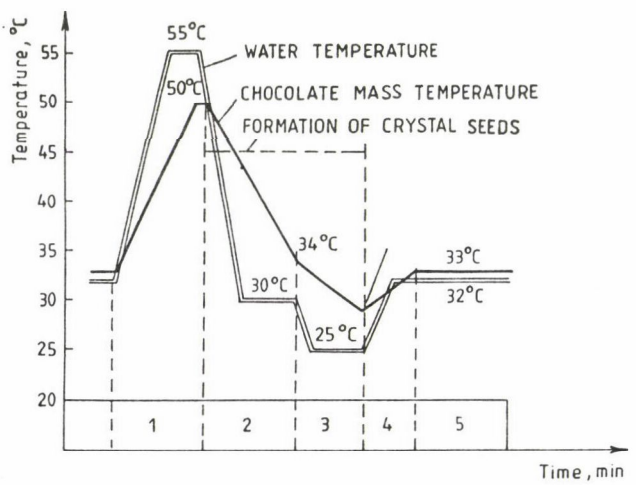

Fig. 11. The KREUTER interval tempering process of chocolate mass. 1: Melting stage; 2: cooling stage I; 3: cooling stage II (super-cooling); 4: heating stage; 5: processing

During the three-stage conventional process (Fig. 9) the chocolate mass is heated to 50 °C to free it from the crystal seeds. Under constant mechanical treatment the mass is super cooled to 27 – 29 °C and, at finally to 31 – 32 °C. At this temperature the further formation of crystal seeds is stopped, and parallel to crystallization, their polymorphic transition to V-polymorphic form takes place. In the same time, the final desired viscosity necessary for the moulding, enrobing or double moulding is attained (KLEINERT, 1979).

KLEINERT (1978) described the four-stage process (Cyclo-thermic process). It operated in the following way (Fig. 10): the melted mass (50 °C) is cooled to 25 °C, to reach the constant viscosity, heated to 33 °C, carefully cooled to 29 °C, again heated to 33 °C, and tempered to constant viscosity. At the end, the chocolate mass is heated to production temperature 35 – 36 °C. The mass is constantly being stirred during tempering.

The products obtained by cyclo-thermic tempering are of better quality compared to the ones obtained by the conventional three-stage procedure. The X-ray diffraction patterns showed that the crystal structure obtained during both mentioned process is in V-polymorphic form, but the concentration and the "Habitus" of the formed crystals are significantly different. Though the results of cyclo-thermic tempering are good, due to complexity of the process, it has not been accepted in larger scale.

The Kreuter interval tempering process (SAWITZKI, 1987) is presented in Fig. 11. The time needed for removing the crystal seeds is shorter, and lasts only for a few minutes.

The cooling (stages I and II) is performed somewhat differently than in conventional and cyclo-thermic processes. DSC (differential scanning calorimetry) is used to determine the moment when the heating should be introduced. This technical-technological innovation enabled the promotion of pre-crystallization process. Namely, the dominated standpoint is that the "key of pre-crystallization" is the exact determination of the moment when the heating of the mass should start to stop the crystal seed formation, and to promote the growth of the crystals i.e. the polymorphic transformations of cocoa butter triglycerides.

With the aim of advancement of cocoa butter pre-crystallization process, the possibility of use of continuous cylinder crystallizers is investigated. These crystallizers are used for the pre-crystallization of cocoa butter and margarine quite successfully. According to WINDHAB and co-workers (1992), the temperature difference between the cooling water and chocolate mass can be higher in these devices. Namely, in all the mentioned devices the temperature difference of cooling water and chocolate mass never exceeds 5 °C. However, during this process the chocolate mass is under intensive mixing, in contact for a few seconds with the cooling surface having the temperature of 4 – 16 °C. This means that the temperature difference between the cooling surface and the mass (24 – 26 °C) is more than 10 °C.

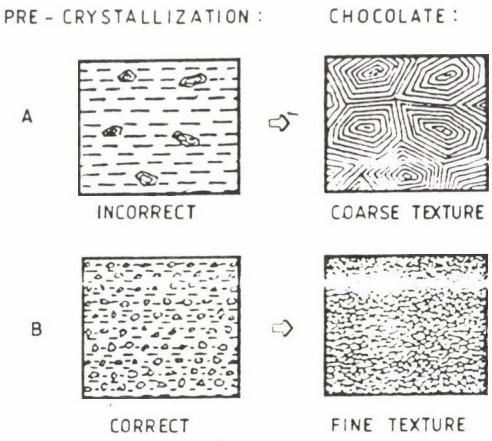

Fig. 12. Texture of chocolate samples obtained by adequate (B) and inadequate (A) pre-crystallization

The investigations proved that even such a great super-cooling results in sufficient concentration of crystals (more than 50%) in V-polymorphic form. The contact time is very short – only a few seconds. The finished product, after proper (correct) solidification, is of good characteristics while stored at 18 °C.

The temperature-time regime of cooling/heating is significantly changing if additives – crystallization accelerators are introduced. Powdered cocoa butter and chocolate are being used for a long time for the improvement of chocolate mass crystallization. It has been proved that high melting triglyceride based additives which are being used lately, are more effective than the powdered cocoa butter (HACHIYA et al., 1989 a, b; KOYANO et al., 1990).

The texture of chocolate samples obtained by adequate and inadequate pre-crystallization process is shown in Fig. 12.

It is obvious that a low crystal concentration is formed during inadequate pre-crystallization, which after further growing and formation of crystal lattice results in a coarse structure. When the optimum crystal concentration is achieved in the pre-crystallization stage (about 4%), the three-dimensional lattice with a fine texture of chocolate is formed (DUCK, 1960; KLEINERT, 1976).

References

AHRENS, A. (1980): Purpose and process technology of automatically-controlled tempering. *Review for Chocolate Confectionery and Bakery*, *5* (4), 3–8.

ANON (1990): *New technic for cacao butter replacers productions*. Company brochure, Karlshamns Oils and Fats AB, Sweden.

BERGER, K. G. (1976): Some theoretical and practical aspects of fractionation. *American Oil Chemists' Society Meeting*, Chicago, pp. 10–18.

CHALSERI, S. & DIMICK, S. P. (1987): Cocoa butter – its composition and properties. *Mfg. Confect.*, *67* (9), 115–121.

CHAPMAN, D. (1965): *The structure of lipids*. Methuen and Co. Ltd, London, pp. 221–310.

CRAVEN, M. B. (1988): Glycerides (Chapter 10) – in: HANAHAN, D. Series Ed. *The physical chemistry of lipids, Handbook of lipid research*. Plenum Press, New York, pp. 345–394.

DIMICK, S. P. & DAVIS, R. T. (1986): Solidification of cocoa butter. *Mfg. Confect.*, *66* (6), 123–128.

DIMICK, S. P. (1991): Principles of cocoa butter crystallization. *Mfg. Confect.*, *71* (5), 109–114.

DUCK, W. (1960): Eine viskosimetrische Laboratoriumsmethode zur Ermittlung des temperierter Kuvertüre vorhandenen Anteils an festem Fett. *Fette Seifen Anstr-Mittel*, *62*, 705–709.

FINCKE, H. (1965): *Handbuch der Kakaoerzeugnisse*. Springer Verlag, Berlin, pp. 329–351.

GARTI, N. & SATO, K. (1988): Crystallization and polymorphic transformation: An Introduction. – in: GARTI, N. & SATO K. *Crystallization and polymorphism of fats and fatty acids*, Marcel Decker Inc., New York, pp. 3–7.

GAVRILOVIĆ, M., JOVANOVIĆ, O. & SAVIĆ, M. (1976–1977): O mogučnosti primene metode mokrog sejanja za odredjivanje finoce cokolade. (On the possibility for the application of wet sieving method for the determination of chocolate fineness.) *Zbornik radova Tehnološkog fakulteta*, (7/8), 47–58.

HACHIYA, I., KOYANO, T. & SAGO, K. (1989a): Seeding effects on solidification behaviour of cocoa butter and dark chocolate. I. Kinetics of solidification. *J. Am. Oil Chem. Soc.*, *66*, 1757–1762.

HACHIYA, I., KOYANO, T. & SAGO, K. (1989b): Seeding effects on solidification behaviour of cocoa butter and dark chocolate. II. Physical properties of dark chocolate. *J. Am. Oil Chem. Soc.*, *66*, 1763–1770.

KLEINERT, J. (1976): Tempering and organoleptics. *Chocolate, Confectionery and Bakery*, *1* (2), 3–7.

KLEINERT, J. (1978): Tempering of melted chocolate masses – Part II. *Rev. Chocolate, Confectionery and Bakery*, *5* (1), 19–24.

KLEINERT, J. (1979): Vorkristallisation von Schokoladeschmelzmassen I. *Zuck.- u. SüsswarWirt.*, *32*, (7/8), 245–248.

KOYANO, T., HACHIYA, I. & SATO K. (1990): Fat polymorphism and crystal seeding effect on fat bloom stability of dark chocolate. *Fd Struct.*, (9), 231–240.

MANING, M. D. & DIMICK, S. P. (1985): Crystal morphology of cocoa butter. *Fd Microstruct.*, *4* (2), 249–265.

SAWITZKI, P. (1987): Interwall – Vorkristallisation. *Zuck.-u. SüsswarWirt.*, *40* (4), 153–154.

SZÁNTÓ, S. & MOHOS, F. (1973): A kakaó és csokoládégyártás X. (Cocoa and chocolate production.), *Édesipar*, *24* (3), 69–77.

TIKVICKI, R. (1991): *Ispitivanje kvaliteta čokoladne mase.* (Milk chocolate mass quality.) B. S. Thesis, University of Novi Sad, Faculty of Technology, Novi Sad.

WILLE, L. R. & LUTTON, S. E. (1966): Polymorphism of cocoa butter. *J. Am. Oil Chem. Soc.*, *43*, 491–496.

WINDHAB, E., NEEDIER, A. E. & ROLFES, L. (1992): Neue Aspekte der Temperier-technik. *"Schoko-Technik '92", International ZDS-Fachtagung*, sic-12, Solingen, Germany.

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ULTRAFILTRATION OF RECONSTITUTED SKIM MILK IN HOLLOW FIBERS AND PLATE-FRAME MODULES

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Ultrafiltration was carried out using hollow fiber Fresenius F 60 module and Sartorius plate-frame laboratory ultrafiltration unit. Experiments were performed at constant concentration and in batch UF mode. Skim milk powder containing 33.4% total protein and 0.31% NPN was reconstituted in distilled water. The effect of transmembrane pressure, temperature, feed flow rate and reconstituted skim milk concentration on flux was investigated.

With the increase of transmembrane pressure, flux of reconstituted skim milk powder by ultrafiltration steadily increased by all investigated temperatures (20, 40 55 °C) and all inlet flow rates. The difference in this effect among samples of various concentrations (8, 10 and 12% TS) was more expressed by lower temperatures, what could be ascribed to the more intense effect of higher RSM viscosity. The dependence of flux from temperature result in straight line, by all inlet flow rates and skim milk concentrations. By constant transmembrane pressure better flux was obtained by higher pH values of reconstituted skim milk (RSM). The experimental data are correlated satisfactorily by the Leveque's equation $Sh = (Re\ Sc\ d/L)^{0.33}$.

Keywords: hollow fiber module, plate-frame module, reconstituted skim milk, ultrafiltration

During the two past decades, ultrafiltration has been used to replace many conventional separation techniques such as centrifugation, evaporation, distillation, etc. In food industry one of the most important application of ultrafiltration is the concentration of milk for the manufacture of dairy products. Industrial significance of ultrafiltration in dairying has stimulated many studies on this subject (CHIANG & CHERYAN, 1986; GLOVER, 1971; PATEL & REUTER, 1986; SETTI & PERRI, 1976). Most of them treat ultrafiltration of native milk, while studies on reconstituted milk and/or skim milk, as pointed out by ABD EL SALAM & SHAHEIN (1989), are lacking. However, there is a necessity nowadays to use reconstituted milk in regular production in many countries. Therefore, the aim of this work was to study the effect

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of operating parameters such as transmembrane pressure, flow rate, temperature, pH and feed concentration of reconstituted skim milk on flux in hollow fiber and plate-frame modules.

1. Materials and methods

Skim milk powder containing 33.4% total protein and 0.31% nonprotein nitrogen (NPN), was obtained from Odžačanka dairy (Odžaci, Yugoslavia). The skim milk was reconstituted in distilled water.

The experimental apparatus as shown in Fig. 1, was very simple with Hemoflow F60 (Fresenius), polysulphone hollow fiber (fiber i.d. 0.2 mm, length 230 mm) and Sartorius laboratory plate-frame ultrafilter (channel height 0.5 mm, length and width 130 mm) with APV, Pasilac GR 61 PP membranes. Ultrafiltration tests were carried out at constant concentration, by recirculating both permeate an concentrate to the feed reservoir and in batch UF mode. Pressure gauges and a regulation valve were used for measuring and controlling inlet and outlet pressure. The transmembrane pressure was varied from 35 to 75 kPa in hollow fiber module, and from 70 to 170 kPa in plate-frame module. The feed flow rates in hollow fiber (HF) module varied from 75 to 126 $l\,h^{-1}$ and in plate-frame (PF) module from 13.5 to 19.4 $l\,h^{-1}$.

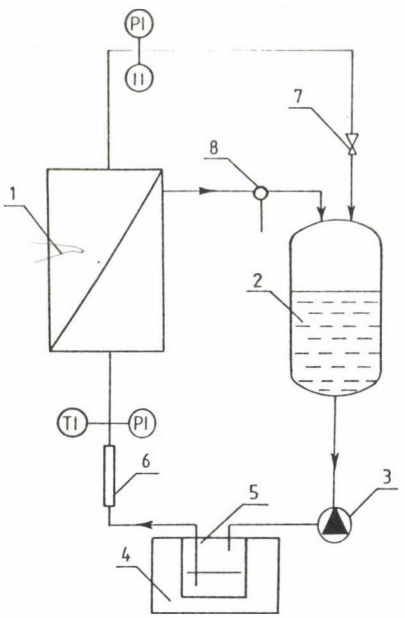

Fig. 1. The experimental apparatus. 1: UF module; 2: feed tank; 3: metering pump; 4: thermostat; 5: compensation vessel; 6: flowmeter; 7: regulation valve; 8: T-cock

Also the effect of pH value on permeate flux in hollow fiber module was investigated. The experiments were carried out at 293, 313 and 328 K, which were maintained using thermostat. The feed concentration was of 8, 10 and 12% total solids (TS).

The permeate flux in all experiments were determined with graduated cylinder and chronometer. The presented data are the average values of three measurements. The reproducibility of these measurements were good, the deviation between parallel experiments were in ±5%.

Chemical composition of skim milk powder, reconstituted skim milk, permeate and retentate were investigated by the following methods:

- total solids by drying at 102±2 °C (PEJIC & DORDEVIC, 1973);
- minerals by igniting at 550 °C (PEJIC & DORDEVIC, 1973);
- lactose by the I.D.F. method (I.D.F., 1974);
- total proteins by Kjeldahl method (PEJIC & DORDEVIC, 1973);
- nonprotein nitrogen (NPN) by Rowland method (ROWLAND, 1938);
- Ca and Mg content according to NTALIANAS and WHITNEY (1964).

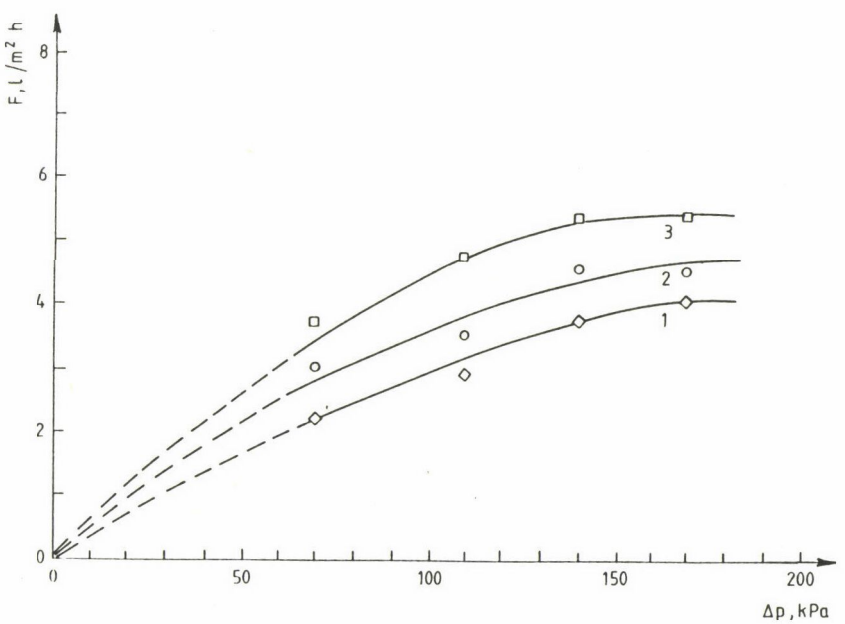

Fig. 2. Permeate flux dependence on transmembrane pressure in plate-frame module at 17.5 l h^{-1} feed flow rate with concentration of 12% TS at different temperatures. 1: 20 °C; 2: 40 °C; 3: 55 °C

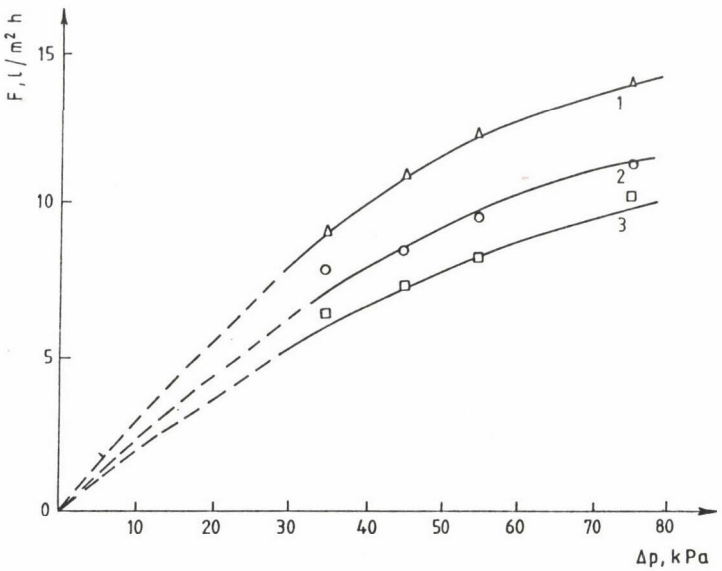

Fig. 3. Influence of feed concentration on permeate flux in hollow fiber module at constant feed flow rate (102 l h^{-1}) and temperature of 40 °C. 1: 8%; 2: 10%; 3: 12%

The above-mentioned experimental methods are accepted in dairy industry and in publications connected to dairy. The measurements were carried out in two parallels, and the standard deviation did not exceed ±5%.

2. Results and discussion

The performance of the hollow fiber module with RSM are shown in Figs. 2–4. The figures show the effect of flow rate and applied pressure at the particular feed concentration–temperature combination on the permeate flux. As evident from Figs. 2–4, in all investigated cases the direct relationship between permeate flux and pressure was observed before the pressure independent region was reached.

Effect of temperature on permeate flux, shown in Fig. 5, is similar to data, obtained by CHIANG and CHERYAN (1986), for skim milk.

One of the most important factors influencing permeate flux is membrane fouling. The effect of pH value on membrane fouling is presented in Fig. 6. As evident from Fig. 6, fouling is actually completed at the begining of ultrafiltration, what is in agreement with data found in literature for skim milk (PATEL & REUTER, 1986).

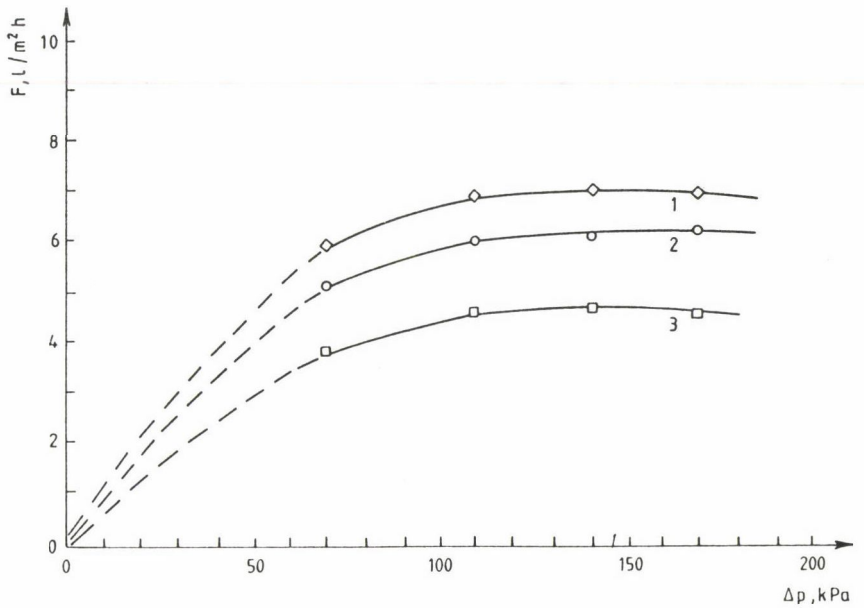

Fig. 4. Feed flow rate influence on permeate flux in plate-frame module at 40 °C and feed concentration of 8% TS. 1: 19.3 l h^{-1}; 2: 17.5 l h^{-1}; 3: 13.5 l h^{-1}

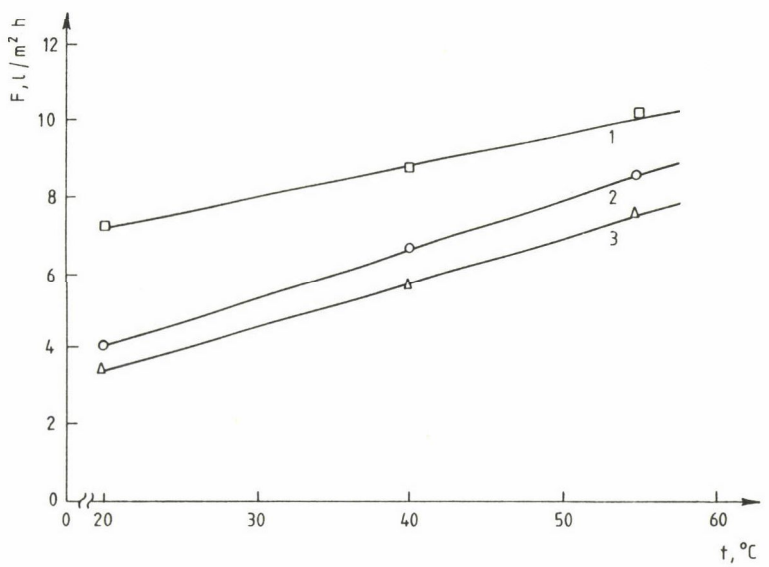

Fig. 5. Permeate flux dependence on temperature in hollow fiber module at constant transmembrane pressure of 35 kPa and feed flow rate of 75 l h^{-1}. 1: 8%; 2: 10%; 3: 12%

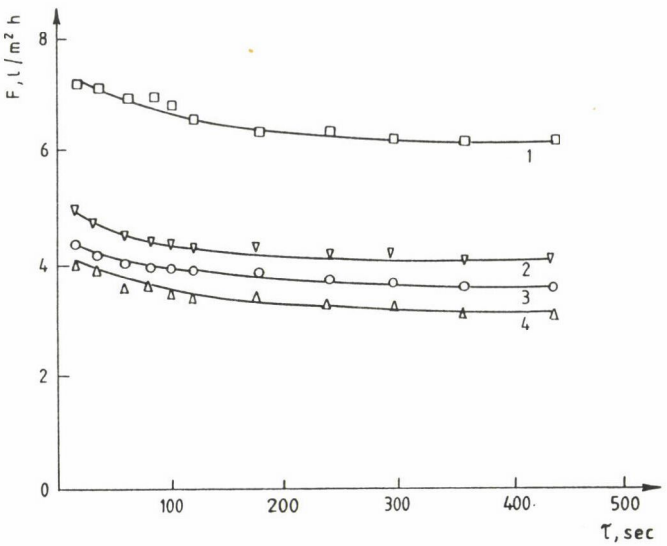

Fig. 6. Effect of pH of milk on membrane fouling in hollow fiber module at constant transmembrane pressure of 35 kPa and feed flow rate of 75 l h^{-1}. 1: 6.6; 2: 6.0; 3: 5.6; 4: 5.2

The mass transfer coefficient (k) (and Sherwood number subsequently) was determined on the basis of the experimental data by using relationship between the flux through membrane (J) and solute concentration according to the equation:

$$J = k \ln (C_B/C_G) \quad (1)$$

where C_B and C_G are bulk and gel concentrations, respectively. In the mass transfer coefficient calculations the experimentally obtained values of limiting fluxes were used, and for the gel concentration literature data, which are around 20%, were used (CHERYAN, 1986). The diffusion coefficient in Sherwood number was calculated for casein using Wilke-Chang equation (TREYBAL, 1976).

The presented experimental data for both modules, as it can be seen from Figs. 7 and 8 may be satisfactorily correlated with Leveque's equation (LEVEQUE, 1928):

$$Sh = 1.62 (Re\ Sc\ d_e/L)^{0.33} \quad (2)$$

which is valid for convective mass transfer in laminar flow channels.

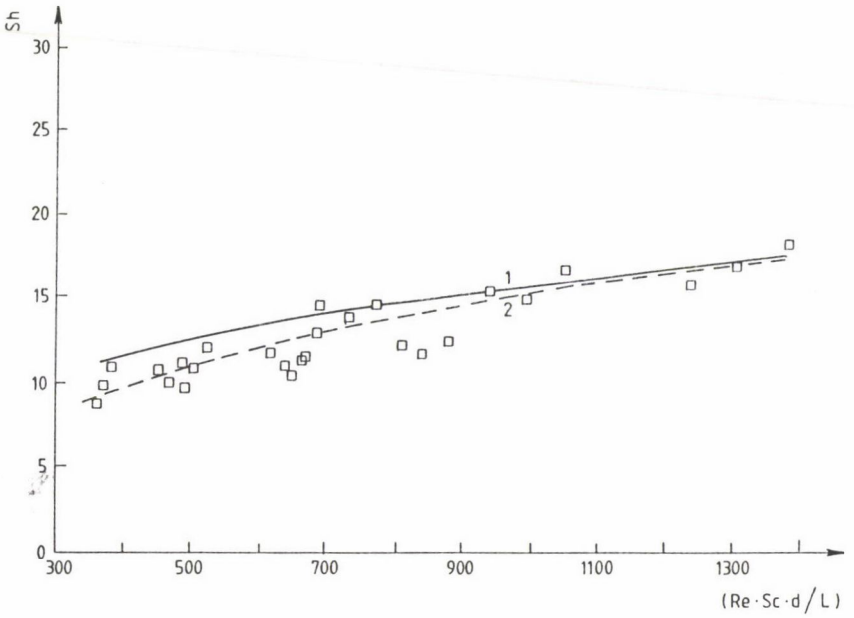

Fig. 7. Comparison of Leveque's equation with experimental data in hollow fiber module. 1: Leveque equ.; 2: regression; □: experimental data

Experimental points are ±15% around the theoretical prediction with mean deviation of 0.26. Better agreement between experimental and calculated data has been reached by using regression separately for hollow fiber and plate and frame data. The obtained correlations are:

- hollow fiber:

$$Sh = 0.55\ (Re\ Sc\ d_e/L)^{0.48} \tag{3}$$

- plate-frame:

$$Sh = 0.46\ (Re\ Sc\ d_e/L)^{0.50} \tag{4}$$

Besides of better agreement of Eqs. 3 and 4 (shown also in Figs. 7 and 8) the disadvantage of those correlations obtained by regression is the validity for the certain domain of measurements given in experimental of this paper.

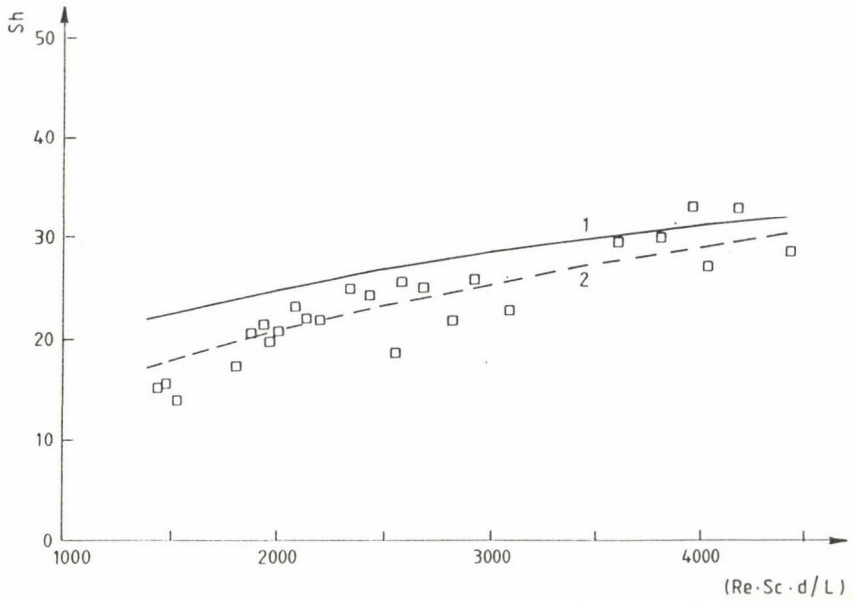

Fig. 8. Comparison of Leveque's equation with experimental data in plate-frame module. 1: Leveque equ.; 2: regression; □: experimental data

The results of chemical composition of retentate and permeate, by varying concentration rate (Fc = 1.5; 2 and 2.5) for experiments in hollow fiber module are shown in Table 1. As was expected, from the known behaviour of natural milk retentate, with the increased concentration rate, total protein, as well as calcium increased, nonprotein nitrogen and lactose decreased, while mineral matters were retained approximately at the same level. The results of RSM concentrating at various temperatures in plate-frame module are shown in Table 2. It is evident from Table 2 that protein concentration is slightly increased with increasing the UF temperature.

3. Conclusions

The experimental results, as it was expected, show similar behavior of reconstitute skim milk compared to native milk. Flux of RSM powder has constantly increased by all investigated temperatures and VF inlet flow rates also increased with the increasing transmembrane pressure. At constant transmembrane pressure higher pH values resulted in better flux. The experimental data for both modules are in satisfactory agreement with the Leveque's equation for convective mass transfer in laminar flow channels.

Table 1

Composition of reconstituted skim milk retentate and permeate as a function of concentration rate
UF temperature: 55 °C (hollow fiber module)

Component	Reconstituted milk C = 10%	F_c = 1.5		F_c = 2.0		F_c = 2.5	
		Retentate	Permeate	Retentate	Permeate	Retentate	Permeate
TS (%)	9.55	11.64	4.43	13.14	5.72	15.60	5.96
Total proteins (%)	3.34	6.01	1.32	7.20	0.77	9.32	0.20
(%/TS)	34.97	51.63	29.82	54.80	13.55	59.70	3.37
Nonprotein N (%)	0.031	0.081	0.014	0.064	0.022	0.052	0.018
(%/TS)	0.33	0.69	0.33	0.49	0.38	0.33	0.31
Minerals (%)	0.79	1.09	0.07	1.21	0.35	1.37	0.45
(%/TS)	8.32	9.30	1.64	9.20	6.12	8.78	7.55
Lactose (%)	4.64	3.89	3.58	4.24	3.68	4.75	3.77
(%/TS)	48.58	33.42	80.09	32.27	64.33	30.45	63.25
Ca/g l^{-1}	1.44	1.77	0.28	2.03	0.24	2.74	0.088
(%/TS)	1.51	1.52	0.63	1.55	0.42	1.76	0.15
Mg/g l^{-1}	0.042	0.073	0.06	0.20	0.087	0.22	0.085
(%/TS)	0.044	0.063	0.14	0.15	0.15	0.14	0.14

Table 2

Composition of reconstituted skim milk retentate and permeate as a function of UF temperature
Concentration rate: 1.5 (plate-frame module)

Component	Reconstituted milk C = 10%	Retentate		
		20 °C	40 °C	55 °C
TS (%)	9.55	10.44	11.04	11.58
Total proteins (%)	3.34	5.36	5.42	5.62
(%/TS)	34.97	51.34	49.09	48.53
Nonprotein N (%)	0.031	0.087	0.094	0.102
(%/TS)	0.33	0.83	0.85	0.88
Minerals (%)	0.79	1.13	1.17	1.08
(%/TS)	8.32	10.83	10.59	9.33
Lactose (%)	4.64	4.06	4.27	4.02
(%/TS)	48.58	38.89	38.67	34.72
Ca/g l^{-1}	1.44	1.54	1.27	3.21
(%/TS)	1.50	1.47	1.15	2.77
Mg/g l^{-1}	0.04	0.09	0.07	0.18
(%/TS)	0.04	0.08	0.07	0.15

References

ABD EL-SALAM, M. H. & SHAHEIN, N. (1989): Ultrafiltration of reconstituted skim milk. *J. Dairy Res.*, *56*, 147–149.

CHERYAN, M. (1986): *Ultrafiltration handbook*. Technomic, Lancaster, Basel, pp. 76–98.

CHIANG, B. H. & CHERYAN, M. (1986): Ultrafiltration of skim milk in hollow fibers. *Fd Sci.*, *51*, 340–344.

GLOVER, F. A. (1971): Concentration of milk by ultrafiltration and reverse osmosis. *Dairy Res.*, *38*, 373–379.

I.D.F. (1974): International Dairy Federation, Document No. 28.

LEVEQUE, M. A. (1928): Les lois de la transmission de chaleur par convection. *Ann. Mines*, *13*, 201.

NTALIANAS, F. A. & WHITNEY, R. L. (1964): Calcein as an indicator for the determination of total calcium and magnesium and calcium alone in the same aliquot of milk. *J. Dairy Sci.*, *47*, 19–27.

PATEL, R. S. & REUTER, H. (1986): Fouling of hollow fibre membrane during ultrafiltration of skim milk. *Milchwissenschaft*, *40*, 731–733.

PEJIC, O. & DORDEVIC, J. (1973): *Methods of analysis of milk and dairy products.* (English transl. of the title) University of Belgrade.

ROWLAND, J. C. (1938): The precipitation of the proteins in milk. *J. Dairy Res.*, *9*, 42–47.

SETTI, D. & PERI, C. (1976): Whey and skim milk ultrafiltration 2. Parameters affecting permeation rate in skim milk ultrafiltration. *Milchwissenschaft*, *31*, 466–468.

TREYBAL, R. E. (1976): *Mass transfer operations*. Mc Graw-Hill, New York, p. 47.

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INTERPRETATION OF THE THERMOLUMINESCENCE PHENOMENON IN MILK PROTEIN CONCENTRATE POWDER

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High-temperature thermoluminescence curves of γ-irradiated milk protein concentrate powder were analysed, and possibilities for the detection of the previous performance of γ-irradiation were examined. The semiconductor physical interpretation of the thermoluminescence phenomenon was taken as the basis of the analysis, since the sample material exhibited features characteristic of organic semiconductors. The activation energies of the processes responsible for the thermoluminescence response were determined by means of numerical curve fitting. The activation energies characterizing the electron traps in the sample were found to be 0.65 and 1.60 eV.

Keywords: thermoluminescence, γ-irradiation, activation energy, numerical curve fitting

For more than a decade, thermoluminescence (TL) has been successfully applied to detect the previous irradiation of a number of foodstuffs (spices, dried vegetables, etc.) (BÖGL & HEIDE, 1991). The energy required for the TL phenomenon is stored in metastable compounds or deficiencies (SWALLOW, 1991) where electrons and ions may be trapped. These trapped charge carriers can be released thermally. TL, characteristic of irradiation or the degree of irradiation, originates primarily during the recombination of released electrons. A plot of the intensity of the detected (visible) light versus temperature gives the TL curve, which is specific for the luminescent material, in the present case a food industrial product.

The TL method was first applied in solid-state physics for the study of different band structures. Exact mathematical descriptions for simple cases have been developed (CHEN & KIRSH, 1981), and the appropriate results could be utilized for analysis of TL curves of other materials.

Features of processes resulting in the TL phenomenon, or trap parameters (e.g. activation energy and frequency factor), can be calculated by the mathematical analysis of TL curves on the basis of special theoretical models. Such analyses are

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also of importance as concerns the interpretation of TL in different foodstuffs, but very few studies of this kind are to be found in the literature.

It has been established (BÖGL & HEIDE, 1991) that, at temperatures above room temperature, the TL curves of spices consist of different numbers of peaks, but the curves have not been subjected to intensive analysis. SANDERSON (1990) has investigated inorganic samples obtained from foodstuffs by a mineral separation procedure, and also samples of the dust adhering to the surface of the products. Such mineral samples can be used in TL studies for dosimetric purposes, but no information on the TL emitted by the products is obtained. (In dosimetric evaluation, the fact that irradiation has been applied can be demonstrated on the basis of the ratio of the areas under the TL curves for treated and untreated samples.)

The present work may remedy the above-mentioned deficiency by studying the TL curves of a model material, milk protein concentrate powder (MPCP), which has been thoroughly investigated before (KISPÉTER et al., 1993; 1994). The aims were to determine the kinetic order and activation energy, which are the chief characteristics of the TL processes in the material under investigation.

Attainment of these aims was made feasible by methods developed for the evaluation of TL results in solid-state physics (CHEN & KIRSH, 1981). Electrical conductivity measurements (KISPÉTER & HORVÁTH, 1987) indicate that food ingredient MPCP can be regarded as an organic semiconductor with amorphous structure. Accordingly, the TL results were evaluated in an appropriate (comparatively simple) band model.

1. Materials and methods

The MPCP chosen as the model material for the present work is used in human nutrition and animal feeding. The powder was produced by a patented method (BABELLA et al., 1987) at the Hungarian Dairy Research Institute (Mosonmagyaróvár, Hungary).

The composition of the MPCP used in the experiments was as follows (KISPÉTER et al., 1993): 75% protein (mainly in the form of caseins, globulins and α--lactalbumin), 10% lactose, 5.5% water, 2% fat and 7.5% ash.

Powder with a mass of 40 mg was pressed at 5 MPa into discs 8 mm in diameter and ca. 0.6 mm in height for TL measurements. Disc samples were irradiated with a ^{60}Co γ-radiation source (5.55×10^{14} Bq) in the dose interval of 2–20 kGy. TL readings were begun within an hour after radiation treatment. Data normalized to the mass of the sample were considered characteristic, and 5 parallel measurements were carried out for each analysis.

TL measurements were performed with an NHZ–203 thermoluminescence dosimeter reader (Central Research Institute for Physics, Budapest, Hungary), as

previously (KISPÉTER et al., 1993 and 1994; KISPÉTER & KISS, 1993). Measurements at high linear heating rates (3 – 12 K s^{-1}) in the dosimeter were possible up to 620 K. To reduce the oxidation of the organic samples, high-purity nitrogen gas was circulated through the sample chamber (at a rate of 2 l min^{-1}).

The measured TL curves were analysed by numerical fitting in order to determine the characteristic parameters of the TL phenomenon taking place in the sample. The intensity of TL for second-order kinetics can be given as follows (HALPERIN & BRANER, 1960; CHEN & KIRSH, 1981):

$$I \alpha \frac{\exp(E/kT)}{[1+(s'Nk/\beta E)T^2 \exp(-E/kT)]^2}$$

where the proportionality factor contains all constants. T is the temperature (in K), β is the applied heating rate (in K/s^{-1}), and k is the Boltzmann constant ($8.616 \cdot 10^{-5}$ eV/K^{-1}).

The parameters to be determined are the activation energy, E (in eV), the frequency factor, s' (in cm^3 s^{-1}), and the concentration of total electron traps, N (in cm^{-3}). In the curve fitting procedure, these parameters are obtained when the square sums of the differences between the function values and the measured data are minimum. (Appropriate initial values of the constants and parameters in the above equation are necessary for the mathematical procedure.) The calculations were performed with SigmaPlot 4.1 software.

2. Results and discussion

TL curves of MPCP obtained at different γ-doses and heating rates were analysed by curve fitting. One representative result of these measurement series is shown in Fig. 1, for a dose of 10 kGy and a heating rate of 6.79 K s^{-1}.

The behaviour of the TL peaks in the heating rate interval 3 – 12 K s^{-1} was investigated in previous experiments (KISPÉTER & KISS, 1993; KISPÉTER et al., 1994). It was found for MPCP that

- each curve contained two peaks,
- the TL intensity and the area under the curve (i.e. the integrated intensity) decreased with increasing β,
- the TL peaks separated from each other with increasing β, and
- the peak temperatures shifted towards higher values with increasing β.

The higher temperature peak can be regarded as characteristic of the irradiation, for it can be detected for a longer time (BÖGL & HEIDE, 1991). To

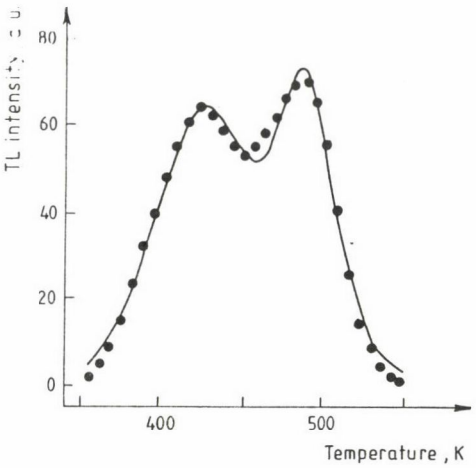

Fig. 1. Result of curve fitting for TL measurement above room temperature (β = 6.79 K s^{-1}, D = 10 kGy). Black circles indicate measured values; standard deviations: s = ±7–9%. The continuous line is the theoretical curve obtained by means of the fitting procedure, (a.u.: arbitrary units)

determine the activation energy relating to this peak, another method – the method of different heating rates – was used earlier (KISPÉTER et al., 1994), similarly with the assumption of second-order kinetics. The method revealed a slight dose-dependence of E in the interval 2–20 kGy, but it cannot be considered to be acceptable because of the overlapping of the TL peaks.

This problem can be solved by application of the method of curve fitting, when at least 30–40 measurement points are involved in the evaluation. However, it should be noted that the intensity of TL depends on the product of s' and N, hence only the activation energy can be given unambiguously by this mathematical procedure. After determining the value of N in another investigation, the value of s' is also obtainable by curve fitting, based on the above-mentioned equation.

The activation energy was determined by curve fitting for measurements made at different heating rates and γ-doses. The calculated energy values are listed in Tables 1 and 2.

Table 1

Activation energies (E) obtained by curve fitting on the basis of TL measurements above room temperature at D = 10 kGy. E_1 and E_2 refer to the lower and higher temperature peaks, respectively

Activation energy	β (K s^{-1})					
	3.85	4.86	5.66	6.79	8.39	11.20
E_1 (eV)	0.69	0.67	0.65	0.69	0.63	0.60
E_2 (eV)	1.57	1.60	1.60	1.61	1.64	1.63

Table 2

Activation energies (E) obtained by curve fitting on the basis of TL measurements above room temperature at $\beta = 6.79\ K\ s^{-1}$. E_1 *and* E_2 *refer to the lower and higher temperature peaks, respectively*

Activation energy	D (kGy)			
	2	5	10	20
E_1 (eV)	0.71	0.64	0.69	0.70
E_2 (eV)	1.57	1.64	1.61	1.59

The mathematical analysis demonstrated that for the investigated MPCP:

- the TL peaks above room temperature relate to second-order kinetics, and thus the measured TL curve can be resolved into two second-order curves;
- the activation energies can be considered to be independent of absorbed γ-radiation dose: $E_1 = 0.66 \pm 0.03$ eV and $E_2 = 1.61 \pm 0.03$ eV.

3. Conclusions

In this work, the band model used for semiconductors was shown to be applicable for interpretation of the TL phenomenon in a chosen food-industrial ingredient MPCP, as model material. With the assumption of second-order kinetics, characteristic activation energies (≈ 0.65 and ≈ 1.60 eV) were determined by curve fitting. These energies indicate the depths of electron traps; accordingly, in MPCP two separate (deep) traps are responsible for the TL above room temperature.

We consider that a knowledge of the activation energy and other trap parameters is necessary if methods are to be developed for the successful detection the irradiation of foodstuffs. For this reason, investigations of this nature have been begun on other products (KISPÉTER & KISS, 1993), and it is planned to extend these investigations to low temperatures (80 – 200 K), primarily for basic research purposes.

*

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References

BABELLA, GY., NOVÁK, Á., JANCSÓ, J., MIKE, F. & MOLNÁR, GY. (1987): *Eljárás teljes tejfehérje-koncentrátumok előállítására.* (Procedure for preparing whole milk protein concentrates.) Hungarian Patent No. 183 726.

BÖGL, K. W. & HEIDE, L. (1991): Detection of irradiation by thermo- and chemiluminescence. – in: THORNE, S. (Ed.) *Food irradiation.* Elsevier Applied Science, London, pp. 19 – 60.

CHEN, R. & KIRSH, Y. (1981): *Analysis of thermally stimulated processes.* Pergamon Press, Oxford, pp. 17 – 59 and 144 – 182.

HALPERIN, A. & BRANER, A. A. (1960): Evaluation of thermal activation energies from glow curves. *Phys. Rev., 117,* 408 – 415.

KISPÉTER, J. & HORVÁTH, L. (1987): Some physical properties of powdered milk and lactalbumin. *J. Non-Cryst. Solids, 90,* 661 – 664.

KISPÉTER, J., HORVÁTH, L. I., BAJÚSZ-KABÓK, K., KISS, L. I. & ZÁHONYI-RACS, P. (1993): Identification of radiation treatment of mineral-enriched milk protein concentrate by complex test protocols. A comparison of thermoluminescence, electron spin resonance and rheological investigations. *Fd Struct., 12,* 379 – 384.

KISPÉTER, J. & KISS, L. I. (1993): The detection of irradiation in the case of protein-containing food-industrial products by thermoluminescence method. – in: SCHREIBER, G. A., HELLE, N. & BÖGL, K. W. (Eds) *New development in food, feed and waste irradiation, SozEp Hefte 16,* pp. 71 – 75.

KISPÉTER, J., DÉKÁNY, I., KISS, L. I. & MAROSI, T. (1994): Investigation of the microstructure in milk protein concentrate powder by small-angle X-ray scattering and thermoluminescence methods. *Fd Struct.* (in press).

SANDERSON, D. C. W. (1990): Luminescence detection of irradiated foods. – in: JOHNSTON, D. E. & STEVENSON, M. H. (Eds) *Food irradiation and the chemist.* Royal Society of Chemistry (Special Publication No. 86), Cambridge, pp. 25 – 56.

SWALLOW, A. J. (1991): Effects of irradiation on food proteins. – in: HUDSON, B. J. F. (Ed.) *Developments in food proteins vol. 7.* Elsevier Applied Science, London, pp. 195 – 229.

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THE INFLUENCE OF PACKAGING AND STORAGE TIME ON THE COLOUR OF DEHYDRATED RASPBERRY

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The influence of six plastic combined packaging materials, packing conditions and storage time on the intensity change of colouring matters in dehydrated raspberry was investigated. Dehydrated raspberry was packed under atmospheric pressure, in vacuum and under nitrogen. A glass ampulla airtightly sealed after filling with raspberry powder was used as a control.

The colour investigation of the packed product was performed immediately after packing and then after 1, 3, 6, 9 and 12 months of storage in two experimental replications.

The colour changes were determined as the sum of adsorption values for colouring matters at 520 and 440 nm. In order to reach the stable conditions and to avoid the influence of pH value on anthocyanins during storage, the measurement of the total spectrum was carried out at pH 1 and pH 4.5.

The results showed the gradual increase of adsorption values at 440 nm during storage, depending on type of packaging material and on conditions of packaging.

Keywords: packaging materials, raspberry powder, colour changing

The usability of packed product, besides its total quality, depends on packing conditions, type and quality of package used and on its protecting properties in relation to the environmental conditions. It has to be mentioned that inappropriate packaging material and inadequate application of packagings affect the quality and shelf-life of the packed product (GVOZDENOVIC, 1981; GVOZDENOVIC et al., 1978; HANLON, 1984; VARSÁNYI, 1985).

The correct choice of materials for packing dehydrated products depends on many factors, as for example on specificity of the product and its starting quality, properties of packaging materials, production and storage conditions. All the factors must be observed, considering the existing interactions and the dynamic system of mutual functional dependences (GVOZDENOVIĆ et al., 1978, 1981, 1982, 1983; GVOZDENOVIĆ & CURAKOVIĆ, 1984; GVOZDENOVIĆ, 1987; LABUZA & SALTMARCH, 1980; LUCAE, 1978; HEISS & EICHNER, 1971).

The most important physico-chemical property of plastic materials is their permeability to light, gases and water vapour, so that such materials, in accordance

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with the protection of packed product, could be considered as foils, which are, more or less, permeable to light, molecules of water vapour and gases. As the dehydrated products are very sensitive to water vapour and oxygen, the permeability of packaging materials is of great importance for the shelf-life of the product (GVOZDENOVIĆ, 1981, 1987; PÖÖKKÖNEN & MATTILA, 1991).

1. Materials and methods

In order to enable an all-inclusive approach to the problem, i.e. to investigate the influence of combined packaging materials on the quality change of raspberry powder, the following combinations were made.

- PAP (50) - PE (25), as the usual combination for the packing of dehydrated food (PAP+PE) – paperboard-polyethylene;
- PAP (70) - ALU (9) - PE (50), (PAP+ALU-PE) – paperboard--aluminium-polyethylene;

Using plastic semipermeable materials and their combinations the following materials were produced:

- F - 320 (25), (PP) polypropylene, protected on both sides with acrylic lacquer;
- BOPP T 25 30 (35) - PE (50), (PP+PE) – polypropylene-polyethylene;
- PET (12) - PE (50), (PETP+PE) – polyesther-polyethylene, and
- PET (12) - ALU (9) - PE (50), (PETP+ALU+PE) – polyesther--aluminium-polyethylene;
- An airtight glass ampulla of special design was used as a control.

Raspberry powder was produced from frozen mush, by spray drying using the semi-industrial sprayer ("ANHIDRO"), without additives in the first experiment and with the addition of starch syrup in the second experiment.

The barrier characteristics of the packaging materials were determined investigating the permeability of light, water vapour and gases.

The light permeability was determined in the range from 200 to 460 nm on the UV spectrophotometer UNICAM SP 800 B.

The water vapour permeability of packaging materials was measured on VAPOR PERMEATION TESTER L-80 (method according to LYSSY, 1974) (DIN 53122).

The gas permeability was measured on PERMEABILITY TESTER L-100 (method according to LYSSY, 1975) (DIN 53380).

After determining the barrier characteristics of the packaging materials the following materials were chosen: PAP-PE, PAP-ALU-PE, PP, PP-PE, PETP-PE and

PETP-ALU-PE. Sacks were made of the mentioned materials and dehydrated raspberry was packed using. AUDION-ELEKTRO packing machine, Type 500.

Three groups of samples were formed:

a) after filling, the sacks were sealed (atmospheric conditions in the packaging),

b) after filling, the air was sucked out, and the packaging was sealed (packing under vacuum) and

c) after filling, air was sucked out, nitrogen was introduced into the sacks and they were sealed (packing in nitrogen atmosphere).

As control samples, glass ampullae were filled with the same amount of dehydrated raspberry and sealed without sucking the air from above the powder. A number of ampullae were wrapped in ALU foil, to determine the influence of light on the colour in the glass ampullae.

The total colour was determined spectrophotometrically, on UV spectrophotometer UNICAM SP 800 B, dissolving 1 g of powder in 50 cm^3 water.

The change of the pH value during storage may have a significant influence on the anthocyanins. To avoid this effect, as well as to achieve stable and reproducible conditions, the measurements of the whole spectrum were performed adjusting the pH value of the raspberry solution to pH 1, where the presence of nondegraded anthocyanins and degradation products can be proved, and to pH 4.5, where the degradation products can be proved (FRANCIS, 1980; MOŠORINSKI, 1972, GVOZDENOVIĆ, 1987).

The colour intensity of anthocyanins, according to CASOLI's method (1967), is expressed as the sum of adsorption values of coloured matters at 520 nm and 440 nm, e.g. as the sum of adsorption maximum for the product and for the product of colour degradation. The CASOLI's (1967) method was modified by measuring the whole spectrum of the product at pH 1 and pH 4.5, and the results were given as the total of adsorption values at 520 nm and 440 nm, e.g. as the colour intensity.

The observation of quality changes of the packed content was performed immediately after the packing and then after 1, 3, 6, 9 and 12 months of storage in duplicate.

2. Results and discussion

2.1. Light permeability

The results of permeability to light investigations are presented in Fig. 1.

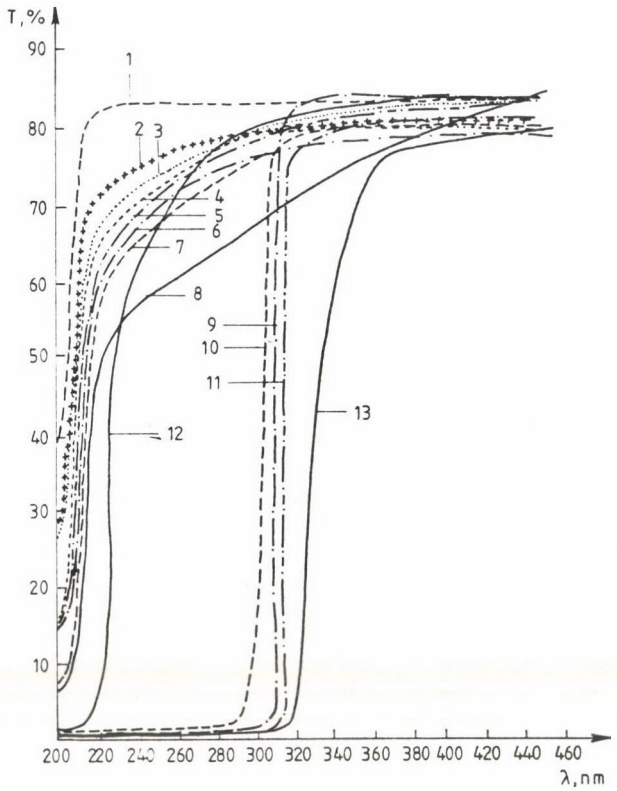

Fig. 1. Permeability of packaging materials to light. 1: F-320; 2: PE 0 033; 3: PE 0 038; 4: PE 0 044; 5: PE 0 045; 6: PE 0 049; 7: PE 0 055; 8: PE 0 100; 9: PETP; 10: BOPPT 2530 + PE; 11: PETP + PE; 12: BOPPT 2530; 13: GLASS

The spectrophotometric investigations of light permeability in the range from 200 nm to 460 nm show that the highest transparency of the investigated materials was found over 320 nm. The influence of kind, combination and thickness of the packaging material is expressed at lower wavelengths. Compared to glass, which is practically impermeable to light till 320 nm, the permeability of monolayer polypropylen BOPP F 320 is the highest, followed by BOPP 2530. The combination of polypropylene and polyethylene (0.050 mm thick) results in decrease of permeability to light. The barrier characteristics of polyesther are significantly better than that of polypropylene and its combination with polyethylene, and they are even better when this packaging material is combined with polyethylene. The permeability of the polyethylene as monolayer, is in a wide range of wavelengths of 220 nm to 320 nm, depending on the foil thickness. Polyethylene (0.050 mm thick) was chosen for the formation of combined materials, since polyethylene 0.100 is inconvenient due to its thickness and smaller elasticity.

2.2. Permeability to water vapour and gases

The permeability to water vapour and gases was also investigated in this work. The results obtained are summarized in Table 1.

Table 1

Permeability of combined packaging materials

Sample	Permeability			
	Water vapour ($cm^3\ m^{-2}\ 24\ h^{-1}$)	Air ($cm^3\ m^{-2}\ 24h^{-1}$)	Oxygen ($cm^3\ m^{-2}\ 24h^{-1}$)	Nitrogen ($cm^3\ m^{-2}\ 24h^{-1}$)
PP	1.31	31.60	70.55	1.53
PP+PE	0.89	2.16	14.87	1.49
PETP+PE	1.48	5.56	6.10	0.79
PAP+PE	3.52	vacuum not reached (porous)		
PAP+ALU+PE	0.05	1	1	1
PETP+ALU+PE	0.05	1	1	1

On the basis of the presented data, it can be concluded that the combinations with aluminium are impermeable to water vapour and are gastight.

Polypropylene, i.e. its combination with polyethylene was better in comparison to polyesther and its combination with polyethylene, relating to water vapour and air permeability. The combination of polyesther and polyethylene showed better protecting properties regarding the oxygen and nitrogen permeability.

The combination of paperboard and polyethylene was found to be porous, so the permeability to gasses could not be measured.

2.3. The changes in the sum of adsorption of raspberry powder

Total spectra of 3 parallel samples, in two experiments (with and without starch syrup addition) were measured at pH 1 and pH 4.5. The mean values obtained at 520 nm and 440 nm respectively, are presented at the diagrams as the sum of adsorption values 520+440 nm (colour intensity) of the product packed in 6 different materials and under 3 different packing conditions (atmospheric pressure, vacuum and under nitrogen). The change in the sum of total spectra was followed during storage (1, 3, 6, 9 and 12 months) (Figs. 2 and 3).

The spectrum measured in the range from 400 to 800 nm is characterized by a maximum at 520 nm. This maximum is characteristic of the product colour and during the storage its intensity changed. The increase of adsorbance at 440 nm was also observed during the investigation, depending on the packaging material applied, and the packing conditions. Our results confirm the findings presented by AMATI, 1984, that the increase of anthocyanins adsorbance in the range from 400 to 440 nm

is the result of increase of coloured degradation products which are formed during the nonenzymatic browning process. In our previous investigations, dealing with TLC separation of raspberry powder anthocyanins, we have found cianidin-3-glucoside and cianidin-3-soprozid. During storage, depending on the applied packing conditions and packaging materials, new peaks were identified after 6–9 months of storage at 254 and 336 nm, applying the densitometry. A new spot appeared over the cianidin-3-soprozid, and a well-recognizable spot, characteristic for degraded anthocyanins at the start of the chromatogram (MOŠORINSKI, 1972; VOJNOVIĆ, 1976; FRANCIS, 1980; GVOZDENOVIĆ, 1987).

2.3.1. First experiment. A slight increase of colour intensity was noticed at pH 1, in all samples in the first experiment, in the first 3-month storage period (curves 1–5). The values increased mostly after 6 months, regardless to the applied packaging material.

After reaching this maximum, in the period between 6 and 9 months the values decreased and after that increased again in all samples (Fig. 2).

Such cyclic changes in colour intensity may be the result of anthocyanin degradation and formation of some products having different spectral properties under these conditions (GVOZDENOVIĆ, 1987; AMATI, 1984; FRANCIS, 1980).

It is obvious from diagrams 1–6 that the intensity of changes varies depending on the protecting properties of the packaging materials and their water vapour and gas permeability, which is in correlation with the results presented in Table 1. Degradation of anthocyanins takes place during the storage, as well as the increase of colour intensity at pH = 1 up to 6 months of storage. The total colour intensity decreased at pH = 1 and that of the degradation products increased at pH = 4.5. Our investigations confirmed the findings of LONCIN (1968), EICHER & KAREL (1972) and HEISS (1980), CHU-HONG TSAI and co-workers (1991), who reported that in case of increased a_w value of the powder, due to the higher water content (as a consequence of the water vapour permeability of the material), the adsorption values decreased, especially at 440 nm. By further moisture increase, the powder reaches the maximal value for nonenzymatic browning (HEISS, 1980), so after 9 months the colour intensity of raspberry powder at pH = 1 and at pH = 4.5 increased.

After 9 months of storage, the increase of values measured at pH = 4.5 was obvious, compared to the values measured at pH = 1, when minimal values were registered. The correlation shows that after 9 months the intensity of anthocyanine natural colours of raspberry decreases and the values for degradation changes increase. This is especially expressed in samples packed in PAP+PE (diagram 1), under atmospheric pressure, where the highest decrease of colour intensity at pH = 1 (curve 3) and the highest increase at pH = 4.5 (curve 8) was registered.

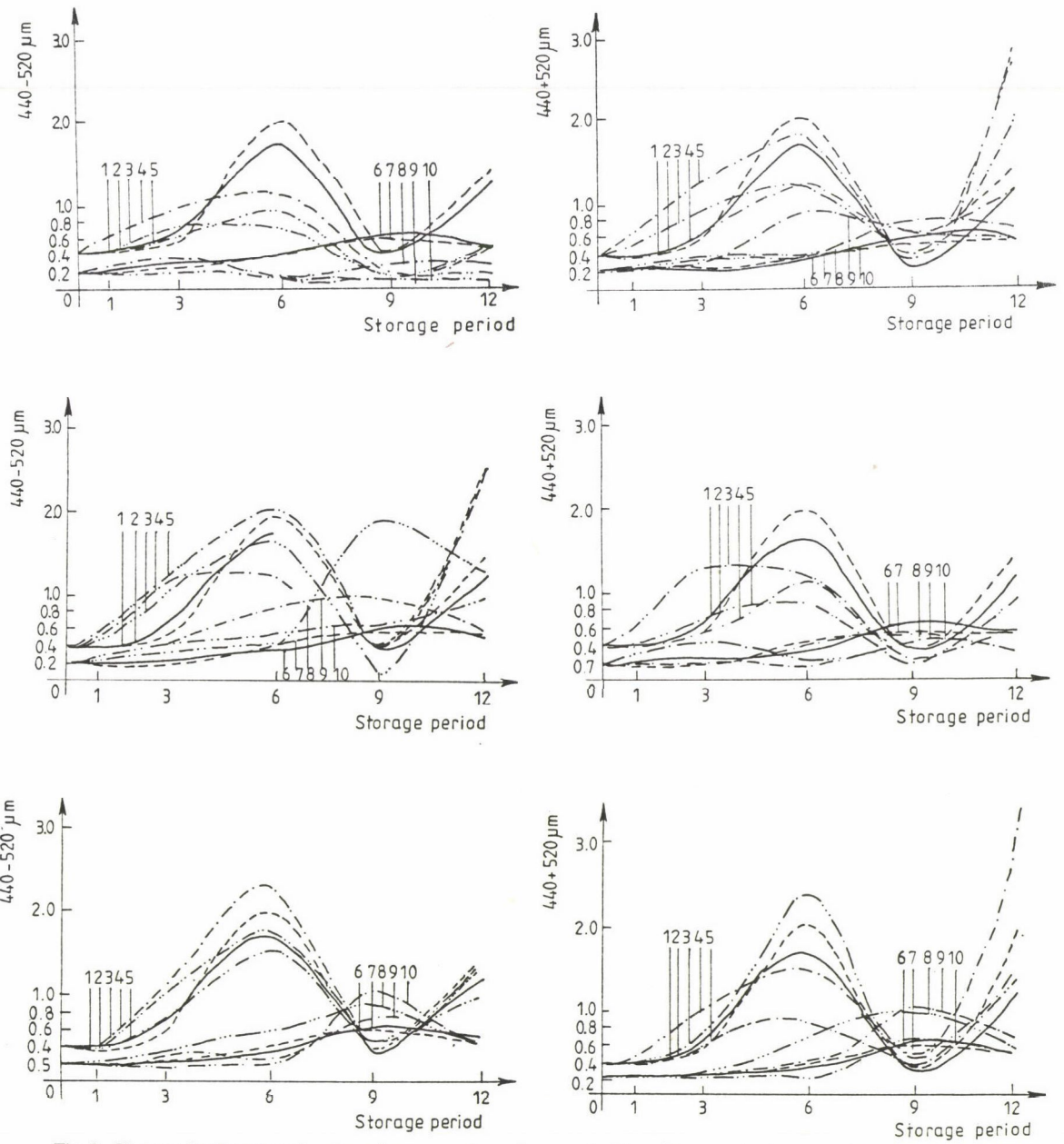

Fig. 2. Changes in the intensity of raspberry powder colour packed in various packaging materials. First experiment. 1: PAP+PE; 2: PAP+ALU+PE; 3: PP; 4: PP+PE; 5: PETP+PE; 6: PETP+ALU+PE. Packing conditions: ampullae with light protection (curves 1, 6); ampullae without light protection (curves 2, 7); under atmospheric pressure (curves 3, 8); under vacuum (curves 4, 9); under nitrogen (curves 5, 10). Experiment at pH=1 (curves 1, 2, 3, 4 and 5); experiment at pH=4.5 (curves 6, 7, 8, 9 and 10)

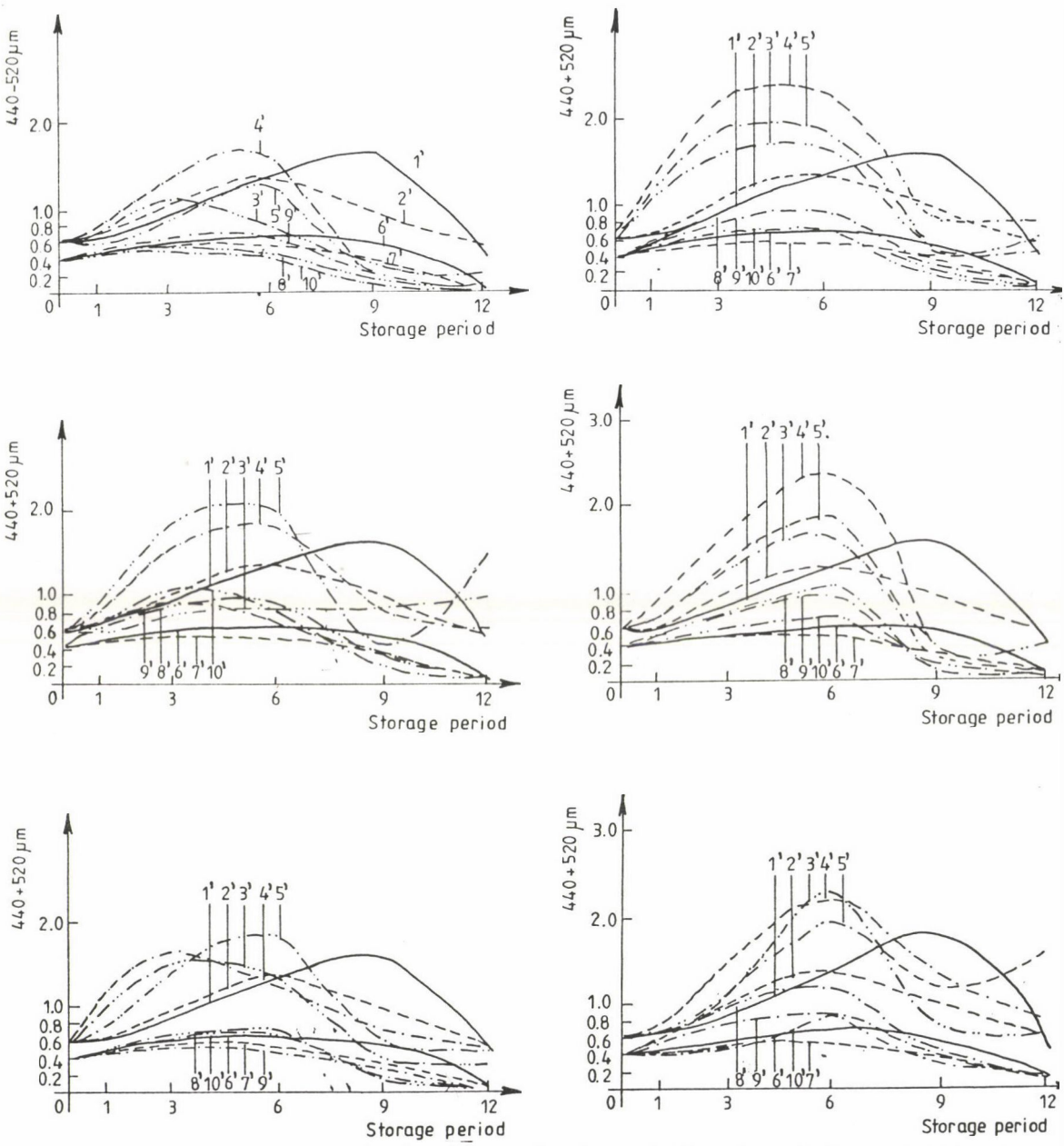

Fig. 3. Changes in the intensity of raspberry powder colour packed in various packaging materials. Second experiment. 7: PAP + PE; 8: PAP + ALU + PE; 9: PP; 10: PP + PE; 11: PETP + PE; 12: PETP + ALU + PE. Packing conditions: ampullae with light protection (curves 1', 6'); ampullae without light protection (curves 2', 7'); under atmospheric pressure (curves 3', 8'); under vacuum (curves 4', 9'); under nitrogen (curves 5', 10'). Experiment at pH = 1 (curves 1', 2', 3', 4' and 5'); experiment at pH = 4.5 (curves 6', 7', 8', 9' and 10')

The same can be observed in samples packed under vacuum, where the decrease of vacuum in packagings was registered. These changes are in correlation with the permeability values, presented in Table 1. Some better colour intensity was determined in samples packed under nitrogen, which confirms the protective function of packing under nitrogen.

The situation seems to be similar in samples packed in monomaterial PP (diagram 3). The protective function of packing under nitrogen is especially expressed during the first 6 months, when the intensity of changes is the lowest at pH = 1 (curve 5). At pH = 4.5, greater increase of values was registered at the 6th month of storage (curve 10), and by the 9th month the values under these conditions (curve 10) reach the maximum for intensity, due to great gas permeability of PP, especially to nitrogen and oxygen (Fig. 2). The same can be stated for the combination PP+PE (diagram 4).

In samples packed in PET+PE under atmospheric pressure (diagram 5, curve 3), the highest changes of colour intensity at pH = 1 were registered already after the first 3 months of storage. The lower changes in samples packed under vacuum and nitrogen point to the protective function of packing conditions (vacuum and nitrogen) and at the same time point out the effect of greater permeability of this combination of materials to water vapour an oxygen, i.e. air and low permeability to nitrogen. Similar trends were also registered by measurements carried out at pH = 4.5 (curve 8).

In samples packed in unpermeable combinations with aluminium foil (PAP+ALU+PE and PET+ALU+PE) after 6 months of storage the maximum changes in colour intensity was registered at pH = 1 in samples packed under atmospheric pressure (curve 3), as well as the highest increase of values at pH = 4.5 after 9 months (curve 8), which indicates the great influence of oxygen on colour changes, because the packing conditions (under vacuum and nitrogen) reduce these changes.

2.3.2. Second experiment. During this experiment (diagrams 7–12 curves 1'–10'), higher values for colour intensity were found, which could be explained by the better starting quality of powder (lower moisture content – 1,2%), as well as by the applied technology of drying with addition of starch syrup. On the basis of the obtained values, measured at pH = 1 (curves 1'–5'), it was found that the increase of colour intensity depending on packaging materials and conditions, reached its maximum at some what lower values, comparing to the first experiment. Between months 6 and 9 the decrease of colour intensity was higher than in the first experiment. The increase of values in the period between 6 and 12 months is much less expressed than in the first experiment. In these investigations no cyclic changes after 9 months were registered, i.e. no increase at pH = 1 was observed. The values at pH = 4.5 were practically stable up to 6 months and after that a lower decrease

was registered, which could be explained by better starting quality of raspberry colour and by the fact that disintegration of anthocyanins occurs somewhat later. The protecting function of packing under nitrogen and vacuum can also be stated by measurements of colour intensity in the second experiment.

From both experiments it is also obvious that light as activator of photooxidative changes has influence on colour intensity. This fact was confirmed by changes in colour intensity under the atmospheric pressure (oxygen content over the product), in samples packed in hermetically sealed ampullae without protection from light, compared to the samples packed in light protected ampullae.

3. Conclusions

On the basis of performed investigations, the following conclusions can be drawn:

In general, cyclic changes of colour characteristics intensity of raspberry powder during storage can be stated. The decrease of colour intensity measured at pH = 1 during the storage between months 6 and 9 is followed in the same period by the increase of colour intensity measured at pH = 4.5. In the period between 9 and 12 months, due to moisture increase, as the result of water vapour permeability of the packaging materials, nonenzymatic degradation products were formed, so the powder being in the sphere of increased activity of nonenzymatic browning shows again an increase of colour intensity at pH = 1 (as stated by the maximal increase of colour intensity after 9 months at pH = 4.5). The dependence of colour intensity on applied combinations of packaging materials and on their water vapour and gas permeability was stated, as well as the protective function of packing under vacuum and nitrogen.

The colour intensity is also influenced by the light as photooxidative activator of the colour changes during storage, which is confirmed by higher changes in colour intensity in samples packed in hermetically sealed ampullae without light protection, where besides light, the packing conditions also affect the changes (oxygen is present over the content).

The starting quality of raspberry powder has also effect on colour intensity, which is proved by lower changes of colour in the second experiment, where better starting characteristics (moisture content) and higher colour intensity at the beginning of storage were registered.

References

AMATI, A. (1984): Versuche zum Abpacken von Wien in Behaltern aus "Tetra Brick". *Verpack. Rdsch.*, *2*, 35, 7–1.

CASOLI, V. (1967): *Ricerche sugli antociani di fragola e di Mirtillo*. Monography of the Experimental Institute for Canned Food Industry. Parma, p. 123.

CHU-HONG TSAI, KONG, M. S. & PAN, B. S. (1991): Water activity and temperature effects on nonenzymic browning of amino acids in dried squid and simulated system. *J. Fd Sci.*, *56*, 665–670.

EICHNER, K. & KAREL, M. (1972): The influence of water content and water activity on the sugar-amino browning reactions in model system under various conditions. *J. agr. Fd Chem.*, *20*, 218–223.

FRANCIS, F. J. (1980): *Current aspects of food colorants*. Paper from the Department of Food Science and Nutrition, University of Massachusetts.

GVOZDENOVIĆ, J., VUJKOVIĆ, I. & CURAKOVIĆ, M. (1978): Ispitivanje propustlijvosti vodene pare i vazduha polietilenskog filma. (Permeability of polyethylene foil to water vapour and air.) *Savremeno pakovanje*, *3*, 36–39.

GVOZDENOVIĆ, J. (1981): *Ispitivanje podobnosti kompleksnih ambalažnih materijala i ambalaže za pakovanje instant paradajza.* (Investigation of composite packaging materials and packagings for the packing of instant tomato.) M. Sci. Thesis, Faculty of Technology, Novi Sad.

GVOZDENOVIĆ, J., CURAKOVIĆ, M. & VUJKOVIĆ, I. (1981): *Pakovanje celera u prahu pod vakuumom u različite kombinacije kompleksne ambalaže.* (Packing of dehidried celery under vacuum in different combinations of composite packagings.) Paper presented at the Serbian Congress of Vacuum Technics, Beograd.

GVOZDENOVIĆ, J., CURAKOVIĆ, M. & VUJKOVIĆ, I. (1982): Primena kombinovanih ambalažnih materijala za pakovanje dehidriranih proizvoda. (Application of composite packaging materials for the packing of dehidried products.) VI Kongres o ishrani naroda Jugoslavije (VIth Congress on nutrition in Yugoslavia), Novi Sad, *Savremeno pakovanje*, *23* (5), 20–24.

GVOZDENOVIĆ, J., CURAKOVIĆ, M. & VUJKOVIĆ, I. (1983): Karakteristike višeslojnih ambalažnih materijala za pakovanje dehidrirane dečje hrane. (Characteristics of the multilayer packaging materials for the packing of dehidried infant food.) Savetovanje Društva za unapretenje ishrane naroda Vojvodine (Conference of the Society for the nutrition advancement in Vojvodina.) Novi Sad. *Hrana i ishrana*, *24*, 255–256.

GVOZDENOVIĆ, J. & CURAKOVIĆ, M. (1984): Pakovanje sušenog voca i povrća. (Packing of dehidried fruit and vegetable.) Zbornik radova za Savetovanje jugoslovenske industrije za preradu voća i povrća. (*Conference of the Yugoslav industry for fruit and vegetable processing*), *Proceedings*, Faculty of Technology, Novi Sad, pp. 153–161.

GVOZDENOVIĆ, J. (1987): *Uticaj osobina semipermeabilne višeslojne ambalaže na promene kvaliteta praha maline.* (Influence of semipermeable composite packaging on the quality change of raspberry powder.) Ph.D. Thesis. Faculty of Technology, Novi Sad, pp. 266.

HEISS, R. & EICHNER, K. (1971): Moisture content and shelf life. *Fd Manufacture*, *53* (May), 35–42.

HEISS, R. (1980): *Verpackung von Lebensmitteln*. Springer Verlag, Berlin–Heidelberg–New York, p. 306.

HANLON, J. (1984): *Handbook of package engineering*. Academic Press, London, New York, San Francisco, p. 560.

LABUZA, T. P. & SALTMARCH, M. (1980): Properties of water related to food quality and stability. –in: ROCKLAND, L. B. & STEWART, G. V. (Eds) *The nonenzymatic browning reaction as affected by water in foods*. Academic Press, London, New York, San Francisco, p. 360.

LONCIN, M. (1968): Influence of activity of water on the spoilage of foodstuffs. *J. Fd Technol.*, *3*, 131–142.

LUCAE, T. (1978): Zertstaubungstrocknung on Frucht- und Gemüse-produkte. *Z. Lebensmittel Technologie u. Verfahrenstechnik*, *7*, 1 – 13.

LYSSY, G. (1974): *Manometrisches Gasdurchlässigkeits*, Massgerät, Bedienungsaufleitung, Zollikon.

LYSSY, G. (1975): *Manual water vapour permeation tester*. Operation manual, Zollikon.

MOŠORINSKI, N. (1972): *Antocijani, prirodne bojene materije*. (Anthocyanins, natural coloured matters.) Tehnološki informativni bilten (Technological informative bulletin.) 86. Jugokonzerva, Beograd.

PÖÖKKÖNEN, K. & MATILLA, M. (1991): Processing, packaging and storage effects on quality of freese-dried strawberries. *J. Fd Sci.*, *56*, 1388 – 1392.

VARSÁNYI, I. (1985): *Packed food quality changes during storage*. Paper presented at Symposium on Aseptic Processing and Packaging of Foods, Proceedings, Tylosand, Sweden.

VOJNOVIC, M. (1976): *Hromatografsko i spektrofotometrijsko ispitivanje aldehida i ketona*. (Chromatographic and spectrophotometric investigation of aldehydes and ketones.) Ph.D. Thesis, Novi Sad University, pp. 167.

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EFFECT OF UV IRRADIATION ON LIPID PEROXIDATION IN EDIBLE FATS

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Free radical reactions generated by ultraviolet light during 20 min and 6 h treatment were investigated in soy oil, sunflower oil, olive oil, margarine and lard. The control samples were not treated. Lipid peroxidation characteristics, fatty acid composition, tocopherol and oxidized derivatives of cholesterol were measured.

Treatment by ultraviolet light for 20 min had no detectable effect on fatty acid composition and tocopherol in the samples. The chemiluminescence intensity, the peroxide value and the thiobarbituric acid reactive substances increased slightly but significantly.

Significant expansion of lipid peroxidation was found after treatment for 6 h. The concentration of free radicals measured by chemiluminometry increased significantly as a consequence of ultraviolet light, as well as the peroxide value and the concentration of thiobarbituric acid reactive substances. Tocopherols decreased considerably in the oils and margarine. Formation of a small amount of oxidized cholesterol derivatives was observed in lard.

The degree of change in lipid peroxidation characteristics was influenced by the fatty acid composition and tocopherol content in fats. Soy oil contains about 7% linolenic acid that is oxidized hundred times faster than oleic acid. In spite of this fact, soy oil did not show an extremely high sensitivity in lipid peroxidation induced by ultraviolet light, since half the amount of tocopherols originally present in oil remained after 6 h.

Strongly significant correlation was observed between chemiluminescence intensity and peroxide value, chemiluminescence intensity and thiobarbituric acid reactive substances (TBARS), peroxide value and TBARS in various edible fats.

Keywords: ultraviolet light, edible fats, lipid peroxidation, chemiluminescence

Atmospheric oxygen has been recognized for more than hundred years as the principal agent responsible for the deterioration of organic materials exposed to air. The parallel role of oxygen, a molecule essential for many forms of life, as a toxic agent for living tissues has been discovered more than a decade ago (MCCORD & FRIDOVICH, 1982). Similarly, light is essential for plants, but sunlight also contains short wavelength ultraviolet photons of high energy which are potentially detrimental

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because of their destructive interactions with cellular molecules, such as amino acids, nucleic acid bases or membrane lipids.

Approximately 4% of the total energy in sunlight occurs in the ultraviolet region, at wavelengths shorter than 400 nm. The intensity of UV irradiation at the Earth's surface varies greatly with season, time of day, latitude, ozone layer thickness, altitudes and cloud cover (LARSON, 1988).

Photochemically damaging events in cells are initiated by the uptake of the electronic energy of a photon by an UV-absorbing molecule. In the UV region of the electromagnetic spectrum, the energy of such photons is sufficient to break certain covalent bonds. Usually, the adsorbed energy induces an excited state of the molecule resulting in a different and often unstable configuration (FEHÉR et al., 1987).

A potential route for the formation of a damaging species of molecules from a photochemically activated state is the transfer of the energy to molecular oxygen. The product of the energy transfer reaction is singlet oxygen, $^1O_2$. Several types of biological molecules are able to be attacked by $^1O_2$, including several amino acids and polyunsaturated fatty acids. Fatty acids with a high number of double bonds may form lipid hydroperoxydes which are likely contributors to damage of the function of cell membranes (ISLAM, 1982; COMPORTI, 1985).

The object of this study was to investigate the effect of direct ultraviolet light on free radical reactions in edible fats. Many foods of plant origin contain highly unsaturated lipids. Lipids of animal origin generally contain lower levels of unsaturated fatty acids, but considerable amounts are still present, especially arachidonic acid. Oxidative degradation of polyunsaturated fatty acids caused by free radical reaction is a major factor limiting the shelf-life of manufactured foods (RAMANATHAN & DAS, 1992; HAMILTON, 1983).

1. Materials and methods

Edible fats of sunflower seeds (Vénusz, Győr), olive (Olio di Oliva, Roma), soybean (Risso Soya Oil, Belgium), lard (Pick, Szeged) and baking margarine (Sennaback, Wien) were purchased from the local supermarkets. The samples were stored in dark place at 4 °C until the beginning of the treatments.

1,1,3,3-tetraethoxy-propane from Fluka and luminol reagent from Sigma Co. were used. All other chemicals were reagents of analytical grade.

Ultraviolet light irradiation was carried out at room temperature by analytical quartz lamp, type AN-4, during 20 min and 6 h; the wavelength of the UV light was 254 nm. The edible oil samples (50 g in Petri dish) were placed under the UV light at a distance of 20 cm. The samples were stirred with a glass stirrer in every 20 min. After the irradiation the chemical analyses started immediately.

The following methods were used for the characterization of changes in edible fats due to free radical reactions generated by UV irradiation.

Direct luminometry was carried out with a CLD-I Medicor-Medilab Luminometer according to ZSINKA and co-workers (1988) to determine the total amount of free radicals in the samples. The luminol reagent solution consisted of 0.7 mmol l^{-1} luminol, 38 μmol l^{-1} hemin, 11.8 mmol l^{-1} Na_2CO_3 adjusted to pH = 10 and deairated with N_2. Two or four hundred μl of the reagent were added to the fat samples of 20 μl. The chemiluminescence intensity in mV was recorded. Maximal intensity was 10 mV.

The detection of peroxide value (POV) was carried out by iodometry according to the HUNGARIAN STANDARD (1981). Thiobarbituric acid reactive substances (TBARS) were measured photometrically at 532 nm after reaction with Fe(II)--ascorbate and the results were expressed as malondialdehyde (OHKAWA et al., 1979), and 1,1,3,3-tetraethoxy-propane was used as standard.

Tocopherols were measured according to JÁKY (1967). Tocopherols being reducing agents react with Fe(III)-ions to form Fe(II)-ions which produce colored product with 2,2-dipyridyl measurable photometrically at 520 nm.

Composition of fatty acids was analyzed by gas chromatography using Carlo Erba Fractovap 2400 with flame ionization detector. Triglycerides were converted into methylesters in the presence of Na-methylate. Fatty acid methylesters were separated with Silar 5 CP and Silar 10 C on Chromosorb using heat program (9 min at 180 °C, and 2.5 °C min^{-1} until 220 °C).

The oxidized derivatives of cholesterol were separated by thin-layer chromatography as described earlier (LEBOVICS et al., 1992).

The statistical analysis was performed on duplicate samples with three replications. Data were analysed using the unpaired Student's t-test, and the linear regression coefficients were evaluated.

2. Results

The chemiluminescence intensity of total free radicals in edible fats is shown in Table 1. The UV irradiation for 20 min resulted in a slight increase in free radicals, but significant expansion was observed after 6 h of irradiation. The amount of free radicals was 50 times higher in the treated lard sample irradiated for six hours than in untreated one, and the increases were fivefold or more in the other fats.

Table 1

Chemiluminescence intensity (mV × 10^3) *in edible fats treated by UV irradiation*

Sample	Untreated			Ultraviolet light irradiation for 20 min			Ultraviolet light irradiation for 6 h		
	$\bar{x}$		SD	$\bar{x}$		SD	$\bar{x}$		SD
Soy oil	127	±	7	157	±	11a	538	±	87a
Sunflower oil	474	±	11	633	±	11a	3190	±	180a
Olive oil	120	±	4	133	±	1a	1080	±	260a
Margarine	67	±	0.3	207	±	9a	1050	±	90a
Lard	20	±	0.2	–		–	999	±	5a

Significant difference in comparison to the untreated sample: a: ($p < 0.001$)

Similar changes were observed in peroxide value (POV). As it is shown in Table 2 the peroxide value was not strongly enhanced in the first 20 minutes of UV irradiation, but after six hours significant increases were observed in every sample. The highest level was found in sunflower oil but it did not achieve the critical value of 50 meqv. peroxide/kg oil, above which the edible fat is not suitable for human consumption according to the HUNGARIAN STANDARD (1981).

Table 2

Peroxide value (mq peroxide/kg sample) *in edible fats treated by UV irradiation*

Sample	Untreated			Ultraviolet light irradiation for 20 min			Ultraviolet light irradiation for 6 h		
	$\bar{x}$		SD	$\bar{x}$		SD	$\bar{x}$		SD
Soy oil	0.75	±	0.05	1.5	±	0.1a	17.9	±	1.6a
Sunflower oil	0.84	±	0.02	2.9	±	0.5a	46.1	±	1.9a
Olive oil	5.59	±	0.12	6.5	±	0.4b	25.9	±	5.3a
Margarine	2.97	±	0.04	3.3	±	0.2	18.9	±	2.0a
Lard	2.21	±	0.06	3.1	±	0.1a	29.0	±	3.1a

Significant differences in comparison to the untreated sample: a: ($p < 0.001$); b: ($p < 0.05$)

As it can be seen in Table 3 the short-term UV treatment had no effect on formation of thiobarbituric acid reactive substances (TBARS) in soy oil, in sunflower oil and in lard, but significant increase was observed in olive oil and margarine. TBARS increased significantly in edible fats after 6 h of irradiation.

Table 3

Thiobarbituric acid reactive substances (TBARS) as malondialdehyde (nmol 100 g^{-1}) *in edible fats treated by UV irradiation*

Sample	Ultraviolet light irradiation for								
	Untreated			20 min			6 h		
	$\bar{x}$		SD	$\bar{x}$		SD	$\bar{x}$		SD
Soy oil	21.1	±	0.7	23.2	±	2.5	26.0	±	1.4b
Sunflower oil	9.3	±	0.3	7.1	±	0.7	16.9	±	0.7a
Olive oil	10.2	±	0.9	15.2	±	2.5c	19.0	±	1.2a
Margarine	16.5	±	0.3	20.2	±	1.0a	25.3	±	0.5a
Lard	13.5	±	1.1	14.1	±	1.7	39.3	±	1.0a

Significant differences in comparison to the untreated samples: a: ($p < 0.001$); b: ($p < 0.002$); c: ($p < 0.01$)

Fatty acid composition of edible fats is shown in Table 4. The ratio of fatty acids changed slightly.

The levels of tocopherol can be seen in Table 5. It seems that tocopherols are sensitive to UV irradiation; after 6 h of irradiation a significant decrease was observed. The method described by JÁKY (1967) measures the tocopherols by its reducing capacity. Keeping this in mind it can be concluded that the amount of tocopherols and/or that of reducing materials were decreased by UV irradiation.

Table 5

Tocopherol content (mg 100 g^{-1} sample) *of edible fats treated by UV irradiation*

Sample	Ultraviolet light irradiation for		
	Untreated	20 min	6 h
Soy oil	106.9	108.9	69.0
Sunflower oil	66.2	64.8	10.1
Olive oil	24.8	32.0	11.4
Margarine	46.8	14.7	11.9
Lard	2.5	2.0	2.0

Oxidized derivatives of cholesterol which are supposed to have atherogenic effect (IMAI et al., 1976; COPPEN, 1983) were not found in untreated lard and after short-term UV treatment. The spots of 7-hydroxycholesterol isomers (7α- and 7β--hydroxycholesterol) were present on the chromatogram at detection limit (0.5 μg) after 6 h of irradiation. The estimated concentration of these compounds in the lard is 1 mg kg^{-1} obtained by visual comparison of colour spots with those of authentic standards of known amounts.

Table 4

Fatty acid composition (g 100 g^{-1} total fatty acids) *of edible fats treated with ultraviolet light irradiation*

Sample	Treatment	C_8	C_{10}	C_{12}	C_{14}	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:1}$
Soy oil	a	–	–	–	–	11.0	–	3.3	23.9	54.5	7.0	–
	b	–	–	–	–	11.0	–	2.9	24.0	55.7	6.3	–
	c	–	–	–	–	11.0	–	2.5	23.6	57.0	5.9	–
Sunflower oil	a	–	–	–	–	6.2	–	4.0	24.5	65.1	–	–
	b	–	–	–	–	6.6	–	3.6	23.0	66.6	0.1	–
	c	–	–	–	–	5.9	–	3.1	22.6	68.3	–	–
Olive oil	a	–	–	–	–	10.4	0.5	1.7	75.7	11.2	0.4	0.1
	b	–	–	–	–	11.6	0.8	1.8	73.0	12.1	0.5	0.2
	c	–	–	–	–	11.8	0.5	1.8	73.8	11.5	0.4	0.2
Margarine	a	0.4	0.2	3.1	1.4	25.3	–	6.3	51.1	9.2	1.9	0.5
	b	0.4	0.2	2.4	0.5	31.7	–	4.7	54.1	5.4	0.5	0.1
	c	0.3	0.3	2.7	1.0	28.8	–	6.1	52.8	6.8	1.0	0.2
Lard	a	–	–	–	1.2	26.5	2.4	15.3	45.2	8.2	0.4	0.6
	b	–	–	–	0.6	26.2	1.6	12.4	51.0	7.9	0.2	0.1
	c	–	–	–	1.1	26.9	2.5	14.1	47.3	7.4	0.2	0.5

a: untreated sample
b: UV irradiated for 20 min
c: UV irradiated for 6 h

3. Conclusions

With respect to lipid peroxidation characteristics, the short-term UV irradiation had only a slight deleterious effect on edible fats. The long-term treatment was significantly effective in generating free radicals, peroxides, aldehydes and in decomposing tocopherols. The significant correlations between some lipid peroxidation characteristics can be seen in Table 6. The results emphasize that the first step of the processes is the formation of free radicals measured by chemiluminescence technique. These unstable radicals react with the fatty acid molecules producing peroxides, hydroperoxides, aldehydes and other degradation products. Tocopherols are able to be attacked by radicals and the degradation products are not effective as natural antioxidants (NIKI, 1987).

Table 6

Linear correlation coefficients between lipid peroxidation characteristics in edible fats treated with ultraviolet light irradiation

Sample	CLI-POV	CLI-TBARS	POV-TBARS
Soy oil	0.99	0.64	0.62
Sunflower oil	0.99	0.96	0.97
Olive oil	0.99	0.83	0.85
Margarine	0.99	0.95	0.95
Lard	–	–	0.99

CLI: chemiluminescence intensity
POV: peroxide value
TBARS: thiobarbituric acid reactive substances

Soy oil contains a relatively high amount (7% of total fatty acids) of linolenic acid ($C_{18:3}$) containing three unsaturated double bonds and this fatty acid has an ability to be oxidized hundred times faster than oleic acid ($C_{18:1}$) (HAMILTON, 1983). Soy oil could be a great target for several oxidizing agents because of its high level of polyunsaturated fatty acids. The results do not support the above-mentioned hypothesis because the amount of tocopherols after 6 h of UV irradiation was nearly 70% of the original level and their antioxidant activity was sufficient to defend double bonds against oxidative degradation.

Sunflower oil reacted strongly to UV irradiation because of its low level of tocopherols and high concentration of polyunsaturated fatty acids. Olive oil has a well-balanced system of oxidizable fatty acids and antioxidative components. Baking margarine and lard have relatively small amount of double bonds but they are easily oxidized because of their stereochemical structure and essentially the lack of antioxidants in the case of lard. It is important to emphasize the presence of probably atherogenic derivatives of cholesterol in lard, resulting in from UV irradiation.

It is essential to mention that the samples were industrial products for human consumption and contained some synthetic antioxidants. These antioxidants (probably BHT, BHA or propyl gallate) could scavenge the free radicals generated by UV irradiation and modify the results observed in this experiment. The amount of synthetic antioxidants originally present in the samples is not known, but their role is important in the defending system of edible fats against lipid peroxidation caused by various external effects (heat, light, UV irradiation).

In conclusion, the ultraviolet light irradiation had measurable effect on lipid peroxidation characteristics of edible fats, but the rate of changes depends on the fatty acid composition, the level of tocopherols or other antioxidants and the time of treatment.

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References

COMPORTI, M. (1985): Lipid peroxidation and cellular damage in toxic liver injury. *Lab. Invest.*, *53*, 599–622.

COPPEN, P. P. (1983): The use of antioxidants. – in: ALLEN, J. C. & HAMILTON, R. J. *Rancidity in foods*. Applied Science Publishers, London, pp. 59–67.

FEHÉR, J., CSOMÓS, G. & VERECKEI, A. (1987): *Free radical reactions in medicine*. Springer Verlag, New York, p. 27.

HAMILTON, R. J. (1983): The chemistry of rancidity in foods. – in: ALLEN, J. C. & HAMILTON, R. J. *Rancidity in foods*. Applied Science Publishers, London, pp. 1–20.

HUNGARIAN STANDARD (1981): Étkezési zsiradékok vizsgálata. Peroxidszám meghatározás. (Test methods of fats. Determination of peroxide value.) M. Sz. 19823-81.

IMAI, H., WERTHESSEN, T., TAYLOR, C. B. & LEE, K. T. (1976): Angiotoxicity and atherosclerosis due to contaminants of USP-grade cholesterol. *Arch. path. Lab. Med.*, *100*, 565–572.

ISLAM, S. J. (1982): The effect of various modifiers on rat microsomal peroxidase kinetics. *Biochem. Pharmacology*, *31*, 1823–1826.

JÁKY, M. (1976): Vitamin E. – in: PARDUN, H. *Analyse der Nahrungsfette*. Verlag P. Parey, Berlin, p. 191.

LARSON, R. A. (1988): The antioxidants of higher plants. *Phytochemistry*, *27*, 969–978.

LEBOVICS, V. K., GAÁL, Ö., SOMOGYI, L. & FARKAS, J. (1992): Cholesterol oxides in gamma-irradiated egg powder. *J. Sci. Fd Agric.*, *60*, 251–254.

MCCORD, J. M. & FRIDOVICH, I. (1982): The biology and pathology of oxygen radicals. *Ann. Med.*, *89*, 122–127.

NIKI, E. (1987): Antioxidants in relation to lipid peroxidation. *Chem. Phys. Lipids*, *44*, 227–253.

OHKAWA, H., OHISHI, N. & YAGI, K. (1979): Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analyt. Biochem.*, *95*, 351–358.

RAMANATHAN, L. & DAS, N. P. (1992): Studies on the control of lipid oxidation in ground fish by some polyphenolic natural products. *J. agric Fd Chem.*, *40*, 17–21.

ZSINKA, Á. J. N., BLÁZOVICS, A. & BIACS, P. (1988): Chemiluminescence phenomena in animal tissues. *Hung. Sci. Instrum.*, *64*, 11–13.

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STUDIES ON THE GROWTH AND ACID PRODUCTION OF PURE AND MIXED CULTURES OF *BIFIDOBACTERIUM BIFIDUM*, *LACTOBACILLUS ACIDOPHILUS* AND *LACTOBACILLUS BULGARICUS*

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Growth and acid production of *Bifidobacterium bifidum*, *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* separately and in combination were investigated. Fermentations were run at 40 and 45 °C, with inocula of 3 and 5% in cow's milk and milk media containing glucose and tomato juice.

Comparing the fermentations, the mixed cultures (*B. bifidum* + *L. acidophilus* and *B. bifidum* + *L. bulgaricus*) resulted in higher acidity and cell count than the single cultures. Addition of the tomato juice to the milk media had a more significant effect on growth and acid production than the addition of 1% glucose. Increasing the inoculum from 3 to 5% increased the acidity and cell count. Comparing the effect of fermentation temperature, growth and acid production rates at 45 °C were higher than those at 40 °C.

Keywords: *Bifidobacterium bifidum*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, mixed culture, nutrient media

Fermented milks containing bifidobacteria are made using either pure strains of these organisms alone or in combination with other bacteria (KURMAN & RASIC, 1988). The microflora of some commercial products are presented in Table 1.

The first commercial process for the manufacture of fermented milks incorporating bifidobacteria was proposed in 1968 (SCHULER-MALYOTH et al., 1968).

Studying the growth and acid production of *B. bifidum* in milk, GOH and co-workers (1986) used a 2% inoculum in reconstituted dried skim milk (9% total solids). They found that increasing the amount of the inoculum from 2 to 5% resulted in an increase in acid production. Similarly, the addition of 0.05% L-cysteine and/or 0.2% yeast extract or tryptone increased acid production. The most significant effect was achieved by adding cysteine and yeast extract. Increasing total solids content of the skim milk from 9 to 12 to 15%, caused acid production to decrease.

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Table 1

Microflora of some fermented milk product containing bifidobacteria (KURMAN & RASIC, 1988)

Commercial product	Microflora involved
Bifidus milk	*B. bifidum*
Bifigurt	*B. bifidum, L. acidophilus, Str. thermophilus*
Biogarde	*B. bifidum, L. acidophilus, Str. thermophilus*
Special yoghurt	*B. bifidum, Str. thermophilus, L. bulgaricus*
Cultura and Cultura drink	*B. bifidum, L. acidophilus*
Mil-Mil	*B. bifidum, B. breve, L. acidophilus*
Progurt	*B. bifidum, Str. lactis subsp. diacetylactis, Str. lactis subsp. cremoris, L. acidophilus*

Comparing the growth and acid production of bifidobacteria and lactic acid bacteria, GOH and co-workers (1987) used a 2% inoculum of brevibacteria in 9% reconstituted skim milk. After 24 h at 37 °C, acid production by *B. bifidum* was similar to that by *B. breve* and greater than acid production by *B. infantis*. The acid production of all spp. increased in presence of 0.05% added L-cysteine--hydrochloride, compared to the addition of 0.2% yeast extract and was highest (1.2% titritable acidity) in the presence of both additives. Applying a 5% inoculum in homogenized whole milk at 37 °C, acid production of *B. bifidum* was less than that of *L. acidophilus*, *L. casei* or *Str. thermophilus*. The highest acid production was found in the case of the mixed culture of *B. bifidum* and lactic acid bacteria.

Studying the growth of *L. acidophilus* in soybean milk, HWA and co-workers (1974) found that soybean milk did not provide for good growth, but the addition of glucose to soybean milk greatly increased growth. Lactose also supported growth, but to a lesser extent, whereas sucrose had no significant effect. The addition of glucose or lactose to soybean milk stimulated the growth of *L. bulgaricus* as well.

The positive effect of glucose and tomato juice addition on the fermentation properties of lactic acid bacteria was reviewed and experimentally studied in our previous paper (BADRAN & REICHART, 1993).

The present paper compares the growth and acid production of *B. bifidum*, *L. acidophilus* and *L. bulgaricus* in milk and modified milk media, using single and mixed cultures of the strains.

1. Materials and methods

The applied materials and a part of methods are summarized in our previous paper (BADRAN & REICHART, 1993).

Experimental method

Fermentation experiments were run in Erlenmeyer flasks containing 200 cm^3 sterilized milk or modified milk media with 3 and 5% inocula at 40 and 45 °C. In the case of mixed cultures of two bacteria the starter volumes were 1.5+1.5%. Temperature was controlled by a water bath with an accuracy of 0.2 °C. Samples were taken from the flasks and total acidity (T.A.), pH and the number of colony forming units (cfu) were determined. The sampling times were 2 h in the case of single cultures and 1 h in the case of mixed cultures. The fermentation were ended after reaching the coagulation point.

Since the effect of the recovery media on cfu was treated in our previous paper (BADRAN & REICHART, 1993) and for the determination of cell numbers Tryptone Glucose Yeast Extract Agar with 1% skim milk (TGE) proved to be the best, this medium was used in the experiments.

2. Results and discussion

2.1. Growth and acid production in milk

The experimental results are summarized in Tables 2 and 3. The values of the means of log cfu were obtained by the logarithmic transformation of the algebrical mean of 3 parallels.

Table 2

Growth of the cultures in milk medium with 3% starter

T	Time	Means of log cfu cm^{-3}				
(°C)	(h)	*B. bif.*	*L. aci.*	*L. bulg.*	*B. bif.* + *L. aci.*	*B. bif.* + *L. bulg.*
40	0	6.53	6.69	6.78	6.59	6.59
	1				6.85	7.11
	2	6.83	7.16	6.95	7.23	7.38
	3				7.38[a]	7.50[a]
	4	7.11	7.35	7.22		
	6	7.29[a]	7.63[a]	7.40[a]		
45	0	6.72	6.65	6.82	6.78	6.80
	1				7.06	7.42
	2	7.01	7.27	7.13	7.44	7.72
	3				7.57[a]	7.67[a]
	4	7.30[a]	7.55[a]	7.41		
	6	7.56[a]	7.95[a]	7.59[a]		

[a]: Coagulated
95% confidence interval for log cfu means = ±0.12

Table 3

Acid production of the cultures in milk medium with 3% starter

T (°C)	Time (h)	Total acidity in lactic acid (%)				
		B. bif.	*L. aci.*	*L. bulg.*	*B. bif. + L. aci.*	*B. bif. + L. bulg.*
40	0	0.20	0.21	0.20	0.20	0.20
	1				0.26	0.25
	2	0.27	0.25	0.27	0.31	0.39
	3				0.55[a]	0.58[a]
	4	0.40	0.37	0.38		
	6	0.57[a]	0.66[a]	0.53[a]		
45	0	0.19	0.22	0.21	0.20	0.19
	1				0.27	0.29
	2	0.29	0.27	0.31	0.36	0.43
	3				0.63[a]	0.64[a]
	4	0.48[a]	0.50[a]	0.40		
	6	0.64[a]	0.84[a]	0.59[a]		

[a]: Coagulated

Table 4

Effect of inoculum percentage, nutrient additives and temperature on the acid production of Bifidobacterium bifidum

T (°C)	Inoc. (%)	Time (h)	Cow's milk		Cow's milk + 1% glucose		Cow's milk + 6% tomato juice	
			T.A.	pH	T.A.	pH	T.A.	pH
40	3	0	0.20	6.45	0.21	6.44	0.23	6.39
		2	0.26	6.25	0.27	6.24	0.29	6.18
		4	0.40	5.74	0.42	5.70	0.45	5.66
		6	0.56[a]	5.38	0.60[a]	5.19	0.64[a]	5.10
	5	0	0.20	6.47	0.20	6.48	0.23	6.36
		2	0.27	6.25	0.28	6.21	0.30	6.12
		4	0.50[a]	5.49	0.52[a]	5.42	0.53[a]	5.40
		6	0.63[a]	5.10	0.66[a]	5.06	0.69[a]	5.02
45	3	0	0.20	6.44	0.20	6.45	0.22	6.39
		2	0.29	6.15	0.30	6.11	0.32	6.06
		4	0.49[a]	5.57	0.50[a]	5.50	0.52[a]	5.46
		6	0.63[a]	5.10	0.67[a]	5.05	0.70[a]	4.97
	5	0	0.21	6.43	0.21	6.43	0.23	6.35
		2	0.34	5.99	0.36	5.98	0.39	5.82
		4	0.55[a]	5.33	0.56[a]	5.31	0.62[a]	5.11
		6	0.68[a]	5.05	0.70[a]	4.97	0.74[a]	4.90

T.A.: Total acidity in lactic acid (%).
[a]: Coagulated

As shown in Table 2 and 3, the rates of growth and acid production of the mixed cultures were higher than those of the single cultures. Times required to coagulation were 6 h at 40 °C and 4 h at 45 °C in the case of the single cultures. Using mixed cultures, these times reduced to 3 h.

The increased growth-rate and acid production of the mixed cultures were based on the symbiotic growth of the two microorganisms.

2.2. Acid production in modified milk media

Acid production of *B. bifidum*, *L. acidophilus* and *L. bulgaricus* in single and in mixed cultures was studied in the following media: cow's milk, cow's milk with 1% glucose and cow's milk with 6% tomato juice. The measured values of T.A. and pH are summarized in Tables 4–8.

Table 5

Effect of inoculum percentage, nutrient additives and temperature on the acid production of Lactobacillus acidophilus

T (°C)	Inoc. (%)	Time (h)	Cow's milk		Cow's milk + 1% glucose		Cow's milk + 6% tomato juice	
			T.A.	pH	T.A.	pH	T.A.	pH
40	3	0	0.20	6.43	0.20	6.41	0.23	6.36
		2	0.25	6.16	0.25	6.15	0.27	6.10
		4	0.34	5.77	0.35	5.75	0.41	5.56
		6	0.64[a]	5.09	0.64[a]	5.08	0.71[a]	4.84
	5	0	0.22	6.40	0.22	6.38	0.24	6.34
		2	0.29	6.13	0.29	6.12	0.31	6.05
		4	0.41	5.55	0.42	5.53	0.50[a]	5.37
		6	0.70[a]	4.69	0.71[a]	4.67	0.89[a]	4.39
45	3	0	0.20	6.43	0.20	6.42	0.23	6.37
		2	0.26	6.04	0.26	6.03	0.30	5.98
		4	0.49[a]	5.38	0.50[a]	5.36	0.53[a]	5.32
		6	0.79[a]	4.70	0.80[a]	4.68	0.85[a]	4.54
	5	0	0.22	6.40	0.22	6.38	0.24	6.34
		2	0.30	6.00	0.30	5.98	0.34	5.93
		4	0.56[a]	5.23	0.57[a]	5.20	0.62[a]	5.15
		6	0.85[a]	4.58	0.87[a]	4.56	0.92[a]	4.47

T.A.: Total acidity in lactic acid (%).
[a]: Coagulated

Table 6

Effect of inoculum percentage, nutrient additives and temperature on the acid production of Lactobacillus bulgaricus

T (°C)	Inoc. (%)	Time (h)	Cow's milk		Cow's milk + 1% glucose		Cow's milk + 6% tomato juice	
			T.A.	pH	T.A.	pH	T.A.	pH
40	3	0	0.20	6.45	0.21	6.46	0.21	6.43
		2	0.27	6.27	0.27	6.26	0.31	6.11
		4	0.37	5.87	0.38	5.82	0.42	5.72
		6	0.52[a]	5.48	0.53[a]	5.45	0.59[a]	5.20
	5	0	0.21	6.44	0.21	6.42	0.23	6.39
		2	0.29	6.20	0.29	6.19	0.33	6.04
		4	0.39	5.83	0.39	5.82	0.45	5.68
		6	0.54[a]	5.40	0.56[a]	5.35	0.63[a]	5.10
45	3	0	0.20	6.46	0.20	6.45	0.21	6.43
		2	0.30	6.26	0.30	6.15	0.33	6.06
		4	0.40	5.75	0.40	5.74	0.47[a]	5.65
		6	0.58[a]	5.20	0.60[a]	5.17	0.65[a]	5.08
	5	0	0.21	6.45	0.21	6.44	0.22	6.40
		2	0.31	6.10	0.32	6.08	0.35	5.96
		4	0.44	5.70	0.46	5.66	0.51[a]	5.50
		6	0.65[a]	5.09	0.67[a]	5.07	0.76[a]	4.80

T.A.: Total acidity in lactic acid (%).
[a]: Coagulated

Table 7

Effect of inoculum percentage, nutrient and temperature on the acid production of mixed culture of Bifidobacterium bifidum *and* Lactobacillus acidophilus

T (°C)	Inoc. (%)	Time (h)	Cow's milk		Cow's milk + 1% glucose		Cow's milk + 6% tomato juice	
			T.A.	pH	T.A.	pH	T.A.	pH
40	3	0	0.20	6.48	0.21	6.48	0.22	6.39
		1	0.25	6.29	0.25	6.28	0.30	6.12
		2	0.30	6.15	0.31	6.08	0.34	6.02
		3	0.54[a]	5.44	0.55[a]	5.40	0.58[a]	5.28
	5	0	0.21	6.45	0.21	6.44	0.23	6.34
		1	0.27	6.24	0.27	6.23	0.32	6.07
		2	0.33	6.03	0.34	6.01	0.36[a]	6.00
		3	0.57[a]	5.30	0.58[a]	5.25	0.61[a]	5.15
45	3	0	0.20	6.46	0.20	6.44	0.22	6.38
		1	0.26	6.23	0.27	6.21	0.30	6.14
		2	0.37	5.85	0.39	5.75	0.41	5.72
		3	0.62[a]	5.14	0.65[a]	5.08	0.70[a]	4.99
	5	0	0.20	6.42	0.21	6.41	0.23	6.34
		1	0.29	6.16	0.30	6.13	0.34	6.00
		2	0.45	5.68	0.49[a]	5.59	0.51[a]	5.49
		3	0.69[a]	5.01	0.73[a]	4.96	0.75[a]	4.86

T.A.: Total acidity in lactic acid (%).
[a]: Coagulated

Table 8

Effect of inoculum percentage, nutrient additives and temperature on the acid production of mixed culture of Bifidobacterium bifidum *and* Lactobacillus bulgaricus

T (°C)	Inoc. (%)	Time (h)	Cow's milk		Cow's milk + 1% glucose		Cow's milk + 6% tomato juice	
			T.A.	pH	T.A.	pH	T.A.	pH
40	3	0	0.19	6.48	0.20	6.47	0.22	6.41
		1	0.26	6.26	0.28	6.22	0.32	6.10
		2	0.38	5.80	0.38	5.84	0.44	5.69
		3	0.58[a]	5.21	0.60[a]	5.18	0.65[a]	5.10
	5	0	0.20	6.44	0.21	6.44	0.22	6.40
		1	0.38	6.08	0.33	6.07	0.36	5.96
		2	0.46	5.65	0.49[a]	5.59	0.52[a]	5.49
		3	0.62[a]	5.12	0.64[a]	5.10	0.68[a]	5.04
45	3	0	0.20	6.47	0.20	6.44	0.22	6.41
		1	0.29	6.18	0.29	6.17	0.34	6.01
		2	0.44	5.68	0.48[a]	5.60	0.52[a]	5.44
		3	0.63[a]	5.11	0.65[a]	5.09	0.71[a]	4.98
	5	0	0.21	6.43	0.21	6.43	0.22	6.39
		1	0.33	6.05	0.34	6.03	0.37	5.92
		2	0.51[a]	5.49	0.52[a]	5.46	0.56[a]	5.33
		3	0.68[a]	5.02	0.69[a]	5.00	0.74[a]	4.91

T.A.: Total acidity in lactic acid (%).
[a]: Coagulated

The effects of the temperature, inoculum size and medium-additives on the acid production of single and mixed cultures were evaluated separately, applying the ANOVA program of the STATGRAPHICS 5.1 program-package. The analysis of variance was performed by involving the acidity values of the 6th hour in the case of single cultures and those of the 3rd hour in the case of mixed cultures. The results are demonstrated by Figs. 1–6.

On the basis of the analysis of variance demonstrated in Figs. 1–6, the following conclusions can be drawn.

The difference between acid productions of the single and mixed cultures was independent of fermentation medium, temperature and inoculum size. The mixed cultures resulted in the highest acid production rate in all treatments.

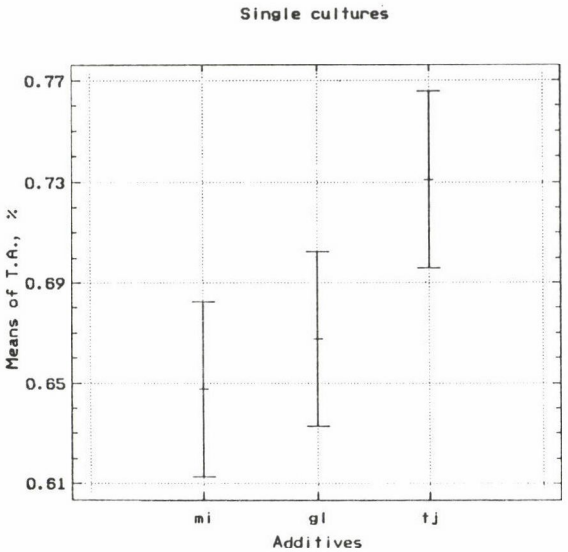

Fig. 1. Effect of additives on the acid production of single cultures (mi: cow's milk, gl: cow's milk with 1% glucose, tj: cow's milk with 6% tomato juice)

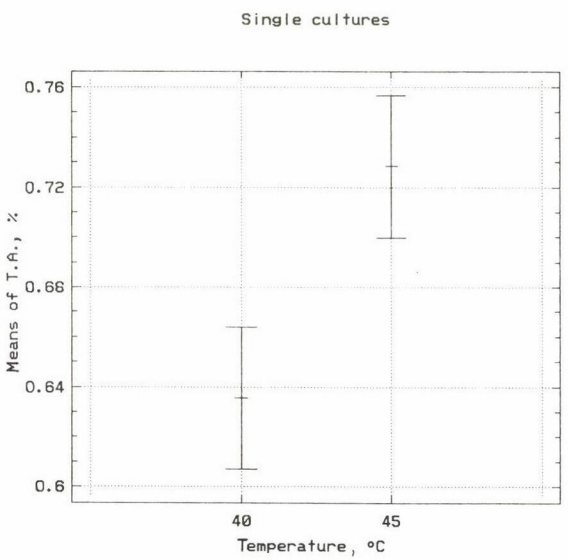

Fig. 2. Effect of the temperature on the acid production of single cultures

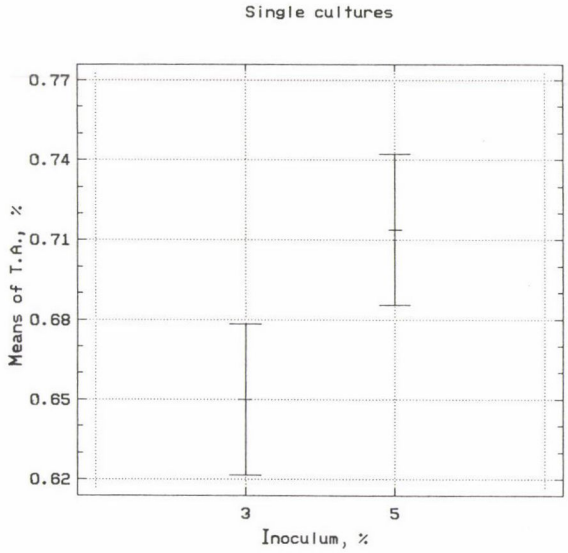

Fig. 3. Effect of the inoculum size on the acid production of single cultures

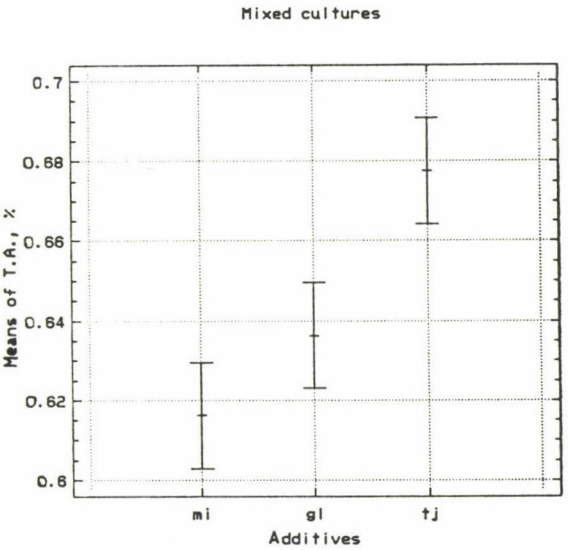

Fig. 4. Effect of the additives on the acid production of mixed cultures. (mi: cow's milk, gl: cow's milk with 1% glucose, tj: cow's milk with 6% tomato juice)

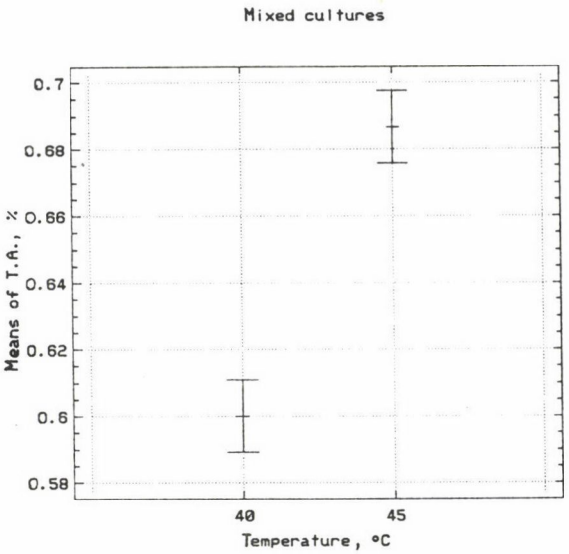

Fig. 5. Effect of the temperature on the acid production of mixed cultures

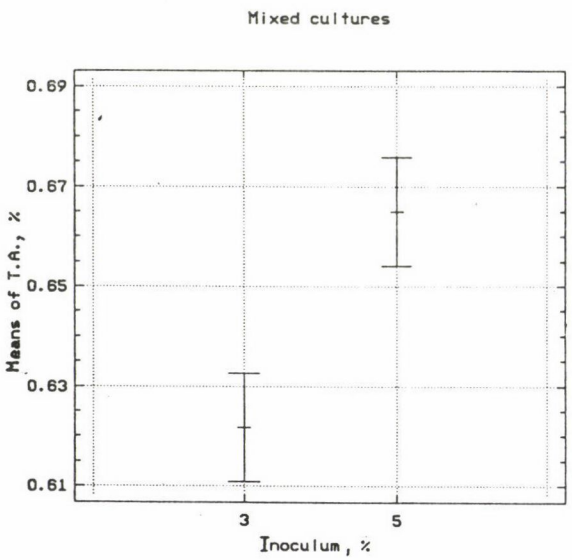

Fig. 6. Effect of the inoculum size on the acid production of mixed cultures

The effects of the medium-additives on acid production of the cultures were equal to the results of our previous investigations with *Lactobacillus acidophilus* and *Streptococcus thermophilus* (BADRAN & REICHART, 1993). Addition of 1% glucose to the milk resulted in a slight (not significant) positive effect on acid production. Addition of 6% tomato juice increased significantly the acid production both in single and mixed cultures. The order of stimulatory effect of the media was independent of the strains, temperature and inoculum size.

The effects of temperature and inoculum size on the acid production were as expected: increasing temperature and inoculum percentage resulted in higher acid production rates.

References

BADRAN, I. I. & REICHART, O. (1993): Comparative study on some fermentation properties of *Streptococcus thermophilus* and *Lactobacillus acidophilus* in milk and modified milk media. – Part I. Effect of the nutrient additives and fermentation temperature. *Acta Alimentaria*, *22*, 359 – 373.

GOH, J. S., KWON, I. K. & KIM, Y. O. (1986): Studies on the growth of *Bifidobacterium bifidum* ATCC 11863. in milk. *Korean J. Dairy Sci.*, *8*, 48 – 56.

GOH, J. S., KWON, I. K., AHN, J. K. & YOON, Y. H. (1987): Fermentation of milk by bifidobacteria, and improvement of usability of *Bifidobacterium bifidum* ATCC 11863 by lactic acid bacteria for the fermentation of milk. *Korean J. Dairy Sci.*, *9*, 211 – 219.

HWA, L. W., LAVANAYA, K. & HESSELTINE, C. W. (1974): Lactic acid fermentation of soybean milk. *J. Milk Fd Technol.*, *37*, 71 – 73.

KURMAN, J. A. & RASIC, J. LJ. (1988): Technology of fermented special products. – in: International Dairy Federation: *Fermented milks science and technology*. pp. 186 – 204.

SCHULER-MALYOTH, R., RUPPERT, A. & MÜLLER, F. (1968): Die Technologie der Bifiduscultur im milchverarbeitenden Betrieb. *Milchwissenschaft*, *23*, 557 – 558.

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FUNCTIONAL PROPERTIES OF ENZYMATICALLY MODIFIED MILK PROTEINS

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Enzymatic modification reactions are mostly suitable for inducing conformational change, that may result in altering the functional properties of the food proteins. In the present work the effect of the enzymatic hydrolysis and enzymatic peptide modification (EPM) and covalent methionine enrichment on the functional properties of milk proteins was investigated. Emulsifying stability of the EPM products tested were very high (ESI = 2.2 or 2.5). Several EPM products of milk proteins did not produce high foam capacity, but these low values are of a stable character. Based on our results, the methionine enrichment and the transpeptidation reaction during the EPM influenced in a particular manner the emulsifying and the foam properties of the protein products.

Keywords: functional properties, enzymatic modification, Met-enrichment, modified cow's milk protein, EPM

Enzymatic peptide modification (EPM) is an available method for improvement of the biological value of milk proteins via covalent methionine enrichment of the protein chains (FUJIMAKI et al., 1977; HAJÓS et al., 1990) and for changing their biological activity (HAJÓS et al., 1993).

TSAI and co-workers (1974) suggested that condensation played role in the enzyme catalyzed reaction, and they found a significant increase in the molecular mass of the product as a result of polycondensation reactions. LORENZEN and SCHLIMME (1992) found that proteolysis of sodium caseinate led to enzyme-induced turbidity of the protein solution as a result of aggregate formation.

LOZANO and COMBES (1992) concluded that the presence of hydroxylated additives increased the role of condensation reaction during the α-chymotrypsin--derived plastein synthesis.

In the course of our earlier studies we have elaborated a special method of this enzyme catalyzed process for designed modification of the amino acid content and

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conformation of food proteins. In these EPM reactions, mainly covalent methionine incorporation and transpeptidation took place (HAJÓS et al., 1988).

Protein chains modified by heat denaturation and proteolysis, amino acid incorporation and transpeptidation may display different functional properties (KATO et al., 1983; 1986). As milk proteins posses good surface activities (KINSELLA & WHITEHEAD, 1987), the question of the changes in the functional properties of the EPM-products is of special interest.

The present study was carried out to determine the functional properties of some enzymatically modified and EPM-treated products of milk proteins.

1. Materials and methods

1.1. Substrates and reference proteins

1.1.1. Casein (Hammersten, Reanal) and an enzymatically prehydrolyzed commercial milk protein concentrate (SR) (Sportrobi, Répcelak, Hungary) were used as substrates, bovine serum albumin and egg-white, as reference proteins.

1.1.2. Enzymes used included α-chymotrypsin (Sigma), Alcalase (NOVO) and papain (Calbiochem).

1.1.3. Hydrolysates: The enzymatically prehydrolyzed product SR without further hydrolysis and casein hydrolyzed by alcalase, α-chymotrypsin and papain, respectively, were used.

1.2. EPM-products

The concentration of the hydrolysates was 20% w/v in the EPM reactions.

A methionine methyl ester : substrate ratio of 1:5 was used for the incorporation in the enzyme catalysed reactions with amino acid enrichment. After incubation, the products with methionine incorporation were simultaneously dialyzed for 2 days through a cellophane membrane against distilled water. The nondialyzable fractions and the EPM products without amino acid enrichment were freeze-dried.

Exopeptidase treatments:

Covalent methionine incorporation in the EPM products with amino acid enrichment was verified by exopeptidase hydrolysis of the protein chains.

Leucine aminopeptidase (LAP) treatment:

A suspension of the EPM-product (5 mg) was incubated with LAP in 100 μl 0.1 mol NH_4HCO_3 buffer. Samples (10 μl) were investigated after

incubating for 0, 10, 30, 60 and 120 min, respectively, at which times each sample was immediately mixed with an equal volume of cc. HCl to stop the enzyme reaction. Free amino acids were separated by thin-layer ion exchange chromatography. A Shimadzu CS 930 TLC Data Recorder DR-2 densitometer was used for quantitative determination.

Carboxypeptidase treatment

The samples were incubated with a mixture of carboxypeptidase A and carboxypeptidase B (1:1). The treatment and the amino acid determinations were the same as in the leucine-aminopeptidase treatment.

1.3. Functional properties

1.3.1. Water binding capacity was measured by capillary method. In the case of partially or fully soluble samples, zero or negative capacities were calculated.

1.3.2. Surface hydrophobicity (S_0) was estimated fluorometrically from the soluble part of the proteins with anilino naphtaline sulphonic acid (ANS) (LUDWIG & LUDWIG, 1985). The S_0-values were calculated from the fluorimetric data of protein solutions of 1 per cent concentration.

1.3.3. Emulsifying activity (EAI) was determined by turbidimetric technique with some modification (LUDWIG & LUDWIG, 1985) of the method of PEARCE and KINSELLA (1978) in accordance with the next equation:

$$EAI = 2.23\, A/\varphi\, c,$$

where A: extinction at 500 nm after 1 min incubation of the emulsion;
φ: ratio of oil in the whole volume;
c: concentration of the protein solution in g 100 ml^{-1}.

1.3.4. Emulsifying stability index (ESI). The period (in hours), necessary to the reduction of extinction (A) to its half.

In the case of bovine serum albumine the value of EAI: 54 m^2g^{-1} and of ESI: 0.92 h were determined.

1.3.5. Foam capacity. Four ml of protein solution (10 mg ml^{-1}) was foamed by an air gas stream of 15 ml min^{-1}. The foam volume was determined in one minute and expressed as foam capacity (FC):

$$FC[\%] = \frac{\text{foam volume} - \text{volume of remaining solution}}{\text{volume of protein solution}} \times 100$$

where: foam volume means the total volume of foam + remaining solution after bubbling the solution by air.

The foam stability (FS) was calculated from the remaining foam volume after "t" minutes:

$$FS_t[\%] = \frac{\text{initial foam volume} - \text{remaining foam volume}}{\text{initial foam volume}} \times 100$$

2. Results and discussion

Table 1 shows some characteristic components of the samples investigated.

Table 1

Some characteristic components of the samples

Samples	Protein (%)	Ash (%)	Water (%)	Methionine[a] (%)
Casein	84			
H1	81	5.6	10.6	
EPM 1/a	84	4.3	9.4	
H2	78	5.8	9.4	
EPM 2				2.1
EPM 2/a		1.2		4.7
H3	84	3.7	8.6	
EPM 3	85	3.2	10.2	1.9
EPM 3/a	85	2.0	12.0	4.8
SR	84	5.1	9.4	
SR H 4	82	5.3	9.6	
EPM 4	87			
EPM 4/a	87		9.0	4.6

[a] Methionine content is given as a per cent of the total amino acids (determined by BIOTRONIK amino acid analyzer)

There are no essential differences in the protein, ash- and water-content of the samples investigated. However, the Met-content of the samples varied considerably because of the covalent methionine enrichment of some EPM-products.

The functional properties of following products were measured.

H1, H2, H3: hydrolysates of casein by alcalase, α-chymotrypsin and papain, respectively

SR: enzymatically prehydrolysed commercial milk protein concentrate

SR H4: α-chymotryptic hydrolysate of SR
EPM 1/a: Met-enriched EPM product of H1 (enzyme catalyst: Alcalase)
EPM 2: EPM product of H2 without amino acid enrichment (enzyme catalyst: α-chymotrypsin)
EPM 2/a: Met-enriched EPM product of H2 (enzyme catalyst: α-chymotrypsin)
EPM 3: EPM product of H3 without amino acid enrichment (enzyme catalyst: papain)
EPM 3/a: Met-enriched EPM product of H3 (enzyme catalyst: papain)
EPM 4: EPM product of SRH4 without amino acid enrichment (enzyme catalyst: α-chymotrypsin)
EPM 4/a: Met-enriched EPM product of SRH4 (enzyme catalyst: α-chymotrypsin)

The data are summarized in Table 2.

The water binding capacity was found to be zero in the case of the hydrolysates H1, H2 and SRH4 and zero or low values in two of the EPM samples without amino acid enrichment: in EPM 2 and EPM 4. These hydrolysates and the two α-

Table 2

Functional properties of substrates and enzymatically modificated proteins[a]

Proteins	Water-binding capacity	S_0	EAI (m^2g^{-1})	ESI (h)	FC (%)	FS (%min^{-1})
Casein	1.84	475	34.0	0.2	421	5.3(10')
H1	0	270	30.0	0.6	450	5.6 (6')
EPM 1/a	0.28	391	19.6	1.6	213	0 (2')
H2	0	203	12.0	2.5	175	0 (1.5')
EPM 2	0	203	13.7	2.2	330	3.5(10')
EPM 2/a	0.52	224	20.3	1.7	337	0.7(15')
H3	1.29	242	43.0	0.4	450	0 (4')
EPM 3	0.80	146	25.5	1.7	50	0 (0.5')
EPM 3/a	1.12	77	21.7	1.8	45	9 (2')
SR	1.32	624	39.0	0.2	413	3 (5')
SR H 4	0	179	31.0	1.4	113	0 (1.5')
EPM 4	0.31	117	17.0	2.5	10	0 (1')
EPM 4/a	0.96	179	18.8	1.4	119	2.2 (2')

[a] Degree of hydrolysis (DH) during the enzymatic treatments was also investigated (HAJÓS et al., 1988)
S_0: surface hydrophobicity
EAI: emulsifying activity
ESI: emulsifying stability
FC: foam capacity
FS %/(min): foam stability

-chymotryptic EPM products (without amino acid enrichment) were fully soluble in water. This is why their water binding capacity values fall down to zero.

The water binding capacity of the methionine enriched EPM products, however, increased significantly (EPM 1/a: 0.28, EPM 2/a: 0.52, EPM 3/a: 1.12 and EPM 4/a: 0.96) in comparison to their hydrolysates.

This essential increase in the water binding capacity of the Met-enriched samples is a straightforward consequence of the hydrophobic character of the methionine built in to the peptide chains.

High water binding capacity was shown only by the two commercial products: casein and SR, although the latter one is a prehydrolyzed protein.

Of the hydrolysates only the papaic hydrolysate of casein was different from the others. This sample (H3) had a higher value (1.29) for the water binding capacity.

The specificity of papain is fairly broad and different from that of the α-chymotrypsin. This fact may account for the irregularly high water binding capacity of the protein chains in the papaic hydrolysate of the casein.

The hydrophobic character (S_0) that was determined from the soluble part of the samples, indicates the high values in case of the substrate proteins casein and SR, and in case of one of the products, the sample EPM 1/a, which is a Met-enriched product prepared by alcalase catalysis.

However, in case of the different proteolytic hydrolysates and of other EPM products, we have not noticed any significant change in the S_0-values of our samples due to the enzyme processes.

As for S_0-values, the sample EPM 3/a showed a special behaviour producing a significantly low S_0-value.

Emulsion activity was found low (34.0) for casein, and the values determined for enzyme hydrolysed and modified products were even lower, in general. The papain hydrolysate sample H3, showed here a different behaviour as well, this was the one of the sample series that had the highest EAI-value (43.0).

The emulsion stability of the enzymatically modified products displayed quite opposite tendencies to the values for emulsion activity. The ESI-values were higher in all samples than the initial casein ESI-values. In sample H3, the papain hydrolysate, where the highest EAI-value had been determined, a relatively lower emulsion stability value (ESI = 0.4) was measured.

Four samples of the products on casein base and modified with α-chymotrypsin produced very significant emulsion stability. These were the followings: H2, EPM 2, EPM 2/a and EPM 4. Their ESI-values were 2.5, 2.2, 1.7 and 2.5, respectively.

An important functional property of proteins and peptid mixtures is their emulsifying behaviour. This is highly influenced by the molecule structure, the position and ratio of hydrophobic-hydrophylic amino acids.

KATO and co-workers (1985) observed, that in case of proteins with great molecular masses, there is a definitive relation between surface hydrophobicity and emulsifying behaviour. This observation could not be extended by our actual investigations to the proteolytic hydrolysates of casein and the EPM-products. LUDWIG and LUDWIG (1985) could not support the observations of KATO and co--workers (1985) with their studies carried out with enzyme modification of *Vicia faba* proteins, either.

Nevertheless, in case of the peptid mixtures tested, another specific property draws attention: the ESI-values of our enzymatically modified products tested are very high. ESI = 2.2 or ESI = 2.5 are so high indices, that even proteins of great molecule masses do not have such emulsion stability.

A most likely explanation of this phenomenon is that in case of hydrolysates and EPM-products, where the degree of hydrolysis is not too high, protein chains are generally so long that they can optimally stabilize the oil drops in aqueous solution. This means, that the relatively few oil drops are closely bound in the emulsion by these peptides. A great role can be attributed to the enzyme specificity beside the molecule size of peptide chains in the development of this phenomenon. The α-chymotryptic hydrolysates and modified products of casein such as H2, EPM 2 and EPM 2/a, have ESI-values of an unusual degree. Nevertheless, the papain catalyzed casein hydrolysate shows strikingly high EAI-value in vain, its emulsion stability is not high at all.

Determining the C- and N-terminal sequence of the peptides, the enzyme specificity basically influences the emulsion stability.

Based on our results, an important foam building property can be attributed to the samples H1, H3 and SR.

Foam capacity of the samples H1 and H3 was slightly higher than that of the initial protein. The foam capacity of the sample SR was near similar to that of the casein.

A significantly higher value for the foam stability of casein was shown only by one of the samples (EPM 3/a). The foam stability of the casein and of the enzyme hydrolysed or modified product prepared from it was in a much lower domain than that of the egg protein (FS: 94/60').

The result that the enzyme hydrolysates and enzymatically modified products of milk proteins do not produce high foam capacity, but their low value capacity is of a stable character, does not support the observations of KATO and co-workers (1985), but agrees of LUDWIG and LUDWIG (1985).

It is quite probable that enzymatic hydrolysis or enzymatic peptide modification can be a way to improve the functional properties of the product, being the size and composition of peptides or their terminal sequence the main targets of modification.

References

FUJIMAKI,. M., ARAI, S. & YAMASHITA, M. (1977): *Enzymatic protein degradation and resynthesis for protein improvement*. Adv. in Chemistry Series, No. 160, pp. 156–184.

HAJÓS, GY., HUSSEIN, S. & GELENCSÉR, É. (1993): Enzymatic peptide modification of food proteins. – in: SCHWENKE, K. D. & MOTHES, R. (Eds) *Food proteins: Structure and functionality.* (4th Symposium on Food Proteins, Reinhardsbrunn, 1992). VCH Verlagsgesellschaft, pp. 82–86.

HAJÓS, GY., MIETSCH, F. & HALÁSZ, A. (1988): Change of degree of hydrolysis in the course of enzymatic peptide modification. *Acta Alimentaria*, *17*, 265–270.

HAJÓS, GY., NÖTZOLD, H., HALÁSZ, A. & LUDWIG, E. (1990): Characterization of peptides enriched in methionine by enzymatic peptide modification. *Acta Alimentaria*, *19*, 73–78.

KATO, A., FUJIMOTO, K., MATSUDOMI, N. & KOBAYASHI, K. (1986): Protein flexibility and functional properties of heat-denaturated ovalbumin and lysozyme. *Agr. biol. Chem.*, *50*, 417–420.

KATO, A., KOMATSU, K., FUJIMOTO, K. & KOBAYASHI, K. (1985): Relationship between surface functional properties and flexibility of proteins detected by the protease susceptibility. *J. agric. Fd Chem.*, *33*, 931–934.

KATO, A., OSAKO, Y., MATSUDOMI, N. & KOBAYASHI, K. (1983): Changes in the emulsifying and foaming properties of proteins during heat denaturation. *Agric. biol. Chem.*, *47*, 33–37.

KINSELLA, J. E., WHITEHEAD, D. M. (1987): Modification of milk proteins to improve functional properties and applications. – in: *International Dairy Congress Dordrecht*, Netherlands, D. Reidel Publishing Co., Dordrecht, pp. 791–804.

LORENZEN, P. C. & SCHLIMME, E. (1992): The plastein reaction: properties in comparison with simple proteolysis. *Milchwissenschaft*, *47*, 499–504.

LOZANO, P. & COMBES, D. (1992): α-chymotrypsin in plastein synthesis *Applied Biochem. Biotechn.*, *33*, 51–65.

LUDWIG, I. & LUDWIG, E. (1985): Untersuchungen zum Emulgierverhalten von enzymatisch modifiziertem Ackerbohnenproteinisolat. *Nahrung*, *29*, 949–955.

PEARCE, K. N. & KINSELLA, J. E. (1978): Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *J. agric. Fd Chem.*, *26*, 716–723.

TSAI, S. J., YAMASHITA, M., ARAI, S. & FUJIMAKI, M. (1974): Polyacrylamide gel electrophoresis of plasteins. *Agric. biol. Chem.*, *38*, 641–644.

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SHORT COMMUNICATION

PRODUCTION AND ANALYTICAL TESTING OF POWDERED GRAPE COLORING AGENT

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During our laboratory experiment we produced grape anthocyanin color-rich powders from table blue grapes grown in Chile using a multi-step procedure of extraction, separation, concentration by solvent destillation and vacuum-drying. The stability of pigment powders was increased by mixing with foodgrade additives. Samples were investigated by photo-acoustic spectrophotometry (PAS), and tristimuli color-measurement. Data obtained from spectrophotometric analysis were processed and evaluated by principal component analysis method, which separated pigment-rich samples according to color-intensity and color-values. Original extracts and re-dissolved powders were analysed by HPLC for anthocyanine-composition and sample identification. Most colorful samples were identified and put into different groups with the same result by using PAS and HPLC thus reflecting well on effectivity of extraction methods and protection by additives.

Keywords: natural coloring agents, anthocyanin stability, HPLC, PAS, PCA model

Coloring, aromatizing and other additives are used in various products, at various places in the food industry. Due to convencience or economical motives, e.g. stability, availability, assortment, production price, etc., the artificial or nature-identical coloring substances are the most popular. However, after thorough toxicological examinations, more and more artificial coloring agents have been detected to have disadvantageous and even harmful effects on human organism (being toxical or causing allergy). The natural coloring agents – being original components in our foods – do not have these undesirable effects, even some of them are definitely advantageous from therapeutical aspects (e.g. French anthocyanin capsules, red beet powder, etc.).

Nowadays, the foods containing pure natural components are highly valuable products being widely demanded for consumption. In order to satisfy this increased demand, intensive research has been carried out world-wide for the production of new natural coloring agents and aroma products. The industrial utilization of natural

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coloring and aroma agents is greatly limited by the instability of these substances. The sensitivity against oxidation of carotenoids, the pH-dependent stability of anthocyanins, or the susceptibility of volatile oils to terpenization are all well known. This is why the applied industrial research put as its priority aim the solution of stability problems.

1. Materials and methods

A rather great amount of pressed grape pomaces remains after the production of red wines in Hungary. This pomace contains 50% of the original red-colored anthocyanin coloring matter. With traditional extraction procedures (KAMPIS, 1985, WALLIN, 1980, LANGSTON, 1985), the coloring matter content can be obtained only in about 60–70%. We tried to elaborate a new method of production by which an anthocyanin coloring powder resisting better to environmental effects, being stable and obtainable in a higher percentage could be produced under gentle conditions with the use of natural substances.

For the production of anthocyanin coloring powder, the skins of Chilian blue table grapes have been used. From this, the coloring matter components were multistage extracted by hydrochloric acid-ethanol or tartaric acid-ethanol extraction procedure at 50 °C temperature.

In case of both coloring matter extractions, the pH was adjusted to 3.0, and then, after a previous test evaporating procedure, concentration was carried out under vacuum to a solid substance content of 33–48% with the help of a Heidolph VV 2000 rotation vacuum evaporator (45 °C water bath temperature, 210 l min^{-1} rotation). The anthocyanin coloring matter granulates were prepared under gentle conditions (10 mbar vacuum, max. 40–50 °C temperature) with a Labor MIM discontinuous vacuum dryer (PAP, 1991). In order to promote vacuum drying of the anthocyanin concentrate and to increase the stability of the coloring granulate obtained, food grade carrier agents were added to the concentrated extracts. Of the permitted carrier- and microcapsulating agents available, maltodextrin and arabic gum were chosen based on the previous testing of solution and of formed viscosity. These were mixed to the concentrates in ratio of 1, 40, 50 and 60% (Table 1), related to the solid substance content of the concentrates and the carrier materials.

After the end of drying, the foam generated was cooled and crushed under the exhauster in a space of reduced humidity content (20% RH) and disintegrated with the help of a metal sieve. The obtained hygroscopic crystal granulates were filled into hermetically closed glass vessels and stored in dark.

Table 1

Additives and their mixing rate

Sample code	Carrier materials	Mixing rate	Type of extraction
B1	maltodextrin	50%	tartaric acid + EtOH
B2	maltodextrin	60%	tartaric acid + EtOH
B3	maltodextrin, arabic gum	49.5%:1%	tartaric acid + EtOH
S1	maltodextrin	50%	HCl + ETOH
S2	maltodextrin	60%	HCl + EtOH
S3	maltodextrin, arabic gum	49.5%:1%	HCl + EtOH
S4	maltodextrin, arabic gum	49%:2%	HCl + EtOH

2. Results

In our analytical testing, HPLC as chromatographic methods, PAS spectroscopic methods and CIELAB colorimeter, an independent non-destructive method were used. Our HPLC analysis was carried out with an equipment provided with Waters 660 MS pump, with injector and program control, and with Waters 990 MS diode series detector. For the separation of anthocyanin components, a 250 × 4.6 mm Waters μ-Bondapak C−18 reversed phase analytical column with 10 μm filling was used. The chromatograms were taken with gradient elution of 1.5 $cm^3\ min^{-1}$ volume flow, with aqueous tri-flour-acetic acid and acetonitril eluent (pH = 2), at 25 min of time period, at three characteristic wave lengths (280, 330 and 530 nm). The spectra corresponding to the peaks of the chromatograms were identified on base of the spectra of delphynidin, cyanidin and peonidin standards (Fig. 1).

During chromatographic separation, six well-distinguishable peaks were identified in case of the tartaric acid-ethanol extracts (Fig. 2) and the hydrochloric acid ethanol extracts (Fig. 3). They represented clearly the delphynidin, cyanidin, peonidin, petunidin, malvidin an pelargonidin grape anthocyanin components being present in South-American grape varieties.

The comparison of the chromatograms of the different samples was carried out on the base of the comparison of heights of the peaks belonging to resp. retention times. As the chromatographic tests were carried out from samples having different coloring matter content, the peak heights obtained for the coloring matter distribution in the samples were altered in relation to a chosen coloring matter sample, proportionally to the total substance content, with multiplication by a norming factor.

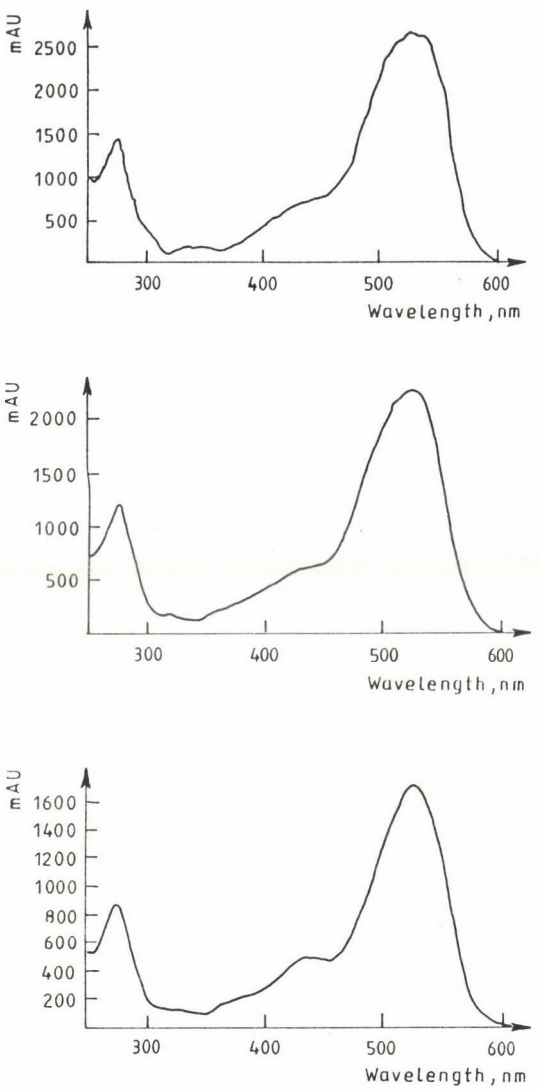

Fig. 1. Standard HPLC spectra of delphynidin (A), cyanidin (B) and peonidin (C)

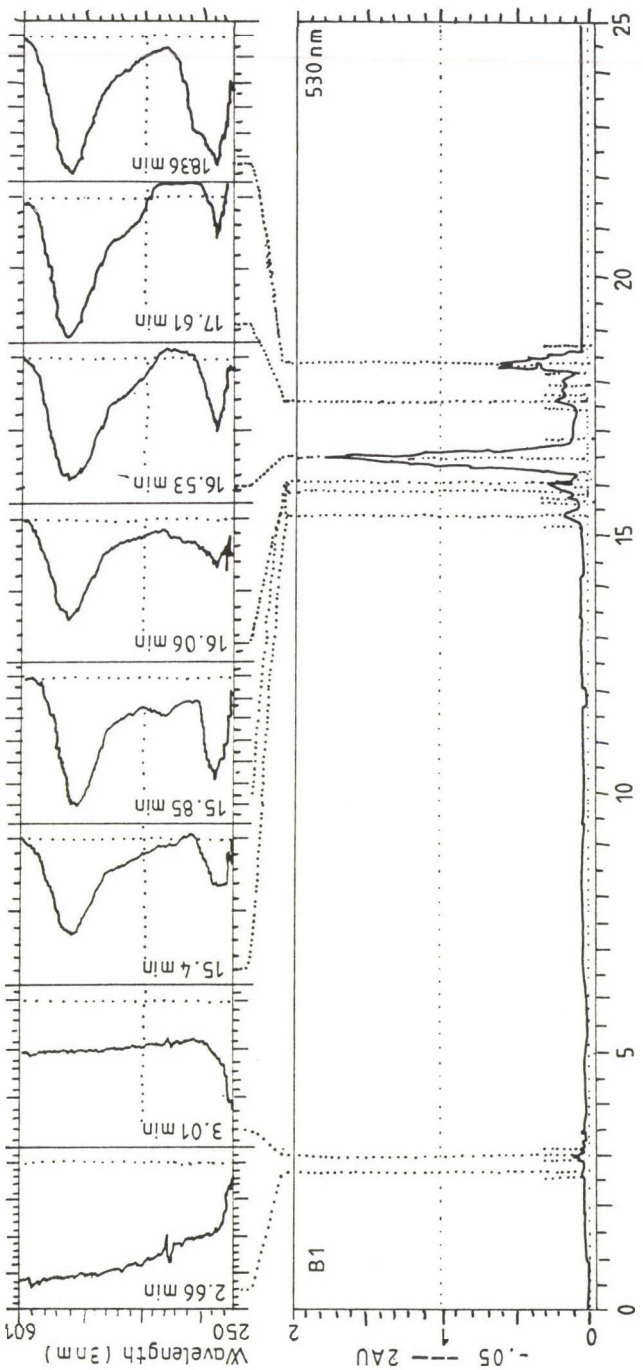

Fig. 2. HPLC chromatogram and spectra of B1 extract obtained by tartaric acid

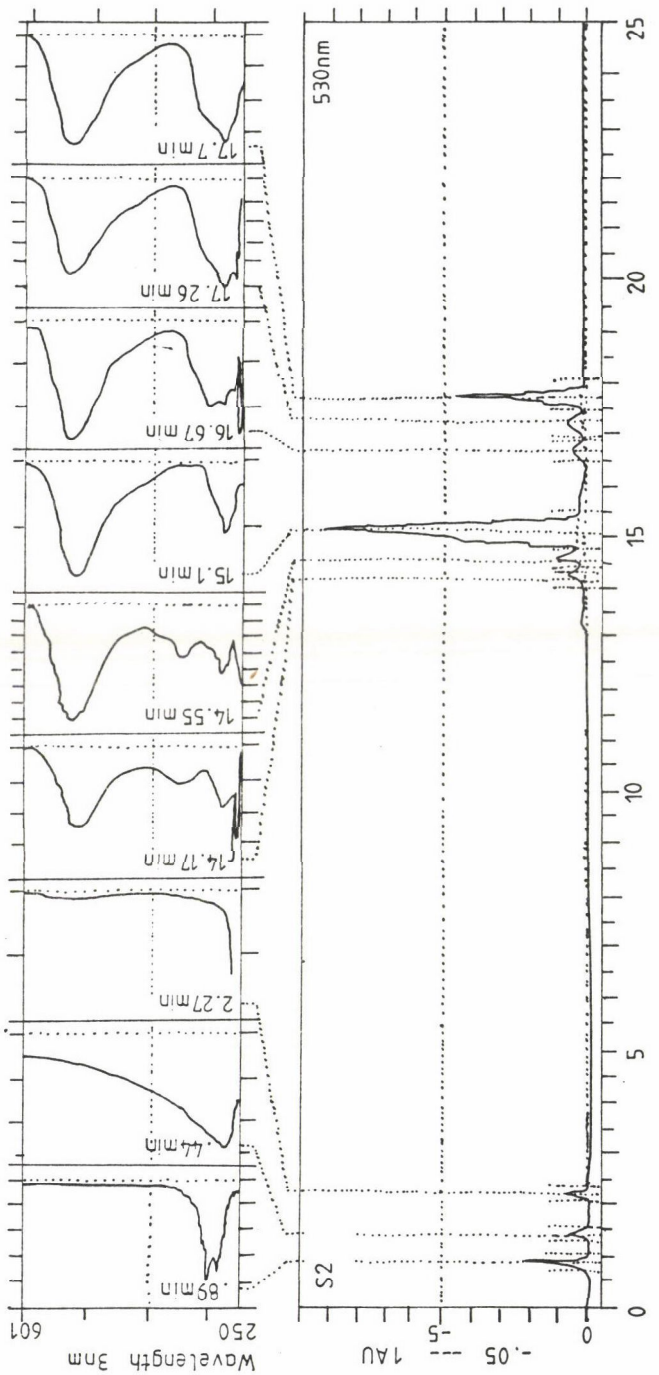

Fig. 3. HPLC chromatogram and spectra of S2 extract obtained by hydrochloric acid

The normed peak heights (P) – thus the chromatograms of the samples – were compared on the base of a quality measuring value created by us, the pattern (M):

$$M_i = \frac{P_{i,\,sample}}{P_{i,reference}}$$

where

$P_{i,s}$ is the i-th peak height contained in the chromatogram of the tested sample,

$P_{i,r}$ is the i-th peak height in the chromatogram of the comparing sample (In our case, the normed peak heights of sample S4 were chosen as reference.)

PAS analysis (NAGEL, 1988; KOCSÁNYI & RICHTER, 1983) was carried out on the pilot photoacustic spectrophotometer being in the possession of the Technical University, Budapest and the Central Food Research Institute (CSX 450 OF xenon lamp, Jobin-Yvon H20FIR monochromator, 2 lock-in amplifiers) with 4 nm pace distance in a wavelength domain between 400–720 nm, with 17 Hz modulation frequency and 1×10^4 signal i.e. 3×10^2 reference signal intensification. As reference spectrum, the spectrum of pure coal substance was taken and the intensity changes of absorptive character of the samples were based upon this (relative PAS signal).

During color measurements with the Momcolor-D tri-stimuli colorimeter, the measuring values being characteristic to the color of the anthocyanin coloring matter granulates were determined by objective photoelectric means with a red reference standard (LUKÁCS, 1982). From the color stimuli components measured (X, Y, Z) the space coordinates of the CIELAB color stimuli measuring instrument were calculated (a*, b*, L*). Based on these coordinates, the difference of the lightness factor of the sample and that of the standard was determined (dL*). Measuring values and coordinates were drawn up in two- i.e. three-dimensional form and the samples were grouped on this base.

3. Conclusions

In our tests it has been found, that there was only slight difference of shade by visual observance between the anthocyanin coloring matter granulates prepared from the two kinds of concentrates. This difference could be the consequence of the extraction i.e. the pH-adjustment and the color changed on the effect of carrier- and microcapsulating agents added in different concentrations to the samples. On the base of the analytical methods applied, we tried to determine the samples containing

Table 2

Quality measurement number of HPLC analysis
(S4 sample as reference)

	M(S1)	M(S2)	M(S3)	M(B1)	M(B1)	M(B1)
Peak 1	0.802	0.454	0.00	1.496	1.295	1.663
Peak 2	0.779	0.611	0.427	1.524	1.118	1.144
Peak 3	0.869	0.816	0.816	1.316	1.033	1.091
Peak 4	0.901	0.706	0.856	2.163	1.455	1.635
Peak 5	0.901	0.824	0.866	1.923	1.213	1.402
Peak 6	1.001	0.854	0.943	1.145	1.017	1.068

the greatest amounts of coloring substances, having the strongest shade of color, to be able to choose the mostly suitable extraction and addition methods.

Of the samples separated on base of the quality attributes of HPLC analysis, the sample B1 prepared with tartaric acid-ethanol extraction and stabilized with 50% maltodextrin carrier agent showed the highest value (Table 2). Besides, on the base of the data, the samples prepared with the two kinds of extraction procedures can be clearly separated. The samples prepared with hydrochloric acid–ethanol extraction got values below 1, and those prepared with tartaric acid–ethanol procedure values above 1.

On base of the PAS measurement spectra, the samples prepared with the different extraction procedures could be well discerned either (Fig. 4). B1 sample showed the highest intensity change of adsorptive character and this corresponded to the results of the HPLC analysis.

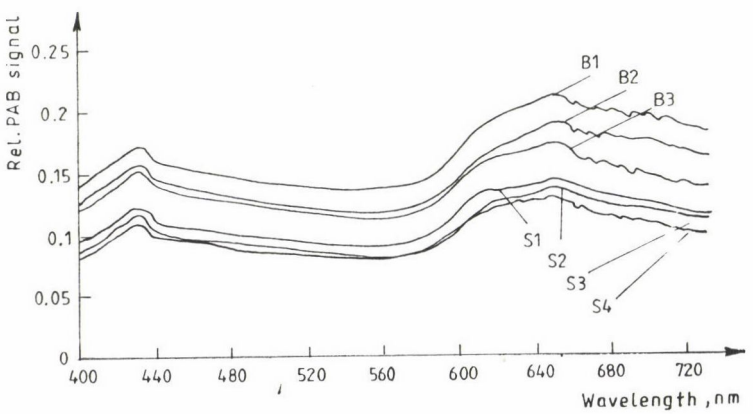

Fig. 4. PAS spectra of coloring samples

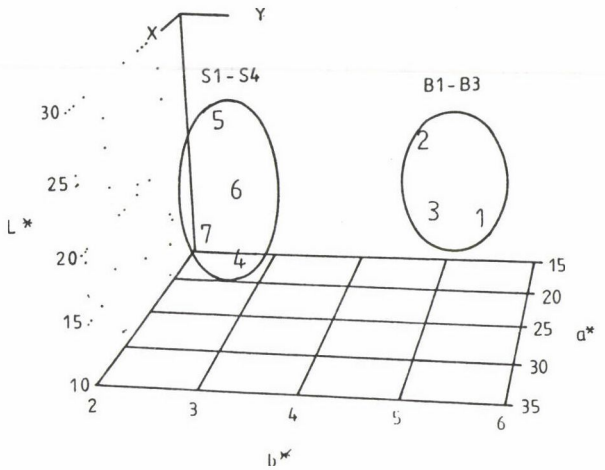

Fig. 5. Results of CIELAB color investigation

On base of the results of the CIELAB colorimetry, the samples prepared with the two kinds of extraction procedures could be well distinguished (Fig. 5) but it was not possible to select the sample containing the greatest amount of coloring substance by this method.

As a summary it can be stated that the grouping of the samples and the selection of the sample having the strongest shade of color and containing the highest amount of coloring substance could be effectuated PAS and HPLC methods as well, with exactly similar results. Both analytical methods relied upon the protective effect of carrier- and microcapsulating agents on coloring substances during drying and the extractive efficiency of extracting agents used for obtaining coloring matters. As the instrumental and material costs (solvents) of the HPLC method are rather high and the test operation is more sophisticated (the powders have to be resolved), and it is more time consuming it would be more reasonable to carry out such tests in the future with the rapid PAS method being less material demanding.

References

KAMPIS, A. (1985): Vörösbor színanyaggyártás Franciaországban. (Red wine pigment production in France.) *Borgazdaság*, *33*, 152 – 154.

KOCSÁNYI, L. & RICHTER, P. (1983): *A fotoakusztikus spektroszkópia (PAS) alapjai és élelmiszeripari alkalmazása.* (The base of PAS spectroscopy and its usage in the food industry.) Lecture notes, Technical University of Budapest, Budapest.

LANGSTON, M. S. K. (1985): *Anthocyanin colorant from grape pomace.* US Patent, No. 4500556.

LUKÁCS, GY. (1982): *Színmérés.* (Colorimetry.) Műszaki Könyvkiadó, Budapest.

NAGEL, E. M. (1988): *Photoakustische Untersuchungen an Pflanzen.* Lecture notes, University of Karlsruhe, Karlsruhe.

PAP, L. (1991): Production of whole fruit juice powders. *Food Technology International Europe.* Abstracts. pp. 141 – 143.

WALLIN, B. K. (1980): *Process of purifying plant anthocyanin colors.* US Patent No. 4211577.

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BOOK REVIEWS

Shelf life evaluation of foods

C. M. D. MAN & A. A. JONES (Eds)

Blackie Academic & Professional, London, etc.,
an imprint of Chapman and Hall, Glasgow, 1994, 321 pages

The new book of the publisher summarized concisely and authoritively information on the shelf life evaluation of foods. The theory and practice of shelf life determination and prediction are divided into two parts. The first part of the book contains the basic principles in 1–5 chapters on 84 pages. The first chapter covers the scientific principles of shelf life evaluation as problems of food determination, evaluation of food quality and the use of sensors to monitor the shelf life of foods. The methodology of shelf life determination is the subject of the second chapter in seven parts. The third chapter shows the principles and practice of shelf life prediction for microorganisms as the development of predictive models, the uses of models and limitations of models. The fourth section gives many practical information about the interaction between the packaging and food quality. The plastic packaging materials for food, the packaging systems, the influence of "scalping" on food, the flavour transfer problems in PET bottles are included. The last chapter of the first part is the preservation technology and shelf life of fish and fish products which leads through the practical part.

The chapters of the second part cover specific commodities and product groups in 6–15 chapters on 229 pages. The sixth chapter is dealing with the shelf life of delicatessen salads and chilled prepared fruits and vegetables. The next is the chapter of chilled yogurt and other dairy desserts which describes the main factors affecting shelf life and shelf life determination of yogurt – as model. The eighth chapter gives a review of modified-atmosphere-packed ready-to-cook and ready-to-eat meat products in five sections. Ambient packaged cakes is the following food item, and then potato crisps and savoury snacks, chocolate confectionary, and ready-to-eat breakfast cereals are analyzed from the aspect of quality changes and consequently shelf life determination regarding to packaging. The thirteenth chapter approaches the storage problems of thermally processed foods in containers other than cans from basic principles as product groups and their characteristic, specific factors affecting shelf life and determination of shelf life. The title of the next chapter is Ambient-stable sauces and pickles. Special attention should be paid to the section of challenge testing and the use of mathematical models. The last, fifteenth chapter is the Frozen foods with parts of how-many industries, Stability of frozen foods (from many aspects), Product characteristics and The cold chain. All the chapters have references and the last part is the index of objects.

The book is a good tool to those working in food industry, in academic and research institutions and it is also be of value to lecturers and students of universities and high schools.

I. VARSÁNYI

Rapid analysis techniques in food microbiology

P. D. PATEL (Ed.)

Blackie Academic & Professional, London, 1994, 294 pages

The food industry, with its diverse range of products is governed by strict food legislation, and microbiological safety has become a key issue. Today, more so than ever, microbiological control during food productions is essential to the manufacture and distribution of safe, wholesome products which meet desired shelf-life. Food manufacturers are demanding analytical techniques that are simple to use, cost effective, robust, reliable and can provide results in 'real time'. The majority of current microbiological techniques, particularly for the analysis of foodborne pathogens, give results that are only a retrospective value. Rapid methods for microbial analysis need to be considered in the context of modern Quality Assurance (QA) systems.

This highly practical book reviews the current status of the rapid or alternative methods from an international perspective, and particular emphasis is on commercially available detection and estimation systems. It contains 10 chapters written by world-wide known experts of food microbiology (D. A. A. Mossel, J. I. Pitt, A. N. Sharpe and others) discussing the history of the rapid and instrumental methodology, the membrane filtration techniques in microbial analysis, the evaluation of commercial kits and instruments for the detection of foodborne bacterial pathogens and toxins, the immunomagnetic, the automated electrical and the luminescent techniques in microbiological analysis of foods, the modern methods for the detection of viruses in foods, and the enumeration of foodborne fungi. This volume gives information on the detection and identification of foodborne microbial pathogens by polymerase chain reaction, and the application of the HACCP approach to managing food safety, the regulatory perspectives and the future directions.

This book is recommended for biochemists, microbiologists and technologists in the food industry and academic, research and public health institutions who are interested in the new generation of rapid methods.

CS. MOHÁCSI-FARKAS

PRINTED IN HUNGARY

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The effect of irradiation of sweet cherries
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RESPIRATION QUOTIENT AND YEAST METABOLISM

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Authors give an extended explanation and a simple mathematical model of the change of respiration quotient (RQ) during carbohydrate based baker's yeast propagation. On the basis of stoichiometric equations and applying the concept of the equivalents of available electrons, useful and simple expressions were deduced on the relationship of RQ to the overall cell yield. It is a widespread misunderstanding that the higher the C-incorporation is the lower the RQ will be. On the contrary, the direction of change and the value of RQ depend not only on the C-incorporation (composition of cells) but on the substrate composition as well. In conclusion, it is shown that RQ is a very informative fermentation variable but to use it as a control parameter is not as easy as usually thought.

Keywords: aerobic and anaerobic yeast growth, carbon/energy source composition, respiration quotient, stoichiometric model of baker's yeast fermentation.

Respiration quotient (RQ) has been widely used as a well-known metabolic characterisation of the gaseous metabolism of aerobic microbes. By definition this is the quotient of the evolved carbon dioxide and the consumed oxygen during the metabolism, both expressed in moles.

This notion can be defined for a given (e.g. total) time of cultivation, i.e. by static way, and in this case the integrated respiration quotient is:

$$RQ = \frac{\Delta CO_2}{\Delta O_2}$$

or more precisely

$$\frac{\int_{t_1}^{t_2} \frac{dCO_2}{dt} \cdot dt}{\int_{t_2}^{t_1} \frac{dO_2}{dt} \cdot dt}$$

or it can be defined infinitesimally as

$$RQ = \frac{\frac{dCO_2}{dt}}{\frac{dO_2}{dt}} = \frac{Q_{CO_2}}{Q_{O_2}}$$

where Q_{CO2} and Q_{O2} are the specific carbon dioxide evolution and oxygen consumption rates, respectively.

Everyone agrees that RQ gives a very good characterisation of the aerobic metabolism. This agreement is general in textbooks. Similarly, it is widely accepted that this is one of the most important variables of the aerobic cultivation and has a special role within the "gateway variables" (i.e. variables that can not directly be measured but can be calculated from other measured ones). This special role has been supported by its appropriateness in controlling fed-batch baker's yeast fermentation when applying carbohydrate as substrate (WANG et al., 1977; NYESTE et al., 1992; BELLGARDT & YUAN, 1991).

However there is no agreement about the significance of the actual values of RQ. What is the real meaning of the magnitude of RQ? How does this magnitude characterise the metabolism? How is this value influenced by other variables of the microbial system or how does this actual value influence the other parameters of the system? We try to answer some of the above mentioned questions in this paper. Significance of RQ was examined in the case of baker's yeast fermentation to find relationships between the parameters of the stoichiometric equation describing the microbial system (yields and composition of microbe and substrate) and the respiration quotient.

1. Relationship between RQ and yield in case of aerobic growth

Let us start with the following stoichiometric equation, which describes the total burning reaction of a carbohydrate (hexose) by molecular oxygen:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

or applying the C mole expressions

$$CH_2O + O_2 \rightarrow CO_2 + H_2O$$

as it was offered by ERICKSON and co-workers (1978).

The RQ value of this process is 1 and, considering the stoichiometry, it corresponds to e.g. the respiration of a microbe without growth. If growth and respiration occur at the same time then the RQ value must be less than 1 because a given part of the C-source incorporated into the new biomass will not be evolved as carbon dioxide. Furthermore if the cultivation is more effective – substrate

utilisation is better i.e. more carbon is incorporated into the cells – the RQ should be even less and lie between 0 and 1. The above cited paper of WANG and co-workers (1977) takes RQ value between 0.9 and 1 in case of this oxidative growth.

Similarly if ethanol is used as C/energy source the stoichiometric equation of the oxidation (without growth) is:

$$C_2H_5OH + 3O_2 \rightarrow 2CO_2 + 3H_2O$$

or

$$CH_3O_{0.5} + 1.5\ O_2 \rightarrow CO_2 + 1.5\ H_2O$$

Now RQ = 0.667 and inferring as in case of glucose the RQ value is expected to be between 0 and 0.667 in aerobic growth. The more effective the substrate utilisation is the less the RQ values will be, we may predict. In an ethanol-based *Hansenula anomala* cultivation (SEVELLA, 1983) the following stoichiometric equation described the system:

$$CH_3O_{0.5} + 0.959\ O_2 + 0.076\ NH_3 \rightarrow 0.489\ CH_{1.85}O_{0.48}N_{0.156} + \\ + 0.511\ CO_2 + 1.16\ H_2O$$

while cultivating *Saccharomyces cerevisiae* on ethanol as C/energy source approximately the next equation was valid:

$$CH_3O_{0.5} + 0.91\ O_2 + 0.075\ NH_3 \rightarrow 0.515\ CH_{1.63}O_{0.52}N_{0.17} + \\ + 0.485\ CO_2 + 1.155\ H_2O$$

In these processes the RQ values are less than 0.67 as expected. However NAGAI (1979) reported the following equation in case of a baker's yeast cultivation on hexose :

$$CH_2O + 0.64\ O_2 + 0.048\ NH_3 \rightarrow 0.325\ CH_{1.72}O_{0.44}\ N_{0.15} + \\ + 68\ CO_2 + 0.787\ H_2O$$

Here RQ = 0.68/0.64 = 1.062 greater than 1. NYESTE and co-workwers (1992) carried out baker's yeast cultivation on molasses and found the following equation:

$$CH_2O + 0.425\ O_2 + 0.089\ NH_3 \rightarrow 0.558\ CH_{1.64}O_{0.52}N_{0.16} + \\ + 0.676\ H_2O + 0.442\ CO_2$$

In this case the respiration process can also be characterised by RQ > 1. Therefore, in contrast with expectations, in the instance of aerobic growth on glucose C/energy source, RQ is greater than 1 while on ethanol it is less than 0.667. Therefore only the latter can be considered as a real upper boundary value. It seems that the prediction of RQ value is not as simple as it is generally thought, and depends on the chemical composition of the consumed C/energy source.

To find relationship between substrate and cell composition and respiration quotient let us consider the following general stoichiometric equation which can be described for any microbe and substrate in the case of aerobic cultivation provided that there is no significant extracellular product formation and the biomass can be represented by its average elementary composition.

$$CH_mO_l + bO_2 + aNH_3 \rightarrow y_cCH_pO_nN_q + cH_2O + dCO_2$$

Material balance equations are as follows:

Carbon balance: $$1 = y_c + d \quad (1)$$

Hydrogen balance: $$m + 3a = y_cp + 2c \quad (2)$$

Nitrogen balance: $$a = y_cq \quad (3)$$

Oxygen balance: $$l + 2b = y_cn + c + 2d \quad (4)$$

Coefficients m and l are known because the composition of C/energy source is supposed to be known and the composition of biomass, p, n, q can be determined experimentally. Applying the material balance equations the coefficients d (carbon dioxide) and b (oxygen) can be expressed and their ratio shows the following form:

$$RQ = \frac{d}{b} = \frac{4 \cdot (1 - y_c)}{y_c \cdot (2n + 3q - p - 4) + (4 + m - 2l)} \quad (5)$$

The two expressions of the denominator in parentheses are the equivalents of available electrons of biomass and substrate, respectively which were introduced by ERICKSON and co-workers (1978). The physical meaning of the equivalents of available electrons is the four fold value of the stoichiometric coefficient of oxygen in the corresponding "burning" equation

$$\gamma_x = 4 + p - 3q - 2n \quad \text{and} \quad \gamma_s = 4 + m - 2l$$

Equation (5) can be taken on the form:

$$RQ = \frac{4}{\gamma_s} \cdot \frac{1 - y_c}{1 - \frac{\gamma_x}{\gamma_s} \cdot y_c} \quad (6)$$

where y_c is the so-called C- mole yield. There is a relationship between y_c and the

true overall yield

$$Y = \frac{y_c \frac{12}{\alpha_x}}{\frac{12}{\alpha_s}} \qquad \text{from where} \qquad y_c = Y \cdot \frac{\alpha_x}{\alpha_s}$$

In these expressions α_x and α_s are the carbon contents of the biomass and substrate, respectively. Putting this formula into equation (6) an expression is obtained showing the relationship between the RQ and the overall yield Y. This equation (7) shows how RQ depends on the substrate as well as on cell composition.

$$RQ = \frac{4}{\gamma_s} \cdot \frac{1 - \frac{\alpha_x}{\alpha_s} \cdot Y}{1 - \frac{\gamma_x}{\gamma_s} \cdot \frac{\alpha_x}{\alpha_s} \cdot Y} \tag{7}$$

Figure 1 illustrates the plot of RQ as a function of Y for some C/energy sources in the case of an "average" yeast which has equivalents of available electrons γ_x = 4.2 and carbon content α_x = 0.5 (4.2 is a good approximation for an "average" microbial cell and so does C content 0.5 [ERICKSON et al., 1978]). This investigation

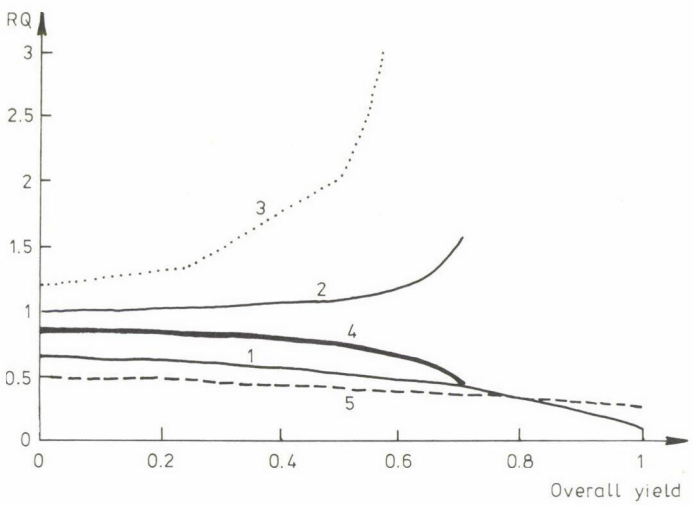


Fig. 1. Relationship between RQ and overall yield coefficient as a function of the substrate composition
1: Ethanol; 2: glucose; 3: pyruvate; 4: glycerol; 5:methane

is partly theoretical because methane obviously can not be utilised by baker's yeast but the other four substrates can be real. Equation (7) results in the RQ = $4/\gamma_S$ when there is no growth just respiration i.e. if Y = 0. These values for the substrates of Fig. 1 are as follows:

pyruvate	1.2
glucose	1.0
glycerol	0.857
ethanol	0.667
methane	0.5

Figure 1 also proves that these RQs are maximum or minimum values depending upon the composition of C-energy source in the case of the complete oxidation of the substrate, i.e. respiration without growth (but no endogenous respiration). In the instances of ethanol, methane and glycerol RQ decreases when yield increases according to our expectations however in the cases of glucose and pyruvate RQ increases. In other words: the improvement of the effectiveness of aerobic growth does not necessarily come together with decreasing RQ. Actually the composition of the substrate determines the direction of change.

2. Relationship between RQ and biomass yield in case of baker's yeast

2.1. Mixed aerobic-anaerobic growth on a carbohydrate

While the above mentioned problems and methods are valid for any aerobic microbe we chose baker's yeast fermentation and its stoichiometric description as an example. However to find a proper expression between RQ and yield or an estimation of RQ in case of a baker's yeast cultivation under real conditions applying a carbohydrate C/energy source we need a more complex approach. Cultivating baker's yeast on sugar one can not talk about pure aerobic growth. The reasons are well-known: *the negative Pasteur- and the Cabtree- effects.*

Existence of these effects means that in an aerobic system below the critical oxygen tension (when the microbe's respiration system is unsaturated with respect to oxygen), the aerobic glucose utilisation switches to anaerobic fermentation very quickly i.e. ethanol forms and growth rate changes. This aerobic-anaerobic switch is the negative Pasteur effect which is characterised by ethanol formation and an increased sugar flux through glycolysis. On the other hand, even well above the critical oxygen level, if the sugar concentration exceeds a given value, aerobic metabolism switches to alcoholic fermentation. This phenomenon is called the Cabtree-effect or catabolic repression (even though it is not sufficient to take the slow enzyme synthesis into account to explain this phenomenon which occurs instantaneously after an increase in sugar concentration).

We are trying to describe a general stoichiometric view of the baker's yeast fermentation in such a way that every "effect" and/or event influencing the respiration quotient and yield can be examined by the same model.

Consider pure aerobic growth on a six-carbon carbohydrate when the stoichiometric equation is:

$$CH_2O + bO_2 + aNH_3 \rightarrow y_cCH_pO_nN_q + cH_2O + dCO_2$$

while alcoholic fermentation is described by:

$$CH_2O \rightarrow 2/3\ CH_3O_{0.5} + 1/3\ CO_2$$

We try to find the result of these two processes corresponding to the reality because both the negative Pasteur-effect and the Cabtree-effect may often occur in a baker's yeast cultivation depending on the value of dissolved oxygen tension and/or sugar concentration. Let α be the part of the sugar utilised purely aerobically for respiration and growth. Then $(1-\alpha)$ means that part of sugar turns into ethanol via fermentation.

Multiplying the two previous stoichiometric equations by α and $(1-\alpha)$ respectively and after summation:

$$CH_2O + \alpha bO_2 + \alpha aNH_3 \rightarrow \alpha y_c\, CH_pO_nN_q + (1\text{-}\alpha)\ 2/3CH_3O_{0.5} + \\ + \alpha H_2O + [\alpha d + (1\text{-}\alpha)1/3]\ CO_2$$

Similarly to the above described procedure the elementary material balance equations for the biogenic elements are the following:

for C: $1 = y_c + d$ implying $d = 1\text{-}y_c$

for N: $\alpha a = \alpha y_c q$ implying $a = y_c q$

for H: $2 + 3\alpha a = \alpha y_c p + 2(1\text{-}\alpha) + 2\alpha c$

implying $c = 1\text{-}y_c/2\ (p\text{-}3q)$

for O: $1 + 2\alpha b = \alpha y_c n + 1/3\ (1\text{-}\alpha) + \alpha c + 2\alpha d + (1\text{-}\alpha)2/3$

implying $$b = \frac{2-(1/2p+2-n-3/2q)\cdot y_c}{2}$$

Respiration quotient can be obtained as the fraction c/b if equivalents of available electrons γ_x of biomass is used again:

$$RQ = \frac{\alpha d + (1-\alpha)\cdot\frac{1}{3}}{\alpha b} = \frac{1-y_c}{1-\frac{\gamma_x}{4}y_c} + \frac{1}{3}\cdot\frac{1-\alpha}{\alpha}\cdot\frac{1}{1-\frac{\gamma_x}{4}y_c} \qquad (8)$$

This expression also includes equation (6) because in case of $\alpha = 1$ only the first term differs from zero and when $\gamma_s = 4$ (i.e. the C/energy source is a carbohydrate) equation (8) is equivalent with equation (6). The true overall yield is

$$Y = \frac{\alpha y_c \cdot \frac{12}{\alpha_x}}{\frac{12}{\alpha_s}} = \alpha y_c \cdot \frac{\alpha_s}{\alpha_x}$$

Substituting y_c into equation (8) we obtain an expression describing the relation between RQ and the true overall yield coefficient which also includes equation (7) as a special case when $\alpha = 1$:

$$RQ = \frac{1 - Y \cdot \frac{\alpha_x}{\alpha_s} \cdot \frac{1}{\alpha}}{1 - \frac{\gamma_x}{4} \cdot Y \cdot \frac{\alpha_x}{\alpha_s} \cdot \frac{1}{\alpha}} + \frac{1}{3} \cdot \frac{1-\alpha}{\alpha} \cdot \frac{1}{1 - \frac{\gamma_x}{4} \cdot Y \cdot \frac{1}{\alpha}} \qquad (9)$$

By the help of equation (9) four possible ways of baker's yeast fermentation can be examined:

- If $\alpha = 0$ and $y_c = 0$ i.e. there is no growth but alcoholic fermentation exists because of the too high level of sugar and/or the partly anaerobic environment then by equation (9) the possible logical result can be obtained, i.e. no oxygen utilisation therefore $RQ = \infty$
- If $y_c = 0$ i.e. there is no growth but aerobic respiration exists and no fermentation (substrate is completely oxidised exclusively for energy production e.g.: when culture medium does not contain N-source) then $RQ = 1$.
- If $\alpha = 1$ i.e. there is no fermentation, the whole sugar is utilised for respiration and growth and the equation (9) forms the next expression:

$$RQ = \frac{1 - Y \cdot \frac{\alpha_x}{\alpha_s}}{1 - \frac{\gamma_x}{4} \cdot Y \cdot \frac{\alpha_x}{\alpha_s}} \qquad (10)$$

which is the special case of equation (7) when the C source is a carbohydrate.

Since $\gamma_x > 4$ always holds (the above mentioned 4.2 can be considered as a good approach) therefore $RQ > 1$ from equation (10).

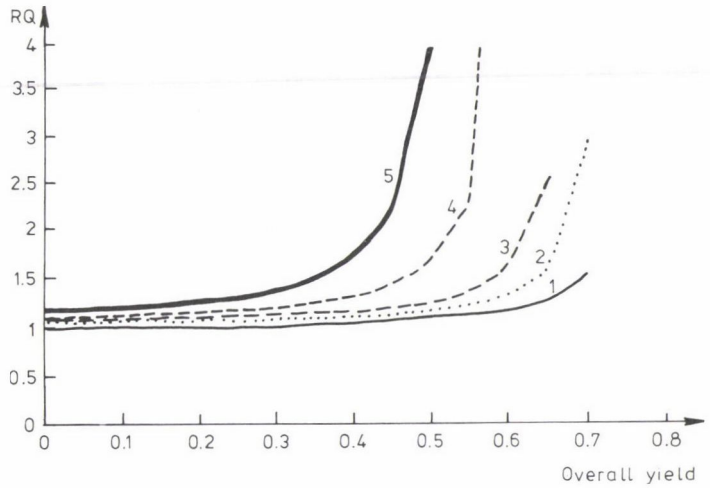


Fig. 2. Influence of α on the RQ - overall yield function. α = ratio of the aerobically utilized carbohydrate to the total sugar consumed. 1: Alpha = 1.0, 2: alpha = 0.95; 3: alpha = 0.9; 4: alpha = 0.8; 5: alpha = 0.7

This result absolutely contradicts the logical expectation as we mentioned above and we wanted to prove: i.e. RQ can be greater than 1 in case of a real baker's yeast cultivation process even if there is no alcoholic fermentation.

– Finally, if $0 < \alpha < 1$, according to equation (9) the respiration quotient again is greater than 1 and increases when α approaches to zero. Figure 2 shows a graphical representation of equation (9). In this figure the decrease of parameter α causes a strong ascent of the corresponding curves around the usual overall yield range (~0.5). In other words, around Y = 0.5, the closer the α value is to 1, the stronger the dependence of the yield on the respiration quotient will be. On the basis of this fact one can understand why baker's yeast technologies take usually their optimum close to RQ = 1 (from upward).

2.2. Simultaneous growth on ethanol and hexose

According to the above shown results there is no baker's yeast cultivation with RQ < 1. Our experimental experience contradicts this for we "measured" RQ < 1 and it was not caused by experimental error. Such a situation may occur and be explained when the biomass grows on the previously produced ethanol as a secondary C/energy source (after a phase of partially anaerobic circumstances or at high sugar level). Let us examine this case, too.

Supposing that ethanol is the only C/energy source, the stoichiometric equation is the following:

$$CH_3O_{0.5} + B\,O_2 + A\,NH_3 \rightarrow Y_c\,CH_pO_nN_q + C\,H_2O + D\,CO_2$$

Applying the notion of equivalents of available electrons and the elementary balance equations the next expression is obtained for the relationship between respiration quotient and yield:

$$RQ = \frac{1 - Y_c}{\frac{3}{2} - \frac{\gamma_x}{4} Y_c} \tag{11}$$

and using $Y_{EtOH} = Y_c(\alpha_{EtOH}/\alpha_x)$ for the true overall ethanol yield then RQ gives the following formula:

$$RQ = \frac{1 - \frac{\alpha_x}{\alpha_{EtOH}} Y_{EtOH}}{\frac{3}{2} - \frac{\gamma_x}{4}\frac{\alpha_x}{\alpha_{EtOH}} Y_{EtOH}} \tag{12}$$

If ethanol is used only for respiration i.e. no growth, then from equation (12) substituting $Y_{EtOH} = 0$, 2/3 is obtained for RQ corresponding to the calculated value from the stoichiometric equation of ethanol oxidation. If $Y_{EtOH} > 0$ i.e. there is growth, then $RQ < 2/3$ always holds as it can be seen in Fig. 1.

We must suppose that there can be a parallel ethanol utilisation even if sugar is the C/energy source and respiration and growth (and less likely fermentation) take place. In this case the following situation occurs.

Respiration – growth – alcoholic fermentation on sugar:

$$CH_2O + \alpha\,NH_3 + \alpha b\,O_2 \rightarrow \alpha y_c\,CH_pO_nN_q + \alpha c\,H_2O + \\ + (\alpha d + 1/3(1-\alpha))\,CO_2 + 2/3(1-\alpha)\,CH_3O_{0.5}$$

Respiration and growth on ethanol:

$$X\,CH_3O_{0.5} + XB\,O_2 + XA\,NH_3 \rightarrow XY_c\,CH_pO_nN_q + \\ + XC\,H_2O + XD\,CO_2$$

where X is the stoichiometric coefficient of available ethanol. The introduction of this notion is necessary because the ethanol which is utilised in the second process, is not necessarily formed at the same time in the first process but it could "previously" be produced and/or its quantity can change because of the ventillation caused by aeration/agitation. Therefore, instead of the formal summation of these two equations another method is chosen. Let us summarise the carbon dioxide production in both processes [numerators of equations (8) and (11)] and oxygen

consumption in these processes (denominators of the equations) and determine their quotients. The overall respiration quotient takes equation (13).

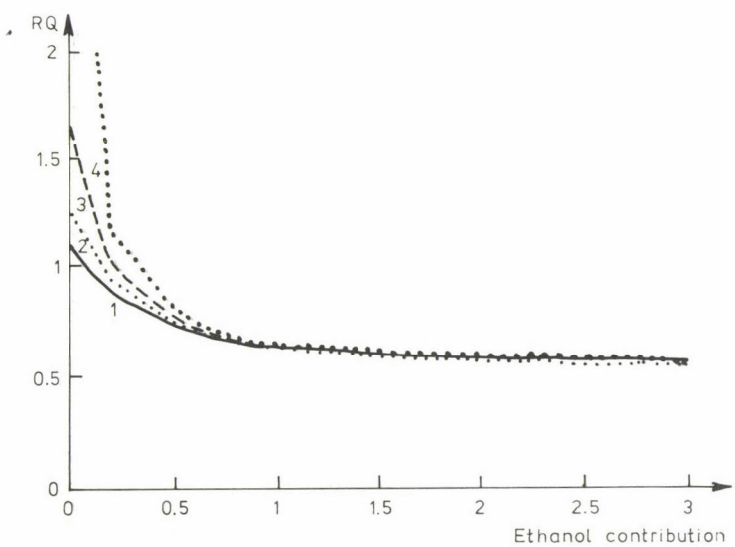
$$RQ = \frac{\alpha(1-y_c) + \frac{1}{3}(1-\alpha) + X(1-Y_c)}{\alpha\left(1-\frac{\gamma_x}{4}y_c\right) + X\left(\frac{3}{2}+\frac{\gamma_x}{4}Y_c\right)} \tag{13}$$

Latter can be rewritten as an expression containing the true overall yields (naturally we have two yields, one for the sugar and the other for the ethanol).

$$RQ = \frac{\alpha\left(1-Y\frac{\alpha_x}{\alpha_s}\frac{1}{\alpha}\right) + \frac{1}{3}(1-\alpha) + X\left(1-Y_{EtOH}\frac{\alpha_x}{\alpha_{EtOH}}\right)}{\alpha\left(1-\frac{\gamma_x}{4}Y\frac{\alpha_x}{\alpha_s}\frac{1}{\alpha}\right) + X\left(\frac{3}{2}-\frac{\gamma_x}{4}Y_{EtOH}\frac{\alpha_x}{\alpha_{EtOH}}\right)} \tag{14}$$

Examining expression (14) the following cases can be distinguishable:

- If $Y_{EtOH} = 0$ i.e. $X = 0$, no growth and respiration on ethanol, then this is the situation that was described by equation (9).This case also implies when $\alpha = 1$.



- If ethanol is the C/energy source i.e. $Y = 0$ and $\alpha = 1$, then the equation results in the form of (12).

Figure 3 shows some simulations when both ethanol and sugar utilisation occur at the same time (respiration, growth and/or alcoholic fermentation). It can be seen that the value of RQ depends on how much the ethanol contributes to the complete process therefore RQ can take on any value (less than one, greater than one) as a function of X and naturally the degree of anaerobicity of the cultivation.

3. Conclusion

By simulations based on experimental experience we deduced some expressions between RQ and overall yield. These expressions have to be taken into account if RQ is used for optimal control of baker's yeast cultivations. It turned out that the RQ control alone around a given value is not sufficient to achieve maximum yield, instead other parameters (media composition or technological variables e.g. dissolved oxygen) have to be considered as well.

*

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References

BELLGARDT, K. H. & YUAN J. (1991): Process models: Optimization of yeast production – A case study. – in: SCHÜGERL K. (Ed.) *Biotechnology*, Vol.4., VCH Weinheim.

ERICKSON, L. E., MINKEVICH, I. G. & EROSHIN, V. K. (1978): Application of mass and energy balances regularities in fermentation. *Biotechn. Bioeng.*, *20*, 1595.

NAGAI, S. (1979): Mass and energy balances for microbial growth kinetics. – in: GHOSE, T. K., FIECHTER, A. & BLAKEBROUGH, N. (Eds), *Adv.in Biochem. Eng.*, Vol.11., Springer, Berlin.

NYESTE, L., SEVELLA, B., SZIGETI, L. & RAJKAI, GY. (1992): Computer controlled fed batch baker's yeast fermentation. Poster presented at VIth Intern. Biotechnol Symp. Aug 16–21, Crystal City, Virg., USA.

SEVELLA, B. (1983): Production of tryptophane by fermentation. PhD Thesis. Budapest.

WANG, H. Y., COONEY, CH. L. & DANIEL, I. C. (1977): Computer aided baker's yeast fermentation. Biotechnol. Bioeng., *19*, 69.

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IMPROVEMENT OF FRUIT COLOUR QUALITY OF PAPRIKA COMBINED TREATMENTS OF Ti(IV) AND HUMIC ACIDS

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Capsicum annuum L. (cv Bunejo) plants were grown under glass and treated with different combinations of humic acids and Ti(IV) ascorbate applied via soil drench of leaf spray. For each treatment, the colour level, yellow/red pigments ratio, lipoxygenase activity, ascorbic acid content and fruit yield were determined. Greater yields, ascorbic acid content and lipoxygenase activity were found in fruits of humic acid-treated plants, compared with the corresponding control plants, but there were no changes in colour or the red/yellow pigments ratio. With Ti treatment, all the measured parameters increased except lipoxygenase activity which decreased dramatically. Apart from the increase in yield, the effects of combined treatments of Ti and humic acids did not result in any improvement of the effects of Ti alone.

Keywords: *Capsicum annuum*, colour, titanium, humic acids, lipoxygenase, ascorbic acid

Titanium is regarded as a beneficial element in plant physiology as it is responsible for maintaining some important metabolic processes (DUMON & ERNST, 1988; CARVAJAL, 1992; CARVAJAL et al., 1994b; GIMÉNEZ et al., 1990; PAIS, 1983). For example, it has been shown that Ti increased the stability of chlorophyll and lutein against photo-oxidative damage in tomato (DAOOD et al., 1987) and the development of the major colour compounds in pepper fruits (MARTÍNEZ-SÁNCHEZ et al., 1993). The formation of the carotenoid pigments in fruits is related to lipoxygenase activity (GROSCH et al., 1977; ESKIN et al., 1977) and to the content of antioxidants which act as competitive inhibitors of this enzyme. Ascorbic acid is one such antioxidant which acts by scavenging active oxygen and thus protects double bonds.

Paprika fruit (*Capsicum annuum*, L.) is one of the oldest and most important sources of natural food colourings. The stability of its pigments has been attributed to a number of factors, including cultivar (ALCARAZ et al., 1991; MARTÍNEZ-SÁNCHEZ et al., 1991), moisture content, stage of ripeness at harvest (KANNER et al., 1979) and antioxidant content (BIACS et al., 1992). However, lipid oxidation has a major effect on fruit colour quality. Lipoxygenases, which are Fe-dependent, requiring 1 mole of

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nonheme iron per mole of enzyme, (CHAN, 1973; ROZA & FRANKE, 1973) can rapidly oxidise polyenes in the presence of linoleic acid and oxygen (GROSCH et al., 1977).

Many workers have shown that solubilised humic acids, in vivo and in vitro, have a positive effect on the growth and metabolic processes of plants (MAGGIONI et al., 1992; VAUGHAN & ORD, 1981). The aim of the work described here was to determine the conditions of growth required to improve paprika fruit colour quality for the manufacture of paprika powder, using titanium and humic acids, two compounds commonly sold as fertilisers in Spain. The work involved treating pepper plants with commercially available humic acids, to optimise nutrition, and titanium, to enhance physiological activity.

1. Materials and methods

1.1. Materials

Capsicum annuum, L. Plants cv. Bunejo were obtained from J.C. Costa (CRIA, Murcia, Spain) and grown under greenhouse conditions. Fertilizer, water and phytosanitary treatments were supplied by a drip irrigation system.

1.2. Treatments

In the greenhouse, located at the CSIC experimental farm (Santomera, Murcia, Spain), 2 plots (204 plants per plot) were given the following treatments:

- **Control:** No humic acids added.
- **Humic acids:** 1.5 mg l^{-1} humic acids and 0.1 g l^{-1} fulvic acids (3 ml m^{-2}, Sinergipron Complex, humic acids 1 g l^{-1}, provided by Probelte S.A., Murcia, Spain). This treatment was applied via soil drench every 15 d during the crop cycle.

These two plots were divided into four subplots containing 51 plants given the following treatments:

- **Control:** No Ti added.
- **Ti via soil drench:** 2 mg Ti l^{-1}, pH 6, [50 ml m^{-2}, Titavit, Ti(IV)-ascorbate complex, 1 g Ti l^{-1}, provided by Nitrokémia, Hungary], applied with humic acid solution.
- **Ti via foliar spray:** 2 mg Ti l^{-1}, pH 6, (10 ml $plant^{-1}$), applied just before the fruit set stage.
- **Ti via soil drench an foliar spray:** 2 mg Ti l^{-1}, pH 6. This treatment was applied as above.

At harvest, 100 fruits per treatment from each plot were sampled. Peduncles

and seeds were detached and samples of the fruit pericarp were washed with 1% BRIJ 35 solution (non-ionic detergent) and then rinsed three times with deionized water.

1.3. Analytical methods

1.3.1. Lipoxygenase activity. Crude extract: The fruit pericarp was cut into short segments and 3 g of this fresh material were homogenized at 4 °C in 9 ml 50 mM phosphate buffer, pH 7.0, containing 1% TritonX – 100 (13, w/v), filtered through 4 layers of nylon cloth and centrifuged at 15000 × g for 15 min.

Enzyme assay: Pure linoleic acid (10 μl) was suspended in 25 ml of 0.1 M sodium tetraborate containing 0.1% Tween 20 by sonication (SEKHAR & REDDY, 1982). One tenth ml of the substrate was shaken vigorously with 2.9 ml of 0.1 M phosphate buffer, pH 4 – 5, in a spectrophotometer cuvette. The reaction was started by adding 0.1 ml of enzyme extract, and the increase in absorbance at 234 nm was measured (DAOOD et al., 1988). A unit of enzyme was defined as the amount which produced an absorbance change of 0.001 a.u. per sec at 234 nm.

1.3.2. Ascorbic acid. Capsicum pericarp (6 g) was homogenized with a Polytron (PCU Drehzahlregler, Kinematica GmbH, Littan-Luzen) in 30 ml 15% metaphosphoric acid (v/v) contained 250 mg PVP (polyvinyl-pyrrolidone) to absorb the interfering pigments, and then filtered through a Millipore filter (A AWP 04700 25 eq AA 0.8 μ). The eluate was processed directly for ascorbic acid determination. As proposed by BEUTLER (1984), L-ascorbic acid and some other substances reduce tetrazolium salt MTT [8-(4,5-dimethylthiazolyl-2)-2,5-diphenyl-tetrazolium bromide] in the presence of an electron carrier PMS (5-methylphenazinium methyl sulfate) at pH 3.5 to form MTT-formazan. For the specific determination of L-ascorbate in a blank sample only the L-ascorbate fraction, as part of all the reducing substances present in the sample, is oxidatively removed by ascorbate oxidase (AAO) in the presence of oxygen. The dehydroascorbate formed does not react with MTT/PMS. The difference in absorbance in the sample minus that of the blank sample is proportional to the quantity of L-ascorbate in the sample. The formation of MTT-formazan was determined by measuring its absorbance at 578 mn. The ascorbic acid concentration was expressed as mg per 100 g fresh weight.

1.3.3. Colour (ASTA, 1968). Dried, ground pericarp (0.5 g) was extracted with 100 ml acetone for 24 h in the dark. Supernantant (5 ml) was diluted to 50 ml with acetone and the absorbance was read at 460 nm against an acetone blank. The colour was expressed in ASTA units:

$$\text{ASTA} = \text{A } 164 \text{ lf w}^{-1}$$

A: Sample absorbance;

If: Deviation factor of the spectrophotometer, which was calculated using a standard 2030 NBS filter, that indicates the relation between the theoretical (At) and real (AR) absorbances at 460 nm;

164: molar extinction coefficient of 1% capsanthin solution in acetone;

w: sample weight.

1.3.4. Yellow/red pigments ratio. Pericarp was extracted as for the ASTA determination. The absorbances were measured at 470 nm for red pigments and at 455 nm for yellow (NAVARRO & COSTA, 1993).

2. Results

With humic acid treatments, there was an increase in fruit yield, compared to controls and a similar result was observed with Ti foliar spray treatments. Furthermore, there was a significant increased in yield between plants treated with humic acids combined with Ti applied via soil drench and foliar spray, and the corresponding controls not treated with humic acids (Table 1).

Table 1

Effect of humic acids combined with different applications of titanium on yield (g per plant) of Capsicum annuum *L. fruits*

	Humic acid soil treatment	
Ti-treatment	No addition	Added
No Ti(IV) applied	98.4 ± 2.9b	112.1 ± 3.1c
Ti (IV) via soil	82.2 ± 2.8a	128.5 ± 3.3de
Ti(IV) via foliar	124.1 ± 3.2d	139.7 ± 3.4f
Ti(IV) via soil and foliar	135.0 ± 3.5ef	130.1 ± 3.2def

Data are the average value from ten individual samples and are expressed as the mean ± se (standard error). Means followed by the same letter are not significantly different at p = 0.05 level (Duncan's test)

There was no consistent effect of humic acid treatment on fruit colour, in the presence of Ti via soil drench or in absence of it (Table 2). A significant increase of this fruit quality parameter was observed with both treatments combined with humic acids. This indicated that humic acid treatments were detrimental to fruit colour quality, when expressed as ASTA units. However, humic acids combined with Ti supplied as foliar sprays or foliar sprays and soil drench, resulted in an increase in the red/yellow pigments ratio compared with all other treatments (Table 3). The data

Table 2

Effect of humic acids combined with different applications of titanium on colour (A.S.T.A.) of Capsicum annuum *L. fruits*

Ti-treatments	Humic acid soil treatment	
	No addition	Added
No Ti(IV) applied	130.10 ± 2.70b	125.00 ± 2.39ab
Ti(IV) via soil	121.66 ± 2.40a	130.11 ± 2.62b
Ti(IV) via foliar	193.08 ± 3.71d	169.32 ± 3.54c
Ti(IV) via soil and foliar	185.93 ± 3.51d	158.68 ± 3.29c

Data are the average value from ten individual samples and are expressed as the mean ± se (standard error). Means followed by the same letter are not significantly different at p = 0.05 level (Duncan's test)

also indicated that Ti supplied on its own as a foliar spray or as a foliar spray and root drench resulted in a greater red/yellow pigments ratio than with Ti supplied by soil drench alone (Table 3). These results indicated that leaf, or leaf/soil, applications of Ti enhanced the concentrations of red pigments (or diminished yellow pigment biosynthesis) and that humic acids intensified this effect.

Ascorbic acid content increased with Ti, humic acids and Ti plus humic acids treatments, independently of the way in which they were applied. However, it was clear that humic acids had an antagonistic effect on the increase in ascorbic acid induced by Ti, the greatest amount being found when Ti was supplied alone as a combined soil and foliar application (Table 4).

Table 3

Effect of humic acids combined with different applications of titanium on red/yellow pigments ratio of Capsicum annuum *L. fruits*

Ti-treatment	Humic acid soil treatment	
	No addition	Added
No Ti(IV) applied	1.026 ± 0.003ab	1.020 ± 0.004a
Ti(IV) via soil	1.033 ± 0.002bc	1.027 ± 0.03ab
Ti(IV) via foliar	1.037 ± 0.003cd	1.056 ± 0.004e
Ti(IV) via soil and foliar	1.039 ± 0.002d	1.052 ± 0.004e

Data are the average value from ten individual samples and are expressed as the mean ± se (standard error). Means followed by the same letter are not significant different at p = 0.05 level (Duncan's test)

Table 4

Effect of humic acids combined with different applications of titanium on ascorbic acid concentration (mg per 100 g f.w.) of Capsicum annuum *L. fruits*

Ti-treatment	Humic acid soil treatment	
	No addition	Added
No Ti(IV) applied	4.37 ± 0.14a	10.72 ± 0.38b
Ti(IV) via soil	14.92 ± 0.85d	12.90 ± 0.76cd
Ti(IV) via foliar	12.78 ± 0.76cd	11.60 ± 0.38bc
Ti(IV) via soil and foliar	19.38 ± 1.03e	15.64 ± 1.00d

Data are the average value from ten individual samples and are expressed as the mean ± se (standard error). Means followed by the same letter are not significantly different at p = 0.05 level (Duncan's test)

Lipoxygenase (LOX) activity decreased dramatically in the presence of Ti, independent of the method of application, whereas, supplying humic acids had the opposite effect (Table 5). The results also seemed to indicate that humic acids and foliar application of titanium resulted in an important decrease in LOX activity, compared to the application of humic acids alone, or in combination with Ti via soil.

Table 5

Effect of humic acids combined with different applications of titanium on lipoxygenase activity (units per g f.w.) of Capsicum annuum *L. fruits*

Ti-treatment	Humic acid soil treatment	
	No addition	Added
No Ti(IV) applied	167.56 ± 8.64d	282.30 ± 7.16f
Ti(IV) via soil	89.14 ± 2.99a	258.82 ± 6.45e
Ti(IV) via foliar	97.36 ± 4.02ab	160.86 ± 5.90d
Ti(IV) via soil and foliar	100.39 ± 3.59bc	110.22 ± 3.83c

Data are the average value from ten individual samples and are expressed as the mean ± se (standard error). Means followed by the same letter are not significantly different at p = 0.05 level (Duncan's test)

3. Conclusions

It has been reported that Ti promotes a higher yield in some varieties of plants when it is applied with the nutrient solution via roots (ALCARAZ et al., 1990; GIMÉNEZ et al., 1990; MARTÍNEZ-SÁNCHEZ et al., 1990), and when it is supplied by leaf spray (PAIS, 1983; FEHÉR et al., 1987; ALCARAZ et al., 1991). However, Ti was

always more effective when supplied as a foliar treatment because of its low mobility in the plant. This is in agreement with the results of the present paper, except achieve a greater increase in yield when Ti was applied as foliar spray combined with humic acid treatment.

Previously (CARVAJAL, 1992; CARVAJAL et al., 1994a) it was demonstrated that the enhancement of both the biomass production and the biological activity of the Ti(IV) ascorbate-sprayed plants was induced by the cation (Ti^{+4}), whereas the anion (ascorbate) did not have any effect. Furthermore, others (MOZAFAR & OERTLY, 1993) showed that the external application of ascorbic acid via roots or leaves did not increase the ascorbic acid concentration in any of the plant organs. With Ti or humic acid application, irrespective of how Ti was applied, a significant increase was observed in the ascorbic acid concentration. However, the combination of Ti and humic acids seemed to have a negative effect.

The decrease in lipoxygenase activity obtained with Ti-treatments, agreed with our previous results with other varieties of *Capsicum annuum*, L. (Negral and Buketen x Albar) (CARVAJAL, 1992; CARVAJAL et al., 1994a). Ascorbic acid inhibits lipoxygenase activity, so the decrease we found might be the result of the high concentration of ascorbic acid produced by the application of Ti. Similar results were found by MARTÍNEZ-SÁNCHEZ and co-workers (1993). It is interesting to note that LOX activity and ascorbic acid content are important in paprika powder production, and that the paprika quality might be improved by the Ti-treatments.

Humic acids did not show any influence on pericarp colour. This effect may act through two pathways. On one hand, yield enhancement may not be associated with an increase in pigment synthesis and a dilution effect could have occurred, but on the other hand, as a result of the increase of LOX activity and the low ascorbic acid concentration, pigments may have degraded. Thereafter, the humic acid treatment might not improve the quality of the pepper for processing as paprika powder. The effect of Ti was, in all cases, beneficial to the plants (applied as foliar spray) but there was no evidence of a synergistic effect when humic acids were combined with Ti.

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References

Asta (1968): *Official analytical method of the American Spice Trade Association.* 2th ed., New Jersey, N.Y.

ALCARAZ, C. F., GIMÉNEZ, J. L., MORENO, A., FUENTES, J. L. & MARTÍNEZ-SÁNCHEZ, F. (1990): Effects of Fe and Ti on yield and fruit quality of *Capsicum annuum* L. plants. – in: *Proc. XXIII Int. Hort. Congr.*, Firenze, Italy, Vol. 2, p. 3359.

ALCARAZ, C. F., GIMÉNEZ, J. L., CARVAJAL, M. & MARTÍNEZ-SÁNCHEZ, F. (1991): Influencias del tipo y de la calidad sobre algunos parámetros de su composición. *Alimentaria, 224*, 35 – 40.

BEUTLER, H. O. (1984): *Methods in enzymatic analysis.* BERGMEYER, H. U. (Ed.) Verlag Chemie, Weinheim, Deerfield Beach/Florida, Basel. 3rd ed. vol. 6, pp. 376 – 385.

BIACS, P. A., CZINKOTAI, B. & HOSCHKE, A. (1992): Factors affecting stability of colored substances in paprika powders. *J. agric. Fd Chem., 40*, 365 – 367.

CARVAJAL, M. (1992): Papel fisiológico del titanio en plantas de pimiento para pimentón (*Capsicum annuum* L.). Doctoral Thesis University of Murcia (Spain).

CARVAJAL, M., MARTÍNEZ-SÁNCHEZ, F. & ALCARAZ, C. F. (1994a): Effect of Ti(IV) application on some enzymatic activities in several developing status of *Capsicum annuum* L. plants. *J. Plant Nutr., 17*, 243 – 253.

CARVAJAL, M., MARTÍNEZ-SÁNCHEZ, F. & ALCARAZ, C. F. (1994b): Effect of Ti(IV) on some indicators of physiological activity in *C annuum* L. *J. Hort. Sci., 69*, 427 – 432.

CHAN, H. W. S. (1973): Soya-bean lipoxygenase: An iron-containing dioxygenase. *Biochim. Biophys. Acta, 327*, 32 – 35.

DAOOD, H. G., BIACS, P. A., FEHÉR, M., HOSCHKE, A., HAJDU, F. & PAIS, I. (1987): Effect of titanium treatment on the development and stability of pigments in tomato fruit. – in: PAIS. I. (Ed.) *New results in the research of hardly known trace elements and the analytical problems of trace element research.* University of Horticulture and Food Industry, Budapest, pp. 69 – 100.

DAOOD, H. G., BIACS, P. A., FEHÉR, M., HAJDU, F. & PAIS, I. (1988): Effect of titanium on the activity of lipoxygenase. *J. Plant. Nutr., 11*, 505 – 516.

DUMON, J. C. & ERNST, W. H. O. (1988): Titanium in plants. *J. Plant. Physiol., 133*, 203 – 209.

ESKIN, N. A. M., GROSSMAN, S. & PINSKY, A. (1977): Biochemistry of lipoxygenase in relation to food quality. *Critical Reviews in Food Science and Nutrition*, pp. 1 – 40.

FEHÉR, M., PAPP, K. L., FODOR, P. & PAIS, I. (1987): The effect of titanium on the uptake of other nutritive elements. – in: PAIS. I. (Ed.) *New results in the research of hardly known trace elements and the analytical problems of trace element research.* University of Horticulture and Food Industry, Budapest, pp. 114 – 127.

GIMÉNEZ, J. L., MARTÍNEZ-SÁNCHEZ, F., MORENO, A., FUENTES, J. L. & ALCARAZ, C. F. (1990): Titanium in plant nutrition. III. Effect of Ti(IV) on yield of *Capsicum annuum* L. – in: BARCELÓ, L. (Ed.) *Nutrición mineral bajo condiciones de estrés.* SPIC-UIB, Palma de Mallorca, pp 123 – 128.

GROSH, W., WEBER, F. & FISHER, K. H. (1977): Blanching of carotenoid by the enzyme lipoxygenase. *Ann. Technol. Agric., 26*, 133 – 137.

KANNER, J., MENDEL, H. & BUDOWSKI, P. (1979): Carotene oxidizing factors in red pepper fruits. *J. Fd Sci., 43*, 709 – 712.

MAGGIONI, A., VARANINI, Z., PINTON, R. & BIASI, M. G. (1992): Humic substances affected transport properties of root membranes. – in: KUBAIT, J. (Ed.) *Humics, its structure role in agriculture and environment.* Elsevier Publishers B & V., pp. 137 – 144.

MARTÍNEZ-SÁNCHEZ, F., GIMÉNEZ, J. L., FRUTOS, M. J., CARVAJAL, M. & ALCARAZ, C. F. (1990): Titanium in plant nutrition. II. Effect of Ti(IV) on germination and initial plant development of

Capsicum annuum L. – in: BARCELÓ J. (Ed.) *Nutición mineral bajo condiciones de estrés*. SPIC-UIB, Palma de Mallorca, pp. 115 – 121.

MARTÍNEZ-SÁNCHEZ, F., GIMÉNEZ, J. L., CARVAJAL, M. & ALCARAZ, C. F. (1991): Influencia del tipo y de la calidad sobre la composición mineral en macroelementos. *Alimentaria*, *223*, 53 – 56.

MARTÍNEZ-SÁNCHEZ, F., NÚÑEZ, M., AMARÓS, A., GIMÉNEZ, J. L. & ALCARAZ, C. F. (1993): Effect of titanium leaf spray treatments on ascorbic acid levels of *Capsicum annuum* L. fruits. *J. Plant Nutr.*, *16*, 975 – 981.

MOZAFAR, A. & OERTLI, J. J. (1993): Vitamin C (ascorbic acid): uptake and metabolism by soybean. *J. Plant Physiol.*, *141*, 316 – 321.

NAVARRO, F. & COSTA, J. (1993): *Estimación del color de una colección de variedades de pimientos para pimentón mediante tres criterios de calidad*. Consejería de Agricultura Ganadería y Pesca de la Región de Murcia.

PAIS, I. (1983): The biological importance of titanium. *J. Plant Nutr.*, *6*, 3 – 131.

ROZA, M. & FRANCKE, A. (1973): Soybean lipoxygenase: An iron-containing enzyme. *Biochim. biophys. Acta*, *327*, 24 – 31.

SEKHAR, B. P. S. & REDDY, G. M. (1982): Studies on lipoxygenase from rice (*Oryza sativa* L.). *J. Sci. Fd Agr.*, *33*, 1160 – 1163.

VAUGHAN, D. & ORD, B. G. (1981): Uptake and incorporation of 14C-labelled soil organic matter by roots of *Pisum sativum* L. *J. Exp. Bot.*, *32*, 679 – 687.

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THE EFFECT OF IRRADIATION ON SWEET CHERRIES

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The effect of irradiation (1, 2.5 and 5 kGy) and packaging (Resinite VF71) on the shelf life of cherries (Prunus avium cv. Germersdorfi óriás) was studied. Cherries were stored for 7 – 26 d (3 – 5 °C, 90% RH). The spoilage, weight loss, texture, sugars and organoleptical properties (color, taste, texture, surface) were determined. The ultrastructure of cells (SEM, TEM, image analysis) and cell wall of the packed and stored (7 d) cherries were investigated.

Irradiation (2.5 kGy and 5 kGy) reduced spoilage after 7 wk of storage at 3 – 5 °C from 60% to 20%. Packaging inhibited spoilage, by 20% (from 50% to 30%), and weight losses nearly completely (from 3 – 5% to 0.2 – 0.3%). Combined treatment (2.5 kGy and packaging) resulted in the best results: spoilage was in average 10%.

Irradiation (2.5 kGy) induced softening ($P > 95\%$). During storage time softening increased ($P > 95\%$) both in the control and in the irradiated cherries. However, panelists could not establish significant differences between texture of control and irradiated cherries.

The ultrastructure of cells in the cherries showed great heterogeneity (cell size, thickness of cell wall, integrity of cell compounds). Cells of the cortex in irradiated cherries were more wrinkled (SEM) than in the control. The middle lamellae of cell wall dissolved moderately in the irradiated (1, 2.5 kGy) cherries (TEM).

Glucose and fructose were detected in cherry. The quantity of these sugars did not change with the increasing radiation dose. During storage fructose concentration increased, glucose concentration decreased.

On the basis of these results irradiation (2.5 kGy) can be applied for extension of the shelf life of cherry. Three times longer storage time (at 3 – 5 °C, 90% RH) can be obtained by irradiation, even longer by using irradiation combined with packaging.

Keywords: sweet cherry, irradiation, packaging, storage, transmission and scanning electron microscopy, spoilage, texture, sugars, starch grains, cell wall

Relatively few publications deal with the shelf-life extension of sweet cherry. Rotting, bruising, discoloration influence the quality of sweet cherry. The rotting can be reduced using spraying with fungicides (dicarboximides-vinclozolin, iprodione) (SEKSE & GJÆRUM, 1987) before or at harvest. Monilia, Botrytis, Penicillium, Rhizopus can cause severe damage. Pre-cooling using cold water has increased the percent ratio of diseased fruits. The duration of the storage can be increased (with 10 or 15 days), if the cherry is stored in controlled atmosphere (CO_2 3 – 5%, O_2

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10–15%). In some countries cherry and sour cherry can be stored (at 1–-0.6 °C) under different circumstances of controlled atmosphere for 10–30 days. The longest period can be reached by high CO_2 (10%) and low O_2 (3%) concentration at –0.5–0.6 °C, 90–95% RH (SASS, 1993).

The texture can be modified by calcium treatment as LIDSTER and co-workers (1978), and KOVÁCS (1980) established several years ago. Softer (lower concentrations of soluble solids, acid and dry matter) fruits were more susceptible to bruising (SPAYD et al., 1986). SEKSE (1987) did not find a correlation between soluble solid contents and cracking susceptibility.

The use of wax coatings on cherry fruit reduced moisture loss during ambient storage, enhanced cuticular diffuse resistance and retarded the incidence of decay. After ambient storage, cherry fruit treated with wax coatings were firmer than the other treatments, but the use of wax did not reduce the incidence of surface pitting, stem discoloration or stem moisture loss. Fruit packed in a poly liner were similar in quality to fruit with wax coatings, but stems on fruit in poly liners had better color and moisture. Cherries stored at 0 °C were significantly firmer than fruit stored at 10 °C. Fruit stored at 4.5 °C had a firmness value that was intermediate to the other storage temperatures. Regardless of wax coating, cherries stored at 0 °C had similar firmness values. After 48 h at ambient temperature no differences in firmness values were noted for the cherries at three storage temperatures (DRAKE et al., 1988).

The effect of irradiation on cherries was studied to extend the shelf-life (SALUNKHE, 1961; MASSEY et al., 1965; O'MAHONY et al., 1985; SASS, 1986; KOVÁCS, 1980; 1994; KOVÁCS et al., 1982), and/or for quarantine purposes (BURDITT & HUNGATE, 1989; JESSUP, 1990). Dose level of gamma radiation applied to various Prunus fruits such as peaches, cherries an nectarines has varied from 0.5 to 4 kGy, 2.5 kGy being the most effective for controlling brown rot in cherries (MOY, 1983).

The aim of our investigations was to study the effect of packaging, irradiation and storage on the macro- and microstructure of cherries.

1. Material and methods

1.1. Raw material, packaging, irradiation, storage

Sweet cherries (*Prunus avium* cv. Germersdorfi óriás) were harvested in July, 1991. The cherries were packed in impregnated paper boxes of $50 \times 100 \times 125$ mm sealed with Resinite VF71 film (permeability for oxygen: 8000 $cm^3\ m^{-2}\ 24\ h^{-1}\ atm^{-1}$, for carbon dioxide: min. 70 000 $cm^3\ m^{-2}\ 24\ h^{-1}\ atm^{-1}$, for water vapor: 100 $g\ m^{-2}\ 24\ h^{-1}$, 25 °C 75% RP) (The Borden Chemical Co., Ltd., UK), and irradiated (1, 2.5, and 5 kGy). Each box contained 350 g cherries. An RH-gamma-30

laboratory Co-60 gamma source (Central Food Research Institute, Budapest) was used, at a dose rate of 4.3 kGy h^{-1}. The storage was carried out at 3–5 °C, 90% RH for different periods between 7–26 days.

1.2. Methods

Spoilage was expressed in the % of original mass remaining after picking out decayed fruits by hand. The softening of cherries was measured by penetrometer (Labor MIM, Hungary). The head (100 g) penetrated into the fruit for 5 s. The softness was expressed as a penetration value × 0.1 mm. Average values were calculated from twenty five replicates. Sugar content was determined by HPLC according to KOVÁCS and KERESZTES (1989). Multifactor analysis of variance was used for statistical evaluation of the data (ANON, 1989). Sensory test: the cherries were scored on a 5-point scale, 5 corresponding to the best, 1 to the poorest sample. Ten trained panelists determined the sensorial properties of cherries. Colour and taste were characteristic for the investigated cultivar, surface (wrinkled or non wrinkled), texture (hard cherry or soft cherry). The average values were ranked according to KRAMER (1960) and evaluated at 95% and 99% probability level.

1.3. TEM and SEM

All transmission (TEM) and scanning electron microscopic (SEM) investigations were carried out with the packed cherries (KOVÁCS et al., 1988).

TEM. Flesh tissue from cherries (about 1 mm^3) was fixed in 5% (v/v) glutaraldehyde solution for two hours at 20 °C temperature, and washed with Sörensen phosphate buffer (0.07 M Na_2HPO_4 and KH_2PO_4, pH 7.2–7.3) three times for 30 min. It was post fixed with 1% osmium tetroxide solution for 2.5 h at 20 °C temperature. Dehydration was carried out in a series of ethanol concentrations (15, 30, 50, 70, 90, 96% and absolute ethanol) each for 10 min then in propylene oxide with absolute ethanol (1:1) or in pure propylene oxide.

The dehydrated tissue was embedded in Durcupan resin (Fluka) and sectioned with a Reichert OMV2 ultramicrotom (Lichtenstein) using an LKB glass knife. The sections observed with TESLA BS 500 (Czechoslovakia) transmission electron microscope, at 10 kV voltage.

SEM. Following dehydration preparation of cherry tissue for TEM, the tissue prepared for SEM were dried using acetone in a Balzers CPD 020 critical point dryer (Lichtenstein) observed with TESLA BS 300 (Czechoslovakia) scanning electron microscope, at 50 kV voltage.

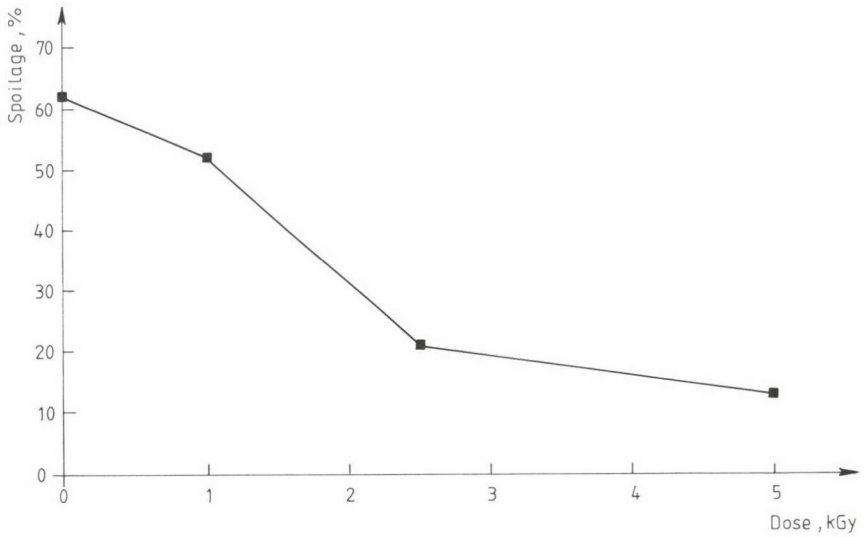

Fig. 1. The effect of irradiation on the spoilage (%) of packed cherries, stored for 7 weeks (2–3 °C, 90% RH)

2. Results

Irradiation (1–5 kGy) reduced spoilage (%) of packed, stored cherry (7w, 2–3 °C, 90% RH) as a function of the increasing radiation dose (Fig. 1). The combined treatment (heat 50 °C, 5 min) and irradiation (1 kGy) reduced spoilage

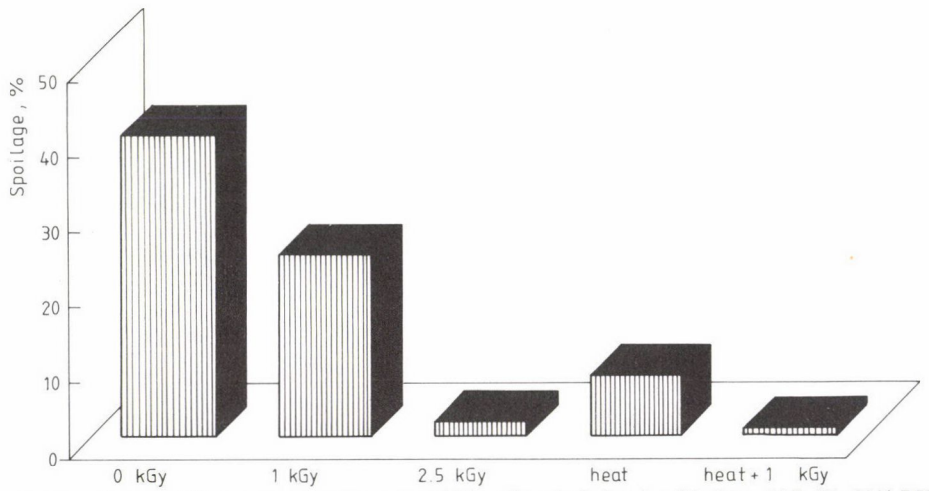

Fig. 2. The effect of different treatments on storability of packed cherries (19 days, 5 °C, 80–90% RH) Heat treatment was carried out by dipping cherries into heated water (50 °C) for 5 min

Table 1

The effect of irradiation on the spoilage of cherries (Germersdorfi óriás) as a function of storage time and years

Year		Storage time (weeks)	Spoilage (%) dose (kGy)		Storage condition °C	RH (%)
			0	2.5		
1	A	3	40.0	0.0	5	80–90
	B	3	28.0	8.0	5	80–90
2	A	4	57.8	18.1	5	95
	B	3	36.8	15.0	5	95
3	A	4	52.3	21.2	5	90
4	A	2	15.8	2.6	5	92–98
5	A	5	43.8	13.9	4–5	90–95
	B	5	31.1	10.4	4–5	90–95
6	A	3	73.3	11.5	5	80–90
7	A	7	65.0	20.0	2–3	90

A: unpacked; B: packed

(%) of packed, stored (19 days, at 5 °C, 80–90% RH) (Fig. 2) cherries, too. One kGy alone reduced spoilage slightly, but in combination with heat treatment resulted in the same result as 2.5 kGy alone. This combination (heat and 1 kGy) was not fruitful in large scale experiment. For that reason other combination (2.5 kGy and packaging) was studied for several years (Table 1) (KOVÁCS, 1994; KOVÁCS et al., 1994). Although the storage times changed from year to year, but the effect of treatment could be seen very well in all cases. Irradiation (2.5-5 kGy) reduced spoilage from 60% to 15-20%. Packaging reduced spoilage from 50% to 30%. Combined treatment (2.5 kGy) and packaging resulted in the best result: spoilage was only 10% in average.

Weight loss of packed cherries after 5 weeks of storage was less than 1%, regardless of irradiation (Fig. 3).

Irradiation induced softening (increasing degree of penetration) ($P > 95\%$) of cherries. The results of texture measurement are presented in Fig. 4. The softening was significantly ($P > 95\%$) reduced by packaging. During storage time (26 d) softening of cherries significantly increased (Table 2).

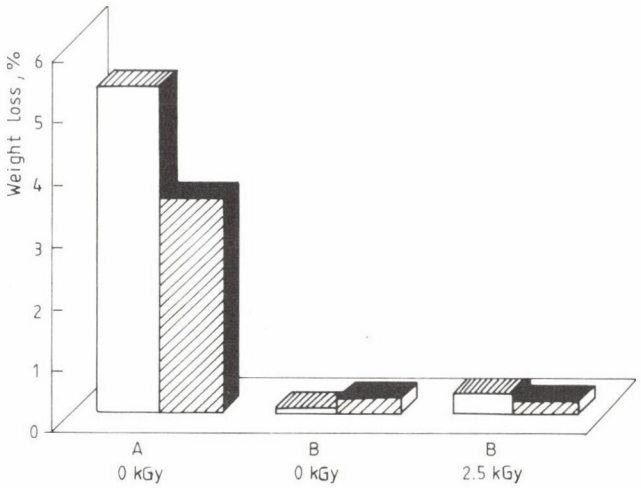

Fig. 3. The effect of irradiation and packaging on the weight loss of cherries (*Prunus avium* cv. Germersdorfi óriás) stored for 3–5 weeks at 3–5 °C, 90–95% RH. (Columns mean an average value of weight loss (% yearly) A: unpacked; B: packed. □: 1st year; ▨: 2nd year

Just after irradiation it was found that the rank sums of irradiated (2.5–5 kGy) cherries were higher than that of the unirradiated or 1 kGy treated cherries (Fig. 5), but the differences were not significant. During storage time the rank sums of texture of cherries increased slightly, within the non-significant range.

Table 2

Multifactor analysis of variance of softening values of cherries (Germersdorfi óriás)

Packaging		Treatments		Storage	
		degree of penetration (× 0.1 mm)			
Unpacked	20.5*	0 kGy	14.8	0 day	4.4
Packed	14.2	2.5 kGy	19.9*	12 days	17.8*
				26 days	29.8*
$LSD_{95\%}$	1.5	$LSD_{95\%}$	1.5	$LSD_{95\%}$	1.8

Storage: 3–5 °C, 90% RH,
n = 60 (2 packagings × 2 treatments × 3 storage times × 5 replications)
Significance level: * ($LSD_{95\%}$)

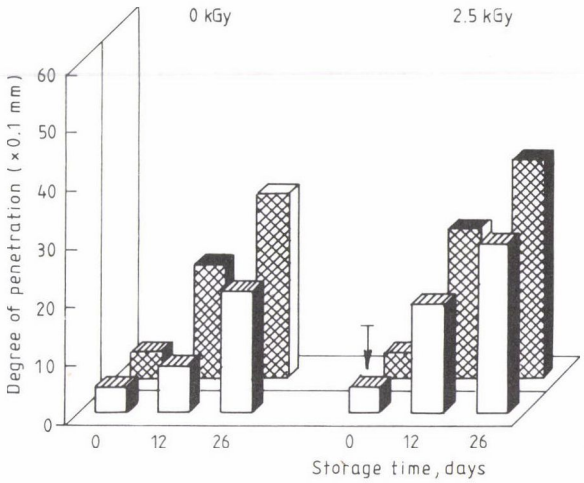

Fig. 4. Change of texture of packed and unpacked cherries (*Prunus avium* cv. Germersdorfi óriás) as a function of radiation dose and storage time (3–5 °C, 90–95% RH) ($LSD_{95\%}$ values: treatment and packaging 1.5; storage time 1.8). □: packed; ▩: unpacked; ↧: before irradiation

Colour of the investigated cherries did not change significantly as a function of treatment, packaging and storage. The surface of irradiated, packed and stored cherry (2.5–5 kGy) shrank significantly less ($P > 95\%$) than that of irradiated unpacked stored cherry.

The sugar (glucose and fructose) content of cherries did not change significantly as a function of radiation dose (1–5 kGy). After 19 day storage fructose content decreased significantly ($P > 95\%$) (Table 3).

SEM and TEM. In Fig. 6 cherry tissues can be seen. The size of cells showed large heterogeneity, vascular bundles placed in concentric circle. In Fig. 7a–d cherry tissues containing high electron dense and strongly vacuolated cells independent of treatments can be seen (TEM).

The results of ultrastructure investigations (SEM) of stored, packed (7 d, 10 °C, 75% RH), unirradiated and irradiated (2.5 kGy) cherries can be seen in Fig. 8a–b. More wrinkled cells were observed in the irradiated cherry tissues than in the control one.

In Fig. 9a–b cell wall of the fresh and stored unirradiated control cherries are presented. Incipient dissolution in the middle of cell wall can be seen. Cell wall of irradiated (2.5 kGy, stored for 7 d) cherries is shown in Fig. 9c. Partial dissolution of

Table 3

Multifactor analysis of variance of soluble sugar content of unpacked and packed cherries (Germersdorfi óriás) as a function of radiation dose after 19 days of storage (3–5 °C, 90% RH)

		Glucose	Fructose
		(g 100 g^{-1} fresh weight)	
Doses	0 kGy	7.19	4.67
	1 kGy	7.44	4.66
	2.5 kGy	7.06	4.45
	5 kGy	7.39	4.45
$LSD_{95\%}$		0.49	0.23
Storage	1 day	7.07	4.64*
	19 days	7.47	4.46
$LSD_{95\%}$		0.55	0.16

n = 24 (4 treatments × 2 storage times × 3 replications)
Significance level: * ($LSD_{95\%}$)

cell wall can be noticed. Cell wall of cherries irradiated with 5 kGy (stored for 9 d) presented in Fig. 9d. The density of the cell wall decreased, indicating that the middle lamellae dissolved.

3. Discussion

Comparing our results (Table 1, Fig. 1–2) with other observations (SALUNKHE, 1961), it is clear, that spoilage of cherries can be reduced by irradiation. The most effective radiation dose was 2.5 kGy for controlling brown rot in cherries (MOY, 1983). According to his results the shelf life of ripe sweet cherries was increased by irradiation doses of 2–3 kGy which delayed the rate of senescence. Further extension of shelf life can be obtained when irradiation is combined with fungicide treatment and packaging (MOY, 1983). According to our results packaging reduced weight loss (Fig. 3), the irradiation and combined treatment (2.5 kGy and packaging) inhibited spoilage successfully (Table 1, Fig. 1).

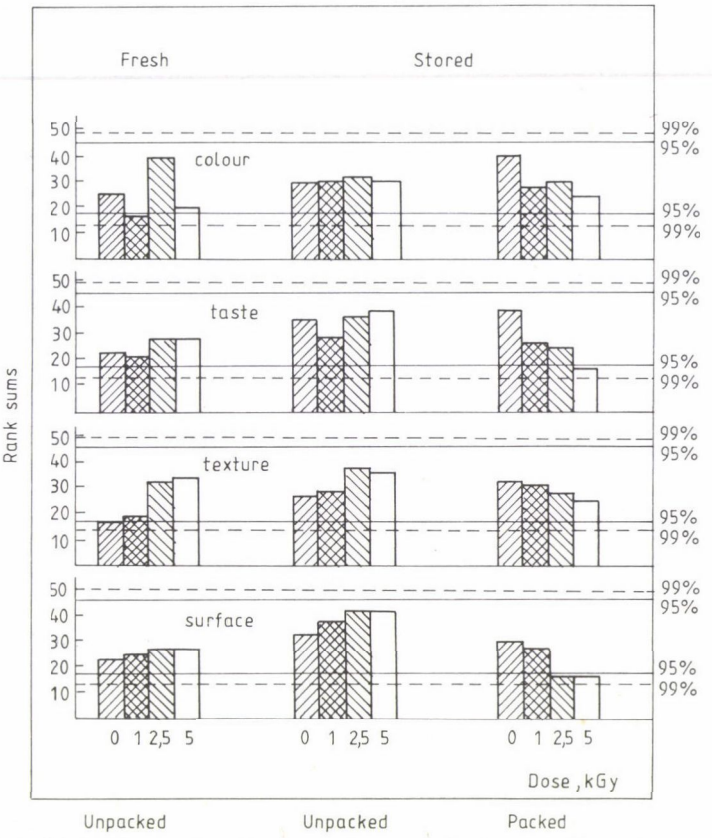

Fig. 5. The effect of irradiation and packaging on the organoleptic properties (colour, taste, texture, surface) of fresh and stored cherries (Germersdorfi óriás) (3–5 °C, 90–95% RH, 26 days)

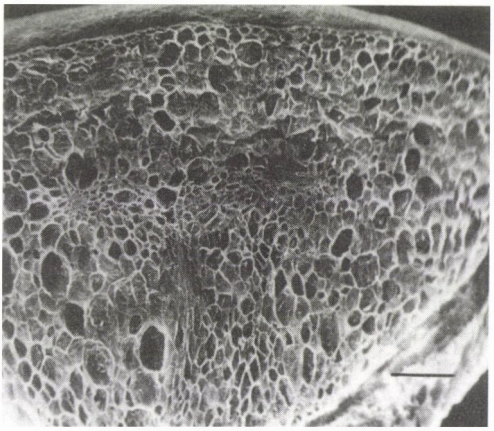

Fig. 6. Structure of fresh cherry tissue (SEM). Bar: 8 μm

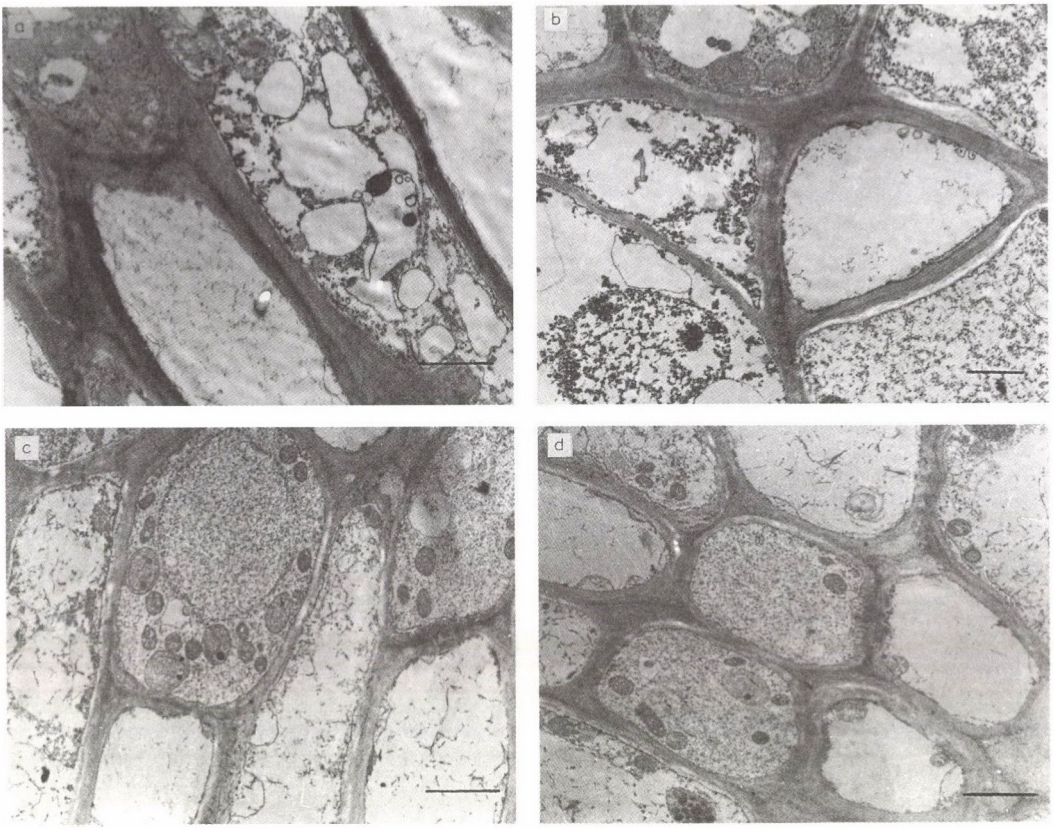

Fig. 7. Tissue structure (TEM) of cherry cortex. a: fresh control; b: stored for 7 d, control; c: stored for 7 d, 2.5 kGy; d: stored for 7 d, 5 kGy. Bars: a: 16 μm; b: 6 μm; c,d: 8 μm

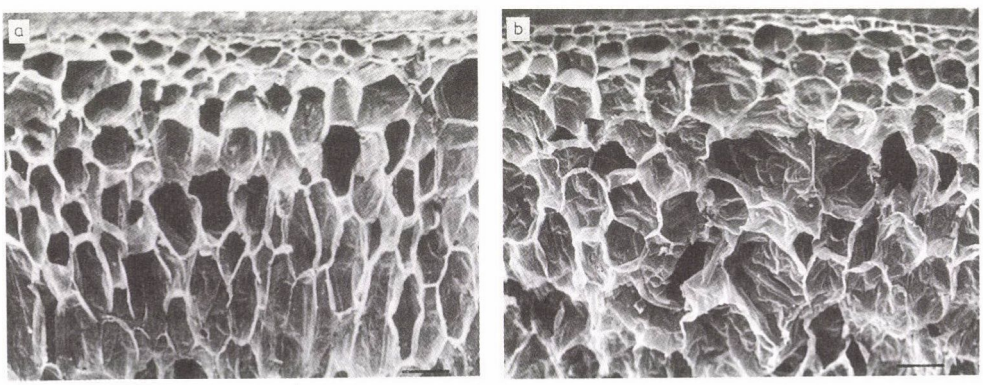

Fig. 8. Cross sections of cell layers (SEM) below the epidermis and cortex of cherry stored for 7 days. a: control; b: stored, 2.5 kGy. Bars: 67 μm

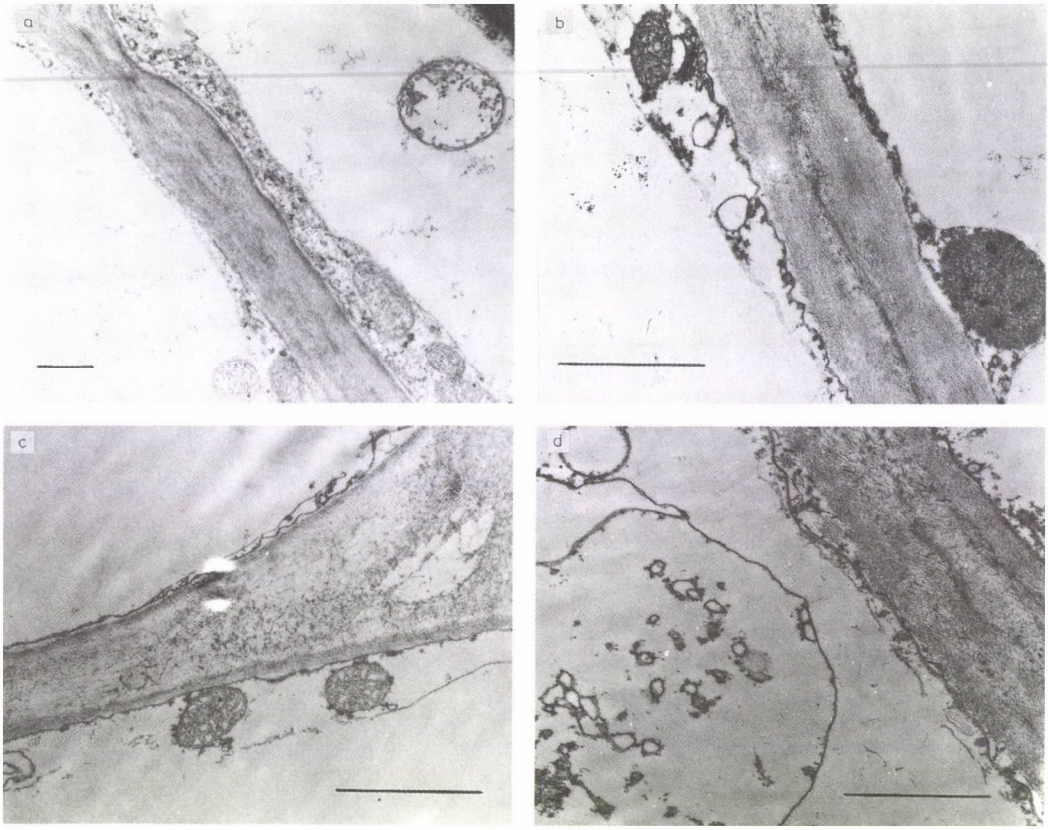

Fig 9. The effect of irradiation and storage on cell wall degradation (TEM) of cherry tissues. a: fresh, 0 kGy; b: stored for 7 d, 0 kGy; c: stored for 7 d, 2.5 kGy; d: stored for 7 d, 5 kGy. Bars: a: 6 μm; b,c,d: 16 μm

Irradiation induced softening of cherry (Table 2; Fig. 4 and 8a–b), but the rate of softening was smaller than that observed in other fruits (see later). Panelists could not find differences between texture of irradiated and control cherries (Fig. 5).

Shape and integrity of compounds of cherry cells (Fig. 6) showed great heterogeneity, which influenced the strength of tissues (Fig. 7a–d). In Fig. 8a–d the shape of cherry tissues, the grouping of cells, which increased the stability of cherry tissue structure against mechanical injury can be observed. The thick wall of cells probably consists of non-degradable polysaccharides (cellulose, hemicellulose), which do not degrade on the effect of irradiation. Further investigation needs to prove this hypothesis. Cells of cortex of irradiated cherries were wrinkled. Irradiation (2.5 kGy) resulted in less degradation of the middle lamellae of cell wall in cherries (Fig. 9a–d),

than it was observed in apple or in pears (KOVÁCS et al., 1988; KERESZTES & KOVÁCS, 1991). Pectin content of apple was 1 – 1.8% of dw, and that of pear was 0.5 – 1.4% of dw (GYÚRÓ, 1990). The softening was attributed to the break down of pectin (KERTÉSZ et al., 1964; MASSEY et al., 1965; SOMOGYI & ROMANI, 1964). In case of cherry, partly the smaller pectin content (0.3 – 0.76% of dw) (GYÚRÓ, 1990), partly the tissue structure of cherry (KOVÁCS et al., 1994) might be responsible for better preserving of ultrastructure of cherry than that of the other fruits.

Irradiation did not influence sugar content of cherry. As a function of storage time glucose concentration increased and fructose concentration decreased (Table 3).

On the base of these and earlier (KOVÁCS, 1980) results irradiation (2.5 kGy) could be proposed for shelf life extension of cherry. Packaging reduced not only the weight loss of cherry, but also influenced the physiological processes by setting up a modified atmosphere (low O_2 content, high CO_2 content) in the boxes. Therefore the combined treatments resulted in even better effect, than the single treatments.

*

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References

ANON (1989): *Statgraphics 5.1* (STSC). Statistical Graphics Corporation, Oregon, USA.

BURDITT, A. K. JR. & HUNGATE, F. P. (1989): Gamma irradiation as a quarantine treatment for apples infested by codling moth (Lepidoptera: Tortricidae). *J. Econ. Entomol., 82*, 1386 – 1390.

DRAKE, S. R., KUPFERMAN, E. M. & FELLMAN, J. K. (1988): 'Bing' sweet cherry (*Prunus avium* L.) quality as influenced by wax coatings and storage temperature. *J. Fd Sci., 53*, 124 – 126, 156.

GYÚRÓ, F. (1990): *Gyümölcstermesztés*. (General pomology.) Mezőgazdasági Kiadó, Budapest, pp. 228 – 229.

JESSUP, A. J. (1990): Gamma irradiation as a quarantine treatment for sweet cherries against Queensland fruit fly. *HortScience, 25*, 456 – 458.

KERESZTES, Á. & KOVÁCS, E. (1991): Ultrastructural effects of ionizing radiation on plant cells. *Scanning Microscopy, 5*, 287 – 296.

KERTÉSZ, Z. I., GLEGG, R. E., BOYLE, F. P., PARSONS, G. F. & MASSEY, L. M. JR. (1964): Effect of ionizing radiations on plant tissues. – III. Softening and changes in pectins and cellulose of apples, carrots and beets. *J. Fd Sci., 29*, 40 – 48.

KOVÁCS, E. (1980): *Combined treatment of fruits and vegetables*. Paper presented at the Annual Meeting of the European Society of Nuclear Methods in Agriculture (ESNA), 25 – 27 August, Debrecen, Hungary.

KOVÁCS, E. (1994): A gyümölcsök makro- és mikroszerkezetének változása sugár- és kalciumos kezelés hatására. (Changes of the macro- and microstructure of fruits as a function of irradiation and calcium treatment.) DSc Thesis, Budapest.

KOVÁCS, E. & KERESZTES, Á. (1989): The effect of irradiation and starch content in Golden Delicious apples. *Fd Structure*, *8*, 67 – 74.

KOVÁCS, E., VÖRÖS, Zs., BENCZE-BŐCS, J. & HORTI, M. (1982): Beszámoló a sugárkezelt gyümölcsök tárolási és árusítási eredményeiről. (Report on results of storability and marketing of fruits.) KÉKI Kollokvium, március 26.

KOVÁCS, E., KERESZTES, Á. & KOVÁCS, J. (1988): The effect of gamma irradiation and calcium treatment on the ultrastructure of apples and pears. *Fd Microstructure*, *7*, 1 – 14.

KOVÁCS, E., BALLA, G. & NESSINGER, A. (1994): The effect of irradiation on sweet cherries. *J. Fd Physics*, *2*, 54 – 58.

KRAMER, A. (1960): A rapid method for determining significance of differences from rank sums. *Fd Technol.*, *14*, 576 – 581.

LIDSTER, P. D., PORRITT, S. W. & TUNG, M. A. (1978): Texture modification of "Van" sweet cherries by postharvest calcium treatments. *J. Amer. Soc. Hort. Sci.*, *103*, 527 – 530.

MASSEY, L. M. JR., ROBINSON, W. B., SPAID, J. F., SPLITTSTOESSER, D. F., VAN BUREN, J. P. & KERTÉSZ, Z. I. (1965): Effect of gamma-radiation upon cherries. *J. Fd Sci.*, *30*, 759 – 765.

MOY, J. H. (1983): Radurization and radicidation: fruits and vegetables. – in: JOSEPHSON, E. S. & PETERSON, M. S.: *Preservation of food by ionizing radiation*. Vol. III., CRC Press, Florida, pp. 86 – 108.

O'MAHONY, M., WONG, S. Y. & ODBERT, N. (1985): Initial sensory evaluation of bing cherries treated with low doses of gamma-radiation. *J. Fd Sci.*, *50*, 1048 – 1050.

SALUNKHE, D. K. (1961): Gamma radiation effects on fruits and vegetables. *Econ. Bot.*, *15*, 28.

SASS, P. (1986): *Gyümölcstárolás*. (Fruit storage.) Mezőgazdasági Kiadó, Budapest, p. 211.

SASS, P. (1993): *Fruit storage*. Mezőgazdasági Kiadó, Budapest, pp. 169, 321 – 322.

SEKSE, L. (1987): Fruit cracking in Norwegian grown sweet cherries. *Acta agric. Scand.*, *37*, 325 – 328.

SEKSE, L. & GJÆRUM, H. B. (1987): Effect of preharvest fungicide sprays on grey mould (*Botrytis cinerea*) of sweet cherries. *Acta agric Scand.*, *37*, 89 – 92.

SOMOGYI, L. P. & ROMANI, R. J. (1964): Irradiation induced textural change in fruits and its relation to pectin metabolism. *J. Fd Sci.*, *29*, 366 – 371.

SPAYD, S. E., PROEBSTING, E. L. & HAYRYNEN, L. D. (1986): Influence of crop load and maturity on quality and susceptibility to bruising of 'Bing' sweet cherries. *J. Amer. Soc. Hort. Sci.*, *111*, 678 – 682.

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COMPARATIVE STUDY OF THE FOAMING PROPERTIES OF PROTEINS USING VERTICALLY AND HORIZONTALLY ARRANGED CONDUCTIVITY CELL

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Foaming properties of four protein preparations (casein, ovalbumin, bovine serum albumin and lysozyme) were measured using different modifications of conductivity method of KATO and co-workers (1983). Although both techniques – horizontally arranged conductivity cell and vertically arranged conductivity cell – may be applied for studying foaming properties, the use of vertical conductivity cell is recommended giving more information about the foam system and being more sensitive. The method gives a better correlation with the data obtained by volumetric method.

Keywords: protein, protein isolate, functionality, foaming properties, conductivity method

The study of functional proteins, protein isolates and protein concentrates gives very useful information both from theoretical and practical points of view. Application of experimental results mentioned above may contribute to the improvement of processing and quality of traditional and novel food products rich in or enriched with proteins (LÁSZTITY et al., 1993).

Among the functional properties of protein preparations foaming properties play an important role. As it is known the foams are collodial systems characterized by finely dispersed gaseous phase and a network of continuous membranes of liquid (solid) phase containing soluble surfactants (generally proteins). Two main procedures may be used for production of foams in laboratory conditions:

– intensive mixing and shaking of the liquid – gas system (YASUMATSU et al., 1972), and

– introduction of the finely dispersed gas through capillary system into the liquid phase. Because many foams have very large interfacial areas, they are often unstable.

There are three main destabilizing mechanisms:

– drainage of the lamella liquid due to the gravity, pressure differences, and/or evaporation: drainage is lessened if the bulk liquid phase is viscous and the surface viscosity of the adsorbed protein film is high,

– gas diffusion from small to large bubbles: such disproportionation results from solubility of gas in the aqueous phase,

– rupture of the liquid lamellae separating gas bubbles: such ruptures results in an increase in bubble size through coalescence, and ultimately lead to a collapse of the foam.

Foaming properties of the proteins are generally characterized by foaming activity index (FAI) or foaming power and foam stability index (FSI). Foaming activity may be measured by volume of the produced foam (LIN et al., 1974; DEV & QUENSEL, 1986) or by quantity of the liquid phase transferred into foam system. Characterization of foam stability could be expressed also in different ways: e.g. quantity of drained liquid during a defined time (ELDRIDGE et al., 1963; BALDWIN & SINTHAVALA, 1974) or decrease of the foam volume (time of foam collapse).

From the point of view of measurement and evaluation of foaming properties of proteins it is important to know the kinetics of foam formation and destabilization. A recent publication (MYEONG-AE-YU & DAMODARAN, 1991a) reports on the studies of kinetics of protein foam destabilization and on the evaluation of a method based on Laplace-theory using bovine serum albumin. The kinetics of destabilization of soy protein foams was also studied (MYEONG-AE-YU & DAMODARAN, 1991b). HAIMING-ZHU & DAMODARAN's more recent study (1994) published the results of investigation of heat-induced conformational changes in whey protein isolate and its effect on foaming properties.

The limited precision and repeatability of the generally used methods stimulated efforts of researchers to find more objective instrumental methods. One of the most well-known instrumental methods was published by KATO and co-workers (1983) based on conductivity measurement in the foam system. Earlier observations carried out in our laboratory showed that the mode of arrangement of electrodes in the apparatus for conductivity measurements of foams influences the results obtained. The aim of investigations discussed in this paper was to study this effect and, based on the results, to improve the methods.

1. Materials and methods

Casein, ovalbumin, bovine serum albumin and lysozyme were used as test materials. The main characteristics of protein preparations are shown in Table 1.

To study the foaming properties a 0.2% solution of proteins was prepared in phosphate buffer (pH = 8.2). 2–2 cm^3 of this solution was transferred to an apparatus (Fig. 1) equipped with both horizontal (A) and vertical (B) electrodes in the conductivity cells. The apparatus is our own production.

For foam formation air was introduced through the glass filter into the solution (air flow 2.2 $dm^3\ h^{-1}$) for 120 s. The conductivity was measured and recorded continuously for 5 min.

Table 1

Characteristics of the protein preparations used

Protein preparation	Characteristics		Producer
Casein	nitrogen	15.6 %	
	moisture	6 %	REANAL
	ash	2 %	(Budapest)
	fat	0.2 %	
	carbohydrate	0.5 %	
	free acids	0.2 %	
Lysozyme (lyophilized)	activity	2×10^5 E/mg	REANAL (Budapest)
Ovalbumin (lyophilized)	albumin	90 %	
	lysozyme	1 %	REANAL
	loss on drying	3 %	(Budapest)
	ash	1 %	
Bovine serum albumin (lyophilized)	albumin	92 %	
	α-globulin (in total protein)	8 %	REANAL
	moisture	5 %	(Budapest)
	ash	1 %	
	fatty acids	0.1 %	

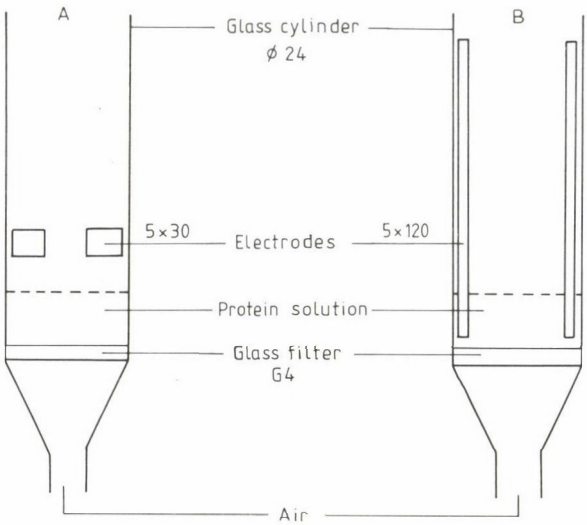

Fig. 1. Conductometric cells for measuring the foam properties of protein preparations with horizontal (A) and vertical (B) arrangement of electrodes

2. Results and discussion

Typical conductometric curves are shown in Fig. 2. The curves obtained using horizontally arranged electrodes were evaluated according to KATO and co-workers (1983). The FAI value was calculated on the basis of the conductivity of the system at the stop of aeration (C_i). The FSI was expressed from the slope ($\Delta t/\Delta C$) of the linear part of the conductivity curve (Fig. 2 A) and the C_0 value was obtained by the extrapolation of the linear part of the curve to zero time (Fig. 2 A).

$$FSI = C_0 \, \Delta t/\Delta C \qquad (1)$$

The numerical results are presented in Table 2. According to the data the FAI is the highest for casein followed, in decreasing order, by bovine serum albumin, lysozyme and ovalbumin.

Regarding to foam stability the bovine serum albumin was found to form the most stable foam followed by casein, ovalbumin and lysozyme. As reported in an earlier work of PUNGOR and co-workers (1990) a very poor correlation was found between these conductometric value and the data of visual evaluation of foam stability. It seems that this contradiction is related first of all to the uncertain determination of the linear descending part of the conductivity curve. According to our view the curve has an exponential character after reaching the maximal value

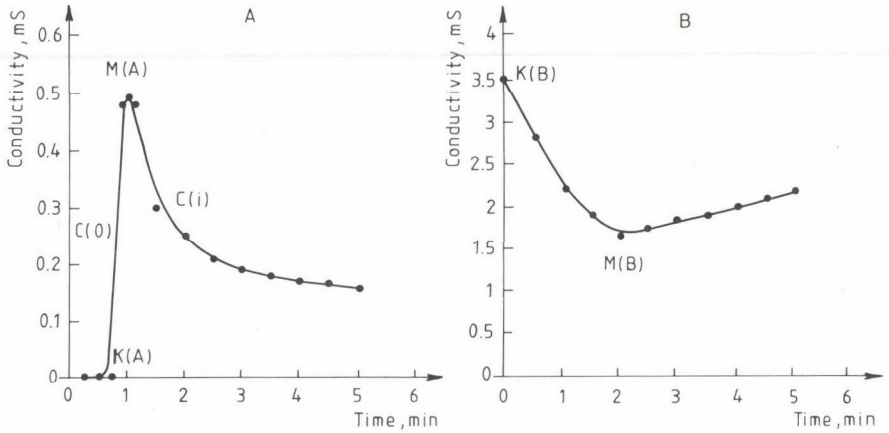

Fig. 2. A: Typical conductivity curves obtained by horizontal electrodes; B: Typical conductivity curve obtained by vertical electrodes

(M(A) in the Fig. 2 A). The trouble mentioned above may be eliminated by using the following equation instead of equation (1):

$$\text{Actual conductivity value: } C(t) = C(i)\, e^{-kt} \qquad (2)$$

In this case the foam stability may be measured by velocity index k instead of FSI value. (It should be noted that the k value represents the rate of foam change and so the lower value of k means a higher stability.)

Table 2

Foaming properties of protein preparations measured by horizontal electrodes

Protein preparation	FAI	FSI	k
	C_i (*mS*)	$C_i \Delta t/\Delta C$ (min)	(l min^{-1})
Casein	0.490 ± 0.015	7.6 ± 0.2	0.145 ± 0.002
Bovine serum albumin	0.460 ± 0.015	9.4 ± 0.3	0.080 ± 0.002
Lysozyme	0.310 ± 0.01	5.1 ± 0.2	0.946 ± 0.002
Ovalbumin	0.061 ± 0.01	5.3 ± 0.2	0.419 ± 0.002

The proposed mode of calculation is confirmed by our observations showing that the visually observed decrease of foam volume is also not a linear but an exponential one and may be expressed as follows:

$$V(t) = V(O)\, e^{-lt}$$

where V(O) : initial (maximal) volume
V(t) : actual volume
l : velocity index (rate of decrease; similar to k value).

So the formal mathematical expression of stability determined by the two methods is the same and is more comparable.

The instrumental determination of stability is more reliable due to the better repeatability and objectivity. This fact makes the detection of slight differences between the investigated proteins possible.

Nevertheless, a more detailed study of measurements made with horizontal electrodes revealed some disadvantages of this method. These may be well demonstrated if we compare e.g. the conductometric curves of casein and bovine serum albumin (Fig. 3).

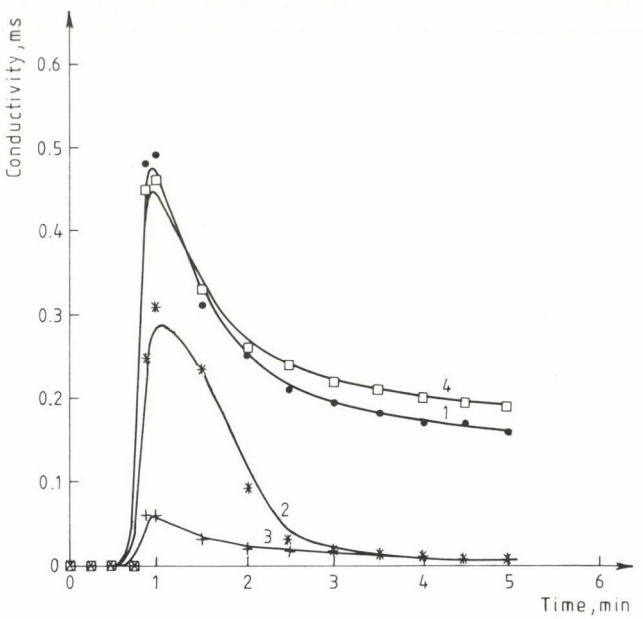

Fig. 3. Characterization of protein foams by data obtained with measuring cell with horizontal electrodes. 1: Casein; 2: lysozyme; 3: ovalbumin; 4: bovine serum albumin

As it can be seen, no significant differences can be observed at any part between the two curves. What could be the cause of this observation?

– The electrodes do not record the events occurring in the total system. The platinium plates of 0.5 cm^3 width are acting as electrodes of a capacitor (condenser) and so they are receptive only to the part of system being in the space between two electrodes. The electrodes do not give information about the events in the space over and under the electrode pair. Although the formation of the foam starts immediately with aeration the change of conductivity (Fig. 2 A) begins only after the upper level of foam reaches the height of the electrodes. After this event the conductivity and the maximal conductivity depends on the quantity of liquid phase transferred to the interelectrode space. This is in correlation with foaming properties of the protein investigated.

– The maximum conductivity is always reached before aeration stops (less than 120 s). This is understandable because the upper level of the foam surpasses after some seconds the height of upper electrode. The decrease in conductivity after reaching the peak of curve (but before stopping of aeration) means that the properties of the foam change in this period. It is suggested that this decrease may be in connection with protein coagulation. Otherwise the conductivity should remain constant after the maximum of the curve. The occurrence of coagulation may be supported by the fact that long time aeration with constant air flow causes a decrease in foam stability.

– If the concentration of the protein solution, the time of foam formation and the air flow are standardized, the total volume of foam depends on the quality of protein. Nevertheless, due to the fact that the foam volume is always higher than the volume controlled by electrodes, no information is obtained about great part of the system. After stopping the aeration only the changes occurring between electrodes are recorded. In addition differences in the status of foam may be observed inter the electrodes and over the electrodes. As a result the data about foam stability do not represent the status of total foam.

To avoid the problems mentioned above LOISEL and co-workers (1993) constructed an apparatus with two pairs of electrodes located at the upper and lower part of instrument and in addition equipped with visual control possibilities with videotechnique and computer aided evaluation of observations.

Our experiments showed that the problems may be solved in a simpler way using vertically arranged electrodes in the apparatus (Fig. 1, B). During experiments all other parameters were the same as used in previous measurements. The typical conductivity curve obtained by using vertical electrodes is shown in Fig. 2, B. Comparing this curve with that shown in Fig. 2, A the following statements could be made:

– The process of foam formations may be followed even in the very beginning. No "dead time" part occurs at the conductivity curve (O – K(A) part of the curve).

– Although a negative slope is typical for the conductivity curve (due to the decrease in the volume of initial protein solution) the signal is three times greater. This is especially important from the view point of measurement precision. The signal to noise ratio is highly improved.

– Using the horizontal electrodes no differences were observed between foaming properties of casein and bovine serum albumin. The vertical electrode arrangement allowed a finer differentiation: the FAI values may be well determined by the difference between initial conductivity and that measured at the minimum point.

– The extreme of conductivity curve is reached at the end of aeration and so the interpretation of FAI values is much easier. As it is seen from Tables 2 and 3 the order of the proteins investigated for foaming properties is not the same. Bovine serum albumin has the best FAI value followed by casein in case of vertical electrodes and it is in contrast to the horizontal arrangement.

– The curve section belonging to the period after stopping the aeration has no break point, it is clearly linear which makes the precise determination of stability (FSI) possible. It means that the use of equation

$$FSI = C_{M(B)} \Delta t_{(B)} / \Delta C_{(B)} \qquad (2)$$

is fully correct. It should be noted that this evaluation gives a good differentiation between ovalbumin and lysozyne, too, supported by visual observations.

Table 3

Foaming properties of protein preparation measured by vertical electrodes

Protein preparation	FAI	FSI
	$C_M = K_B - M_B$ (mS)	$C_M = \Delta t / \Delta C$ (min)
Bovine serum albumin	2.18 ± 0.02	14.8 ± 0.1
Casein	1.66 ± 0.02	11.9 ± 0.1
Lysozyme	1.26 ± 0.01	1.8 ± 0.1
Ovalbumin	0.57 ± 0.01	7.0 ± 0.1

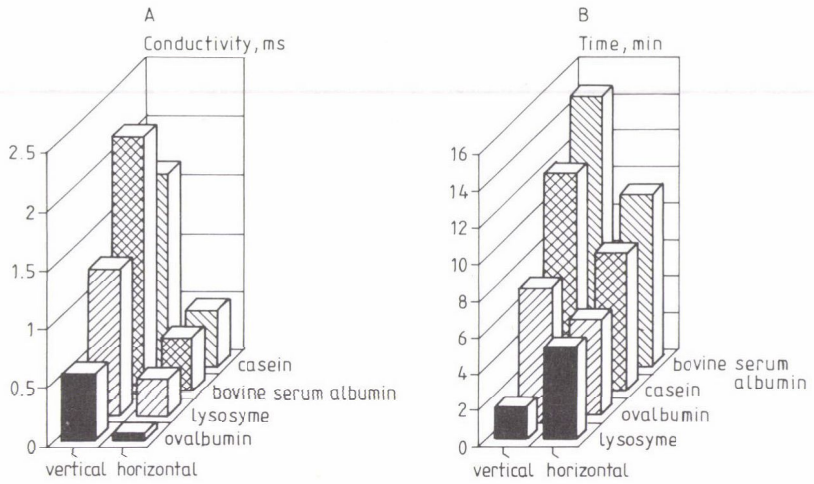

Fig. 4. A: FAI (foam activity index) values of protein preparations, measured by horizontal and vertical electrodes; B: FSI (foam stability index) values of protein preparations, measured by horizontal and vertical electrodes

The advantages of vertical electrodes is well demonstrated by Fig. 4, with the comparison of the two methods.

It is obvious that using horizontal electrodes the stability may be correctly evaluated with exponential mathematical model and in the case of vertical electrodes with linear equation. The vertical arrangement gives a better precision and higher correlation ($r = 0.97$) than the horizontal arrangement ($r = 0.82$).

References

BALDWIN, R. E. & SINTHAVALA, S. (1974): Fish protein concentrate foam. *J. Fd Sci.*, *39*, 880–882.

DEV, D. K. & QUENSEL, E. (1986): Functional and micro-structural characteristic of linseed (*Linum usitatisimum* L.) flour and a protein isolate. *Lebensm.-Wiss. und Technol.*, *19*, 331–337.

ELDRIDGE, A. C., HALL, P. K. & WOLF, W. J. (1963): Stable foams from unhydrolized soybean protein. *J. Fd Technol.*, *17*, 120–123.

HAIMING-ZHU & DAMODARAN, S. (1994): Heat-induced conformational changes in whey protein isolate and its relation to foaming properties. *J. agric. Fd Chem.*, *42*, 846–855.

KATO, A., TAKAMASHI, A., MATSUDOMI, N. & KOBAYASHI, K. (1983): Determination of foaming properties of proteins by conductivity measurements. *J. Fd Sci.*, *48*, 62–65.

LÁSZTITY, R., TÖMÖSKÖZI, S., NAGY, J. & PUNGOR, A. (1993): The surface properties and other functional parameters of cereal germ proteins. – in: SCHWENKE, K. D. & MOTHES, R. (Eds) *Food proteins, structure and functionality.* VCH Publishers, Weinheim-New York, pp. 310–315.

LIN, M. Y., HUMBERT, E. S. & SOSULSKI, F. W. (1974): Certain functional properties of sunflower meal products. *J. Fd Sci.*, *39*, 366 – 370.

LOISEL, W., GEUGUEN, J. & POPINEAU, Y. (1993): A new apparatus for analysing foaming properties of proteins. – in: SCHWENKE, K. D. & MOTHES, R. (Eds) *Food proteins, structure and functionality.* VCH Publishers, Weinheim-New York, p. 320.

MYEONG-AE-YU & DAMODARAN, S. (1991a): Kinetics of protein foam destabilization: Evaluation of method using bovine serum albumin. *J. agric. Fd Chem.*, *39*, 1555 – 1562.

MYEONG-AE-YU & DAMODARAN, S. (1991b): Kinetics of destabilization of soy protein foams. *J. agric. Fd Chem.*, *42*, 1562 – 1567.

PUNGOR, A., TÖMÖSKÖZI, S. & LÁSZTITY, R. (1990): Investigation of the foam characteristics of protein. *J. Fd Physics*, *54*, 19 – 28.

YASUMATSU, K., SAWADA, K., MORITAKA, S., MISAKI, M., TODA, J., WADA, T. & ISHII, K. (1972): Whipping and emulsifying properties of soybean products. *Agric. biol. Chem.*, *36*, 719.

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METABOLISM AND NONABSORPTION OF SOYBEAN HYPOCOTYL SAPONINS IN THE RAT MODEL

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Soybean saponins are soybean glycosides of which new soybean saponins, containing the 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one(DDMP) moiety have recently been detected. Soybean saponins which lost this moiety have already been found to posses physiological functions. The new saponins, called DDMP-saponins, are also expected to exhibit physiological characteristics. However, no digestive experiments for DDMP-saponins or non-DDMP-saponins have been conducted until this study. Therefore, trials have been carried out using rats fed on soybean hypocolyl diets which contain significant quantities of saponins. As a result of analyses using HPLC and TLC, it was found that no saponins were present in urine, in blood nor in liver. However, aglycones, non-DDMP-saponins and small amounts of DDMP-saponins were detected in faeces. These results indicated that soybean saponins are not absorbed in alimentary canal of the rats but rather are hydrolyzed into aglycones.

Keywords: Absorption, saponins, soybean, triterpenoids

Soybeans are important legume that have been utilized for oil production in the food industry. Soybean saponins, one of the glycosides present in soybeans, were initially regarded as a waste product, but recently hypocholestemic (OAKENFUL et al., 1981), antioxidant properties (OHMINAMI et al., 1984), antitumor-promoting (KONOSHIMA et al., 1991) and HIV infection inhibitory properties (NAKASHIMA et al., 1989) have been reported.

Glycyrrhizic acid, a sweet saponin present in liquorice, is well known to have pharmacological activities (AKAMATSU et al., 1991) and several authors have investigated the metabolism of glycyrrhizic acid (ITOH et al., 1985 and OZAKI et al., 1990). Both glycyrrhizic acid and soybean saponins have similar aglycones and contain D-glucuronic acid conjugated to the C–3 position of each aglycone.

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Recently, new soybean saponins called DDMP-saponins, having the 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one(DDMP) moiety, have been detected (KUDOU et al., 1993) and these new saponins are also expected to exhibit physiological characteristics.

Considering these findings, we were interested to investigate the digestibility of soybean saponins. Because the intake of the soybean saponins, especially DDMP-saponins was unknown, the levels of soybean saponins in rat's blood, urine, faeces and liver were initially analysed.

Soybean saponins can be divided into 3 groups, group A, B and E, on the basis of their aglycone. Soyasapogenols A, B and E, respectively. Soybean saponin group A has been found to be distributed only in hypocotyl together with soybean saponin group B. Soybean hypocotyls were, therefore, used as the source of soybean saponins after deactivating antinutritional enzymes, such as Trypsin inhibitor, in this study.

1. Materials and methods

1.1. Animals and diets

Ten 9-week-old rats (male of Wistar strain, purchased from Funabashi Farms, Chiba Japan) were individually housed in sanitized, stainless, and screen bottom cages. Lighting in the animal room was on a 12 h light:dark cycle (light on 08:00–20:00). The temperature was maintained at 20±4 °C with relative humidity at 40–70%.

The rats were fed on control diet for 2 weeks and then the experimental diet containing soybean hypocotyls for the next 2 weeks. In the experiment, a diet of 13.8% corn starch (10% of the total diet) was substituted for soybean hypocotyls. The food and fresh water were given ad libitum. Food consumption and body weights were measured daily. Faeces and urine were collected daily. Body weights were also recorded daily. At the end of the experiment rats were sacrificed and weights of organs were compared between the each feeding period (control diet vs. hypocotyl rich diet; experimental diet). In starvation experiments, ten 9-week-old rats (male of Wistar strain, purchased from Japan Charles River, Yokohama Japan) were housed together and were given only water ad libitum for one week. The rats were then fed on the experimental diet containing soybean hypocotyl for seven days.

1.2. Urine collection

Urine was collected for 24 h in dry tubes, and urine weight was recorded daily. The obtained urine was filtered through a 6 μm filter (Toyo No. 1. Adavantec) and the solution lyophilized and subjected to glycoside analyses.

1.3. Faeces collection

Faeces were collected when food was given and lyophilized daily. The lyophilized samples were subjected to glycoside analyses.

1.4. Blood analysis

Blood was obtained from the tail vein using heparinized 21 gauge needles and 1 cm^3 syringes at the end of each feeding period. The plasma was separated by centrifugation at 6000 × g for 20 min at 4 °C and then subjected to glycoside analyses.

1.5. Glycoside extraction

Lyophilized urine, lyophilized milled faeces and liver, and blood plasma were dispersed with four volumes of 70% aqueous ethanol containing 0.01% EDTA overnight at 5 °C. After filtration through paper, the filtrate was evaporated to dryness under reduced pressure below 40 °C and dissolved in water:1-butanol (1:1). After centrifugation, the butanol layer was analysed by HPLC and TLC.

1.6. Glycoside detection

HPLC was performed on a YMC-pack (7 μm, 10.0 ø × 250 mm, Yamamura Chemical Laboratories Co., Ltd, Kyoto Japan) and ODS-AM303 (5 μm, 4.6 × 250 mm, Yamamura Chemical Laboratories Co., Ltd, Kyoto Japan) using acetonitrile:water:acetic acid:EDTA (39:60.96:0.03:0.01) for soybean saponin group B detection. The instrument used was a Waters HPLC system, comprising a U6K injector. Model 510 pump and Model 991J Photodiode Array detector (Waters, Japan Millipore Ltd.). DDMP-saponins were directly identified by measuring the UV spectra at 205–320 nm with the Photo Array detector (Waters, Japan Millipore Ltd.).

TLC was conducted on a Kieselgel 60 F-254 plate (Merck Co. Ltd.) using chloroform:methanol:water (65:35:10, v/v lower layer) for saponin detection and benzene:dioxane:acetic acid (20:5:1, v/v) for aglycone detection. The components on the TLC plate were visualised by spraying with 10% sulfuric acid and heating at 110 °C for 10 min.

2. Results

2.1. Growth and food consumption

Table 1 shows the composition of soybean hypocotyl and the composition of each diet is given in Table 2. The rats were fed with control diet for 2 weeks and then with the experimental diet for the next 2 weeks. In the experiment, a diet of 13.8%

Table 1

Composition of soybean hypocotyl

Moisture	8.4%	Heat vacuum dry
Protein	36.6%	Kjeldahl method
Fat	14.0%	Chloroform-methanol extraction
Fiber	3.5%	Improved Bennerug-Storman method
Ash	3.8%	Dry ashing
Carbohydrate	33.7%	100-above listed components

corn starch (10% of the total diet) was substituted for soybean hypocotyl. During repletion period, the food intake of the animals was similar, averaging 39.6 g/day and 38.0 g/day for the period of the control diet and the experimental diet, respectively (Table 3). Although slightly higher average food intake was observed for the group on the control diet, there was no significant difference (chi-square statistical analysis) in the food efficiency during the two feeding periods (Table 3). In order to investigate the influence of the high soybean hypocotyl diet, weight of organs after each feeding period was compared (Table 4). There was no significant difference in their weights and no difference was observed in the configuration of their organs (chi-square statistical analysis). It was concluded that the hypocotyl rich diet had no effect on the rats' growth.

Table 2

Composition of the diets (g/100 g diet)

Ingredient	Control diet	Experimental diet
Casein	20	20
Corn starch	72	62
Soybean oil	2	2
Salt mixture[1]	4	4
Vitamin mixture[2]	1	1
Vitamin mixture[3]	1	1
Soybean hypocotyl		10

[1]: Harper's salt mixture;
[2]: Harper's vitamin mixture (water-soluble);
[3]: Harper's vitamin mixture (oil-soluble).
The rats were fed with control diet for 2 weeks and then with the experimental diet for the next 2 weeks. In the experiment, a diet of 13.8% corn starch (10% of the total diet) was substituted for soybean hypocotyls

Table 3

Food intake and food efficiency

	Control diet	Experimental diet
(A) Food intake ($g \cdot d^{-1}$)	39.6 ± 4.3[a]	38.0 ± 2.7[a]
(B) Weight gain ($g \cdot d^{-1}$)	5.5 ± 2.3[a]	4.7 ± 5.4[a]
(C) Food efficiency ($B \cdot A^{-1}$)	0.14	0.12

[a] Values are means ± standard deviation

2.2. Urine, blood and liver analyses.

The results of TLC and HPLC analyses showed that neither saponins nor aglycones were detected in the blood obtained during the experimental diet (Figs. 1 and 2). Urine and liver extracts were also analysed by TLC and HPLC and neither saponin nor aglycones were detected.

On the assumption that saponin detection was impossible, 1 μM of isolated soyasaponin Bb in blood was subjected to TLC analysis. As shown in Fig. 3, the band of soyasaponin Bb can be visualised. In the case of analyses of blood and liver extracts, samples were concentrated 100 times for TLC analyses. Therefore 0.01 μM was the limit of detection for each saponin.

Table 4

Comparison of organs

Organs	Control diet[a]	Experimental diet[a]
Lung	1.16 ± 0.15	1.28 ± 0.18
Heart	1.24 ± 0.35	1.01 ± 0.41
Lien	0.65 ± 0.19	0.76 ± 0.11
Liver	14.17 ± 3.28	10.29 ± 3.69
Adrenal gland	0.53 ± 0.12	0.32 ± 0.18
Kidney	2.84 ± 0.16	2.45 ± 0.21

[a]: Values are means ± standard deviation

These results suggest that soybean saponins were unable to be absorbed by the rat model.

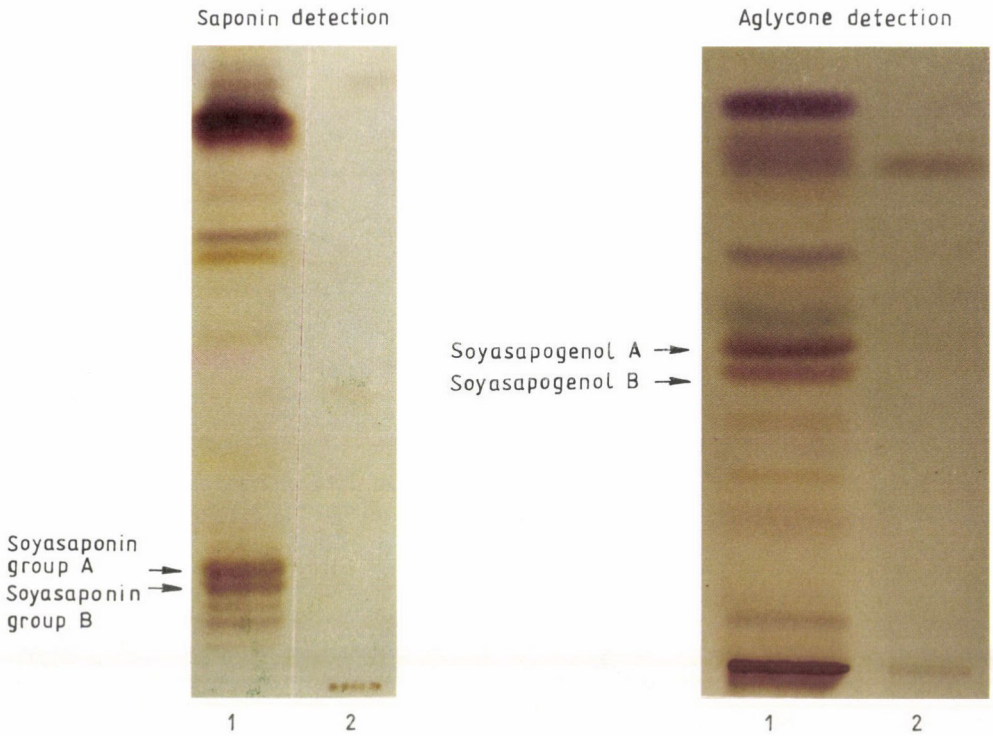

Fig. 1. TLC patterns of blood extracts. 1: Controls of soybean glycosides and aglycones; 2: blood extracts obtained during soybean hypocotyl rich diet (experimental diet). TLC was conducted on a Kieselgel 60 F-254 plate (Merck Co. Ltd.) using chloroform:methanol:water (65:35:10, v/v lower layer) for saponin detection and benzene:dioxane:acetic acid (20:5:1, v/v) for aglycone detection. The components on the TLC plate were visualised by spraying with 10% sulfuric acid and heating at 110 °C for 10 min

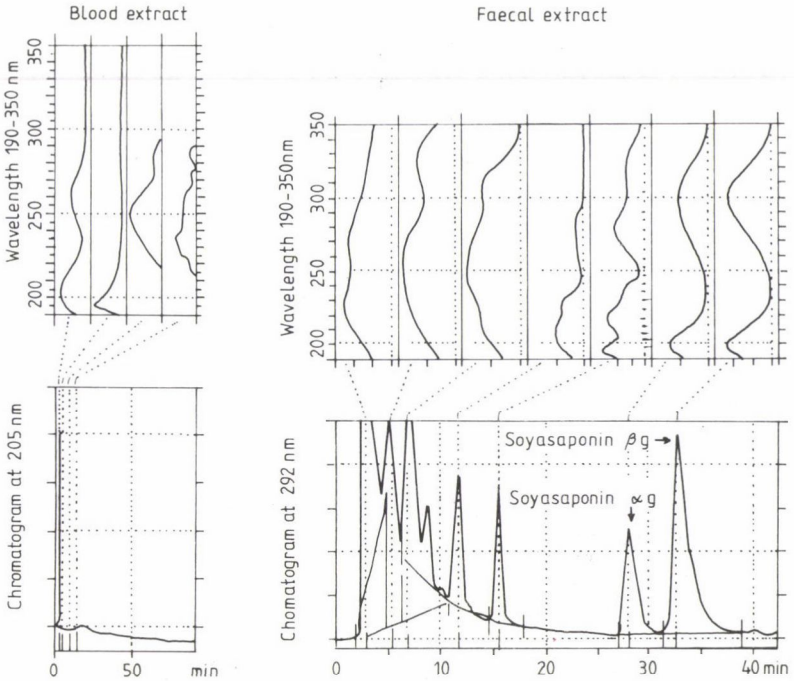

Fig. 2. HPLC patterns of blood and faecal extracts. 70% aqueous ethanol fractions from blood and faeces obtained during the experimental diet were dissolved in water:1-butanol (1:1). After centrifugation, the butanol layer was analysed by HPLC

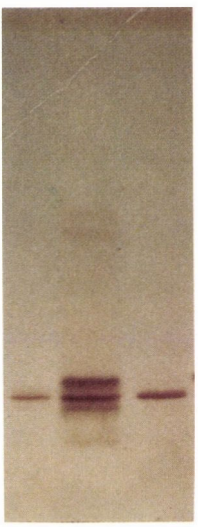

Fig. 3. TLC patterns of soyasaponins. 1: Soyasaponin Bb in blood extract; 2: control of soyasaponins; 3: control of soyasaponin Bb

2.3. Faecal analysis

No soybean saponins were found in the faeces obtained from the control group. Faeces obtained from the experimental diet were analysed by TLC (Fig. 4A). Small amounts of soybean saponin groups A and B were detected. Major bands were found at higher Rf position which were estimated to correspond to their aglycones. The presence of soyasapogenols A and B was confirmed by TLC analyses with aglycone detections (Fig. 4B). The thin layer chromatogram of the substance revealed two additional unknown major bands at higher Rf position. HPLC analysis was also conducted for DDMP-saponin detection (Fig. 2) and two DDMP-saponins (soyasaponins αg and βg), exhibiting a maximum absorbance at 292 nm, were detected. These results suggest that most, but not all, of the soybean saponins were hydrolyzed into aglycones in the rat alimentary canal.

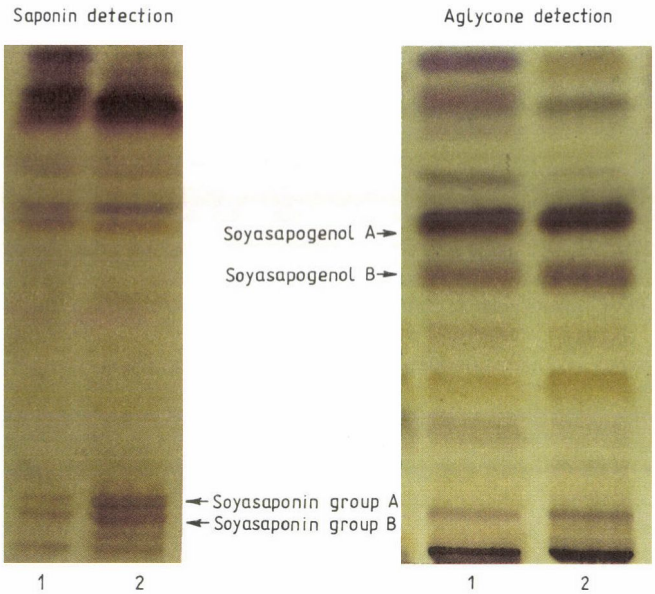

Fig. 4. TLC patterns of faecal extracts. 1: Faecal extract obtained during the experimental diet; 2: controls of soybean glycosides and aglycones

3. Discussion

Neither soybean saponins nor their aglycones were detected in blood and urine. On the assumption that saponins were accumulated in the liver, liver extract was analysed but no saponins were detected. It was also estimated that after

starvation, saponins had a possibility to be absorbed. However no saponins were detected in blood, urine and liver. Soybean saponin absorption was also tested by everted sac method (data not shown) and those components were not detected when the outer Tyrod's solution was analysed.

Soybean saponin aglycones were found in faeces. It is supposed that in the large intestine, some intestinal bacteria may produce enzymes and saponins may be hydrolyzed into their aglycones.

The DDMP (2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one) moiety of DDMP-saponin may be involved in physiological activities. However, at present, we are unable to detect the hydrolyzed DDMP moiety. We are now attempting to determine whether soybean saponins prevent mouse fibroblast cells from damage of hydrogen peroxide and soybean saponins seem to inhibit the cellular injury (data not published). Therefore, soybean saponin physiological functions are still expected even if the whole molecule is not absorbed.

4. Conclusion

When the rats were fed on the experimental diet containing soybean saponins, no saponins and aglycones were detected in urine nor in blood. However, soyasapogenols A and B, non DDMP-saponins and small amounts of DDMP-saponins were detected in faeces. These results suggest that soybean saponins are not absorbed and most soybean saponins are hydrolyzed into aglycones in the alimentary canal.

Abbreviations

HPLC (high pressure liquid chromatography), TLC (thin layer chromatography) and EDTA (disodium ethylenediaminetetraacetated, dihydrate)

*

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References

AKAMATSU, H., KOMURA, J., ASADA, Y. & NIWA, Y. (1991): Mechanism of anti-inflammatory action of glycyrrhizin: Effect on neutrophil functions including reactive oxigen species generation. *Planta Med.*, *57*, 119–121.

ITOH, S., AKASAKA, N., HASHIMOTO, Y., ISHIBASHI, M. & MIYAZAKI, H. (1985): Quantitative analysis of glycyrrhizin and glycyrrhetinic acid in plasma after administration of FM-100 by using gas chromatography-selected ion monitoring. *Yakugaku Zasshi*, *105* (12), 1150–1154.

KONOSHIMA, T., KOZUKA, M., HARUNA, M. & ITO, K. (1991): Constituents of leguminous plants. XIII. New triterpenoid saponins from wistaria brachybotrys. *J. Nat. Prod.*, *54*, 830–836.

KUDOU, S., TONOMURA, M., TSUKAMOTO, C., UCHIDA, T., SAKABE, T., TAMURA, N. & OKUBO, K. (1993): Isolation and structural elucidation of DDMP-conjugated soyasaponins as genuine saponins from soybean seeds. *Biosci. Biotech. Biochem.*, *57* (4), 546–550.

NAKASHIMA, H., OKUBO, K., HONDA, Y., TAMURA, T., MATSUDA, S. & YAMAMOTO, N. (1989): Inhibitory effect of glycosides like saponin from soybean on the infectivity of HIV in vitro. *AIDS*, *3* (10), 655–658.

OAKENFUL, D. G. (1981): Dietary fiber, saponins and plasma cholesterol. *Fd Technol. Aust.*, *33*, 432–435.

OHMINAMI, H., KIMURA, Y., OKUDA, H., ARICHI, S., YOSHIKAWA, M. & KITAGAWA, I. (1984): Effects of soyasaponins on liver injury induced by highly peroxidized fat in rats. *Planta Med.*, *46*, 440–441.

OZAKI, Y., NOGUCHI, M., KAMAKURA, H. & HARADA, M. (1990): Studies on concentration of glycyrrhizin in plasma and its adsorption after oral administration of licorice extract and glycyrrhizin. *Yakugaku Zasshi*, *110* (1), 77–81.

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DETERMINING GLUCOSE CONTENT IN JUICES WITH BIOSENSOR

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An enzyme-based biosensor has been developed for determining glucose content of juices. The biosensor consists of two parts, the enzymatic reaction with glucose oxidase was completed in a thin-layer enzyme cell, while the hydrogen peroxide produced was detected in an amperometric cell.

After optimizing the system, the linear measuring range was between 0.1–5.0 mmol l^{-1} glucose (without using dialyser) or up to 0.5 mol l^{-1} when using dialyser. Juice samples were measured both with reference method and with biosensor, and the results showed a correlation of 0.993. On the base of the results it can be stated, that the constructed measuring system is suitable for determining glucose in fruit juices routinely.

Keywords: glucose determination, juices, biosensor

Biosensors open a new area for quality control, first of all in the technology automatization. Great number of papers concerning the research and application of biosensors were published in the literature in the last few years. Different types of instruments have been developed for determining various substrates (glucose, sucrose, lactose) in food (WISEMAN, 1985). Using biosensors, the control of the fermentation and biotechnological processes has been solved by measuring alcohol, ascorbic acid, amino acids, etc. (TREVAN, 1980). The freshness of meat and fish was also checked by biosensor (WATANABE et al., 1984).

Recognizing the perspectives of biosensors we attempted to introduce this technique in the quality control of juices.

Determination of glucose content is one of the most important routinely performed tests for quality control in fruit drink processing. Generally the glucose content is determined by gas chromatography or by enzyme-photometric method. These methods are expensive and time consuming. The aim of our present work was to study the possibility of constructing a biocell to determine glucose content in soft

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drinks. With this method many disturbing effects could be eliminated, the determination is very quick with a small demand for chemicals.

For enzymatic glucose measurement the following reactions can be used:

$$\text{D-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$H_2O_2 \rightarrow O_2 + 2e^- + 2H^+$$

The biosensors based on glucose oxidase usually measure the H_2O_2 generated or the change in the O_2 concentration. Several authors reported on the development of biosensors for glucose determination.

GUILBAULT and LUBRANO (1973) developed a glucose electrode by coupling the insolubilized glucose oxidase to an electrochemical sensor. The electrode consisted of a metallic sensing layer covered by a thin film of immobilized glucose oxidase held in place by means of cellophane.

YAO (1983) constructed a chemically modified enzyme membrane electrode by cross-linking glucose oxidase with bovine serum albumin using glutaraldehyde. The surface of the platinum electrode was silanized with 3-aminopropyltriethoxysilane.

BROOKS and co-workers (1987/88) improved the enzyme immobilization by using periodate-oxidized glucose oxidase and an alkyl amine electrode coating. A computer controlled analytical system was developed for fermentation monitoring.

DEMURA and ASAKURA (1989) immobilized the glucose oxidase in *Bombyx mori* silk fibroin membrane by physical treatment, it was stretched without any chemical reagents.

MALE and LUONG (1993) developed a FIA biosensor system for determination of glucose from urine, blood and foodstuffs. Glucose oxidase was immobilized onto porous aminopropyl glass beads in an enzyme column. In the system there was an anion exchange column to remove the interfering substances.

The authors pointed out the problems arising from the determination of glucose, such as the difficulty in immobilizing the enzyme, the short stability of the sensor and the insufficient selectivity of the measurement.

To avoid these disadvantages we tried to optimize a thin-layer enzyme-cell for glucose determination in fruit juices. This type of cell has been successfully applied for analysis of maltose in fermentation broth (VÁRADI et al., 1993) and alcohol in beer (VÁRADI & ADÁNYI, 1994).

1. Materials and methods

1.2. The measuring set-up

The measuring system (Fig. 1) consisted of a buffer reservoir, a peristaltic pump (Minipuls 3, Gilson Co., France, the flow rate can be changed by varying the tubings of different diameter, Elkay Products, Inc., Boston, USA), an injection valve (with 20 μl sample loop, Rheodyne Inc., Cotati, CA, USA), a dialyser (it was used only when samples were measured) and a thin-layer enzyme cell. The enzyme cell was placed in a thermostat. For detection of the amperometric current a flow-through amperometric detector was used. A platinum wire of 0.4 mm diameter and 5 mm length was used as a measuring electrode while the reference electrode was a Ag/AgCl electrode. The polarization potential was ensured and fixed (+600 mV) by a polarograph (OH – 105, Radelkis, Budapest, Hungary).

The thin-layer enzyme cell developed by us consisted of two Plexi blocks (A, D), a protein membrane for immobilizing the enzyme (C) and a Teflon plate of 0.5 mm thick (B). The Teflon plate is supplied with a channel of 2.5 mm diameter and 120 mm length. The protein membrane (Pigs' small intestine) was washed in a buffer solution (pH 6), tightened on the Plexi block and dried. The enzyme solution was applied on the membrane and was immobilized with glutaraldehyde solution. While the solution is flowing through the channel of Teflon plate the enzyme reaction is taking place.

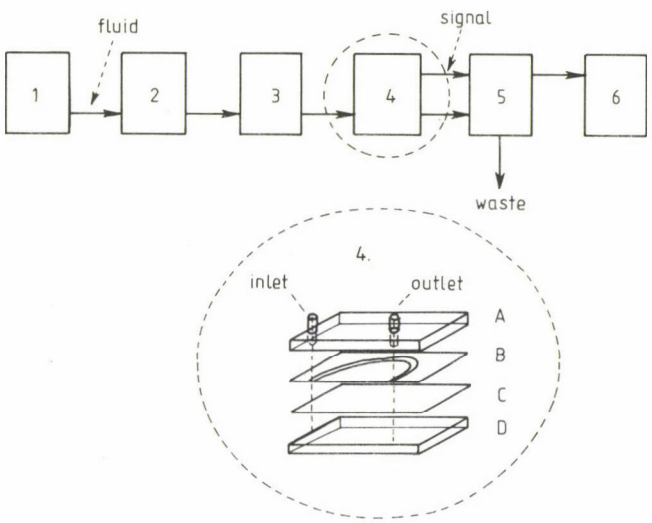

Fig. 1. Measuring set-up. 1: buffer reservoir; 2: peristaltic pump; 3: sample injector; 4: thin-layer enzyme cell; 5: electrochemical detector; 6: computer; A, D: plexi blocks; B: teflon plate; C: protein membrane

1.2. Reagents

Lyophilized glucose oxidase (GO, EC 1.1.3.4., 25 U mg^{-1}) from *Aspergillus niger* was obtained from Sigma (St. Louis, MA, USA).

All other reagents were commercially available and of analytical grade.

1.3. Samples

Twenty-eight different fruit juices were bought. They were produced by 9 different factories. The glucose content of the samples was also determined with a reference UV method (D-glucose/D-fructose UV-test, Boehringer, Mannheim GmbH, Germany).

2. Results and discussion

2.1. Immobilization of the enzyme

To get a stable enzyme-layer the enzyme (GO), the bovine serum albumin (BSA) and glutaraldehyde (GA) was used in different concentrations as it is shown in Table 1.

It was experienced by preparing cells that using too much bovine serum albumin in the enzyme solution caused instability of the enzyme layer, furthermore after drying it could be easily damaged and peeled off. If the glutaraldehyde solution was not thick enough, the enzyme-layer could be easyly washed out, the fixing was not effective enough and the lifetime of the cell was only a few days. Using too much enzyme did not seem to improve the activity of the cell. In fact, putting 15 mg glucose oxidase into the solution caused the detachment of the fixed layer during the measurement and it plugged the system.

Table 1

Composition of enzyme- and glutaraldehyde-solutions

No.	Enzyme solution (0.2 ml)		GA solution (0.05 ml)
	GO (mg)	BSA (mg)	GA (%)
1	5.0	5.0	2.5
2	7.5	5.0	2.5
3	10.0	2.5	1.5
4	10.0	2.5	2.5
5	10.0	5.0	1.5
6	10.0	5.0	2.5
7	12.5	5.0	2.5

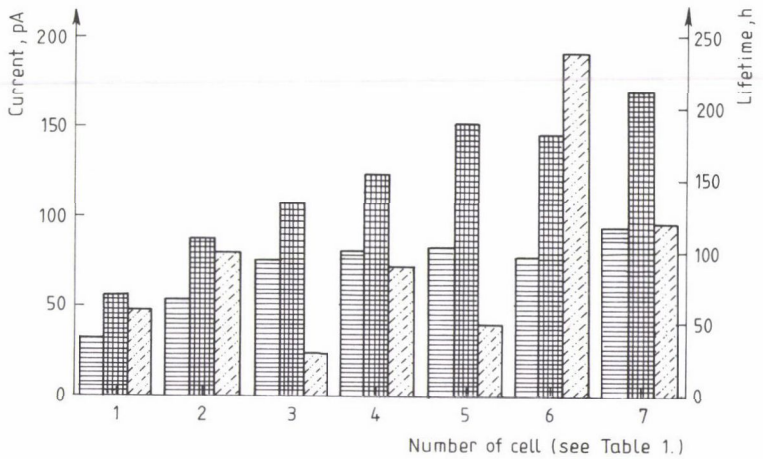

Fig. 2. Characteristics of enzyme cells.
▤: 0.5 mmol l^{-1} glucose; ▦: 1.0 mmol l^{-1} glucose; ▨: lifetime, h

Using the thin-layer cells the amperometric signals of glucose standards were examined along with the progress of the reaction by measuring the quantity of the hydrogen peroxide developed. The characteristics of the experimental cells were summarized in Fig. 2.

The difference between the cells 1 and 2 was the quantity of the enzyme. In case of immobilizing more enzyme the peaks became higher, the lifetime was longer (60, 100 h). The difference between the cells 3 and 4 was the concentration of glutaraldehyde, and there was no significant difference between the heights of the amperometric signals, whereas the lifetime of the cell 3 was only 30 h while that the cell 4 was 90 h. There was more bovine serum albumin fixed in the cells 5 and 6 than in the cells 3 and 4. The higher quantity of bovine serum albumin only a little increased the height of the peaks, but the lifetime of the cells was longer than in the previous cases. The glucose signals of the cell 6 were not the highest, but the cell held its stability for 240 h. In case of cell 5 the enzyme was washed out quickly and it worked only for 50 h. In cell 7 the quantity of glucose oxidase was more than in cell 6, so higher amperometric signals were measured, but the lifetime was only 120 h. According to the results the most stable thin-layer cell is to be made with 10 mg glucose oxidase, 5 mg bovine serum albumin in 200 μl buffer solution, and immobilisation with a glutaraldehyde solution of 2.5%.

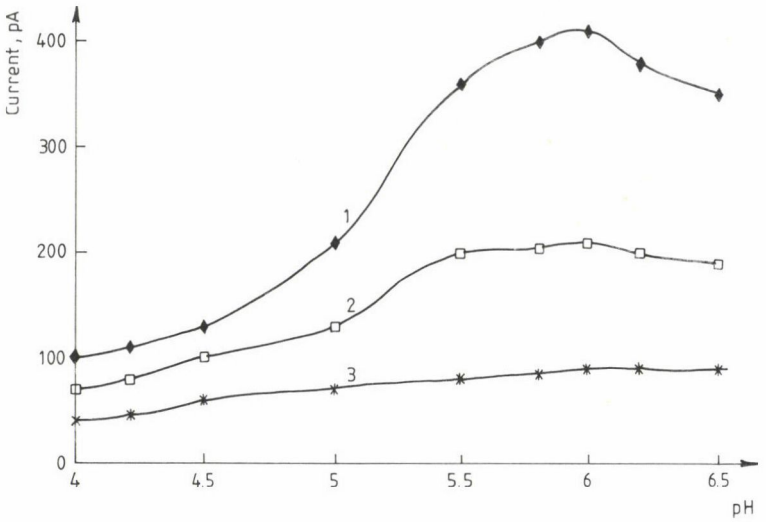

Fig. 3. Effect of pH value on glucose determination. 1: 5.0 mmol l^{-1} glucose; 2: 2.5 mmol l^{-1} glucose; 3: 1.0 mmol l^{-1} glucose

2.2. Optimization of biochemical and electrochemical reactions

To study the effect of pH value glucose standards (1.0, 2.5, 5.0 mmol l^{-1}) and samples were measured in acetate buffers (0.2 mol l^{-1} Na-acetate, pH adjusted by 0.2 mol l^{-1} acetic acid) of various pH-values (4.2, 4.5, 5.0, 5.5, 5.8, 6.0, 6.2, 6.5). The effectivity of the glucose oxidation as a function of pH is shown in Fig. 3. The optimal pH value for measuring glucose was found to be at about pH 6.

The effect of temperature on the enzymatic reaction was also investigated. The temperature of the thermostat was increased from 25 °C up to 45 °C. The highest peaks were obtained at higher temperatures, but the linearity of the measurement was accepted at 32 °C (Fig. 4).

Investigating different flow-rates the results have proved to be optimal at a flow-rate of 0.32 ml min^{-1} (Fig. 5). In case of slower flow-rates the sample remains longer in the thin-layer reactor. Due to this fact the reaction is complete, but the curves are wider and less sharp. While at higher flow-rate peaks are narrower, the carry-over is reduced, but as the residence time in the cell is short there is only a limited amount of glucose reacting with the enzyme.

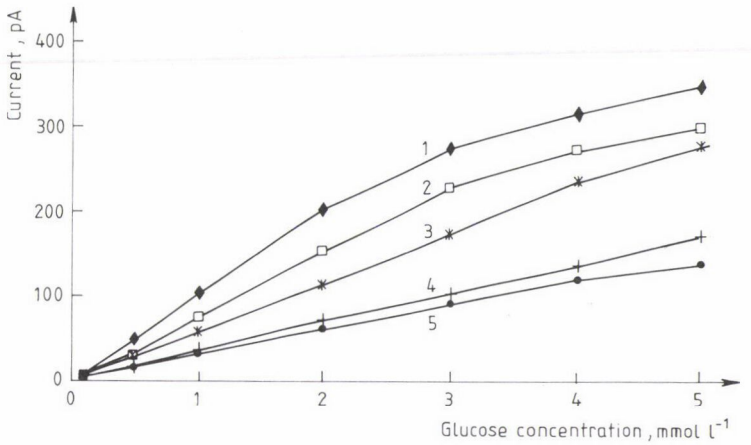

Fig. 4. Effect of temperature on glucose determination. 1: 45 °C; 2: 42 °C; 3: 37 °C; 4: 32 °C; 5: 25 °C

2.3. Study of the effect of other carbohydrates

The effect of different substrates present in soft drinks was studied using optimal parameters for the measurement. To eliminate the disturbing effect of big molecules present in the juices, and to have continuous dilution a flow-through dialyser was inserted into the flowing system (the dilution of the dialyser was about 1 to 100). The effect of D-fructose, D-galactose, maltose. saccharose, and D-xylose was

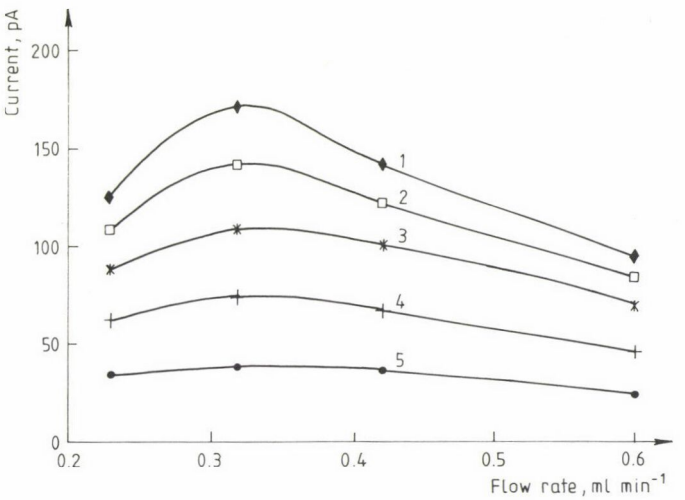

Fig. 5. Effect of flow rate on glucose determination. 1: 5.0 mmol l^{-1} glucose; 2: 4.0 mmol l^{-1} glucose; 3: 3.0 mmol l^{-1} glucose; 4: 2.0 mmol l^{-1} glucose; 5: 1.0 mmol l^{-1} glucose

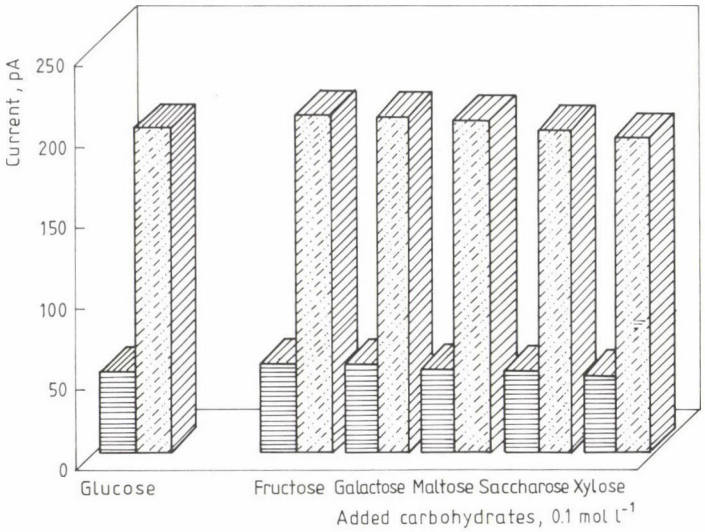

Fig. 6. Effect of other carbohydrates on glucose determination.
▤: 0.025 mol l^{-1} glucose; ▨: 0.1 mol l^{-1} glucose

tested. The substrates were added in 0.1 mol l^{-1} concentration to the glucose standards. The effect of different carbohydrates added to glucose stock solutions (0.025 mol l^{-1} an 0.1 mol l^{-1} glucose) is shown on Fig. 6. It can be seen, that their presence did not change the results more than by 5%.

2.4. Lifetime of the thin-layer cell

The system was used intensively for a longer period, to determine the lifetime of the thin layer cell and the number of samples to be measured. The samples were systematically interspersed with standards (0.05 mol l^{-1} glucose) so that the relevant standardization could take place. The amperometric signals of these samples were taken as a function of time and are illustrated in Fig. 7.

After preparation of the thin layer cell, it takes about an hour while the badly fixed glucose oxidase washed down and the activity of the cell became constant. In the next smooth part of the curve the samples are to be measured with proper accuracy, even though the activity of the cell is decreasing very slowly.

This symptom did not affect the results, because standards are measured time after time and the glucose concentrations of different samples are compared with. In Table 2 the amperometric signals of glucose standards are collected at 3 different periods. In each period 15–15 standards were measured and the standard deviation was calculated.

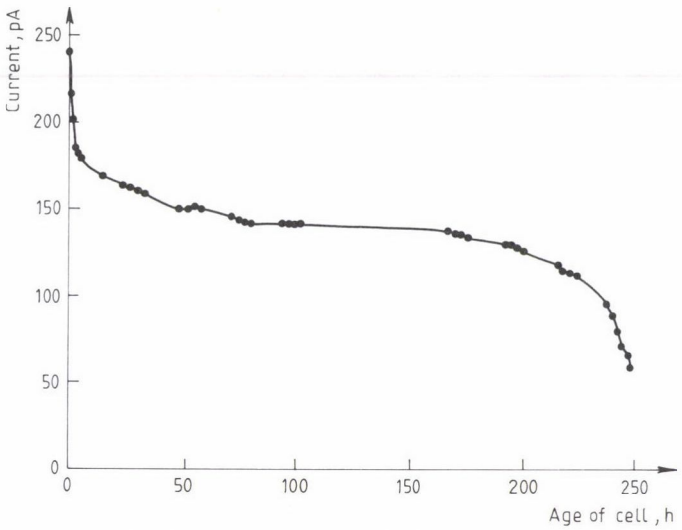

Fig. 7. Activity of enzyme cells. 0.05 mol l^{-1} glucose

In the last period the amperometric signals (after about 240 h) decreased quickly. At this time the rate of the reaction was determinated by enzyme reaction and not by diffusion as earlier. The enzyme cell ran down, the measurements could not be carried out.

2.5. Characterization of the immobilized enzyme

The immobilized enzyme layer was studied under the optimized conditions in the flow system. Using different length of thin-layer cells, the residence times were measured and found to be 1.8, 3.3, 5.1, 7.1, 10.6 and 12.4 s. After determining the quantity of hydrogen peroxide produced during these periods the reaction rate was calculated. As a comparison hydrogen peroxide (0.05 mmol l^{-1}) was also measured.

Table 2

Statistical parameters of glucose determination

Age of ceil (h)	40 – 60	80 – 100	180 – 200
Average current (pA)	149.7	142.2	128.8
Std dev. (pA)	±2.6	±1.72	±2.51

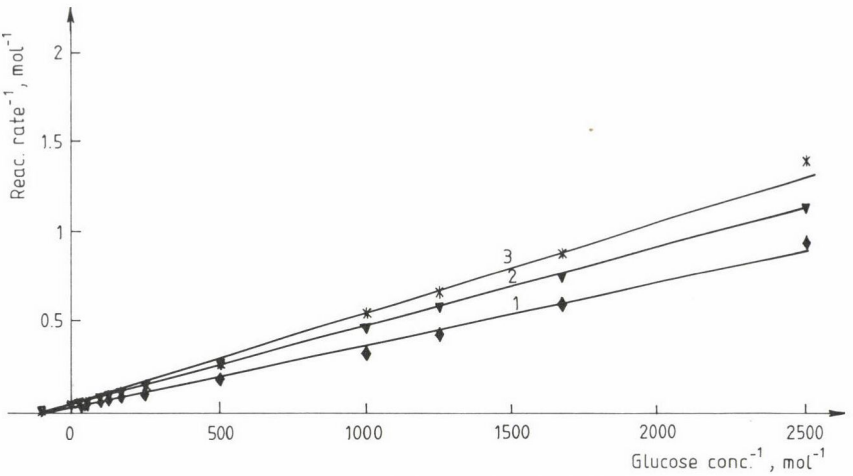

Fig. 8. Lineweaver – Burk plots. 1: 42 °C; 2: 37 °C; 3: 32 °C

Collecting the data by computer the peak area was calculated to eliminate the differences in the widths of the curves. The concentration range of the standards was used up to 0.05 mol l^{-1} to reach that range, where the reaction rates already do not increase at all (in this case dialyser was not used, so this concentration was higher as before). After calculating the reaction rate from the generated quantity of hydrogen peroxide and from the residence time, and plotting it against the original concentration of glucose, the Michaelis constant (K_M) and the $0.5 \times V_{max}$ were determined. Because the maximal reaction rate can not be exactly determined, the value of K_M is uncertain. Using the Lineweaver-Burk equation the graphic plot gives the estimation of the apparent Michaelis constant and that of the maximum rate (Fig. 8). The average of the apparent Michaelis constant in our case was 9.56 mmol l^{-1}. This result is very close to the data published in the literature by SCHELLER and SCHUBERT (1992), who applied different immobilization procedures.

2.6. Determination of glucose content in juice samples

Glucose concentrations of 28 juice samples were measured. From one sample always three parallels were taken and measured in triplicate by both the biosensor developed and the reference UV photometric method. The average concentration ($\bar{x}$) and the deviation (s) measured by both methods are summarized in Table 3.

Table 3

Glucose content of juices (in mol l^{-1})

Drink	Producer	Photometry		Biosensor	
		$\bar{x}$	s	$\bar{x}$	s
White Grape Drink	BB Ltd.	0.185	0.007	0.195	0.009
Blue Grape Drink	BB Ltd.	0.177	0.011	0.173	0.004
Apple Drink	BB Ltd.	0.203	0.015	0.212	0.005
Peach Nectar	BB Ltd.	0.119	0.008	0.118	0.004
Yess! Lemon	Globus Plc.	0.271	0.017	0.261	0.006
Yess! Apple	Globus Plc.	0.063	0.006	0.061	0.003
Yess! Orange	Globus Plc.	0.144	0.009	0.168	0.004
Yess! White Grape Drink	Globus Plc.	0.097	0.004	0.096	0.002
Ribena Black Currant Juice	Globus Plc.	0.363	0.022	0.349	0.004
Ribena Raspberry Drink	Globus Plc.	0.375	0.010	0.391	0.008
Ribena Orange-Apricot Drink	Globus Plc.	0.358	0.009	0.385	0.016
Hohes C (Orange)	Sió-Eckes Ltd.	0.114	0.002	0.118	0.004
Orange Nectar	Sió-Eckes Ltd.	0.079	0.006	0.080	0.004
Villiam Pear Nectar	Sió-Eckes Ltd.	0.139	0.008	0.146	0.004
Grape Drink	Sió-Eckes Ltd.	0.189	0.009	0.201	0.006
Apple Drink	Sió-Eckes Ltd.	0.144	0.010	0.146	0.008
Apricot Nectar	Sió-Eckes Ltd.	0.131	0.008	0.132	0.008
Apple Drink	DEKO	0.288	0.006	0.283	0.004
Grape Drink	DEKO	0.262	0.011	0.261	0.005
Sunclub, Apricot Nectar	Napsugár Ltd.	0.229	0.012	0.242	0.008
Soya & Sour Cherry Cocktail	Napsugár Ltd.	0.164	0.002	0.168	0.004
Apple Drink	Garden Ltd.	0.267	0.008	0.250	0.006
Grape Drink	Garden Ltd.	0.295	0.014	0.289	0.004
Top-Joy Apple Drink	Olympos Ltd.	0.195	0.003	0.201	0.004
Top-Joy Red Grape Drink	Olympos Ltd.	0.271	0.010	0.278	0.007
Top-Joy Light Grape Drink	Olympos Ltd.	0.228	0.008	0.234	0.004
Happy Day Orange Juice	Rauch GmbH	0.161	0.002	0.168	0.004
Orange Juice	Pfanner	0.137	0.002	0.138	0.008

The correlation between the methods demonstrated in Fig. 9 was very good, the correlation coefficient (r) was 0.993.

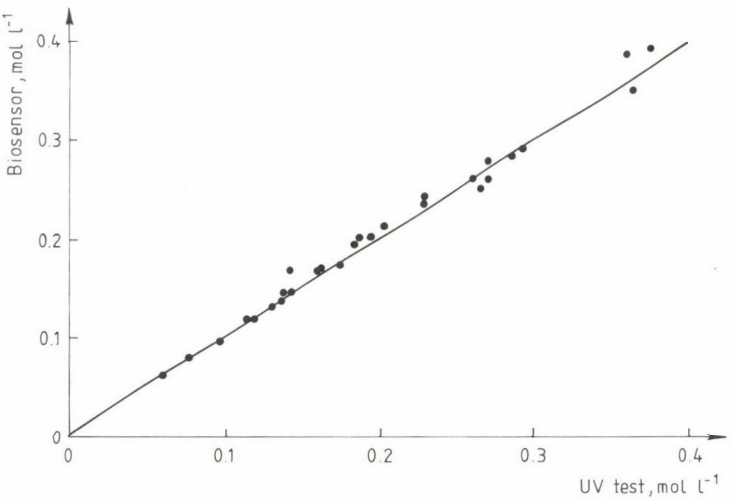

Fig. 9. Correlation between glucose measurement with reference method and with biosensor. $y = 0.003 + 1.001\ x$, $r = 0.993$

3. Conclusions

On the basis of the experiments the proper conditions for immobilization of GO enzyme was established. The enzyme cell constructed proved to be stable during measurements.

Defining the optimal parameters for enzyme and electrochemical reaction the determination of glucose content in fruit juices was possible by the help of this type of biosensor. The linear measuring range was between 0.005 and 0.5 mol l^{-1} glucose, when dialyser was used. The accuracy of the determination was 0.008 mol l^{-1} in case of juice samples. The lifetime of the enzyme cell was about 250 working hours.

Consequently it can be stated that the application of enzyme-based biosensors offers a new possibility for the quality control of fruit drink processing.

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References

BROOKS, S. L., ASHBY, R. E., TURNER, A. P. F., COLDER, M. R. & CLARKE, D. J. (1987/88): Development of an on-line glucose sensor for fermentation monitoring. *Biosensors*, *3*, 45–56.

DEMURA, M. & ASAKURA, T. (1989): Immobilization of glucose oxidase with Bombyx mori silk fibroin by only stretching treatment and its application to glucose sensor. *Biotechn. Bioeng.*, *33*, 598–603.

GUILBAULT, G. G. & LUBRANO, G. J. (1973): An enzyme electrode for the amperometric determination of glucose. *Analytical chim. Acta*, *64*, 439–455.

MALE, K. B. & LUONG, J. H. T. (1993): Improvement of the selectivity of an FIA amperometric biosensor system for glucose. *Biosensors & Bioelectronics*, *8*, 239–247.

SCHELLER, F. & SCHUBERT, F. (1992): *Biosensors*. Elsevier Science Publishing Co., p. 60.

TREVAN, M. D. (1980): *Immobilized enzymes*. Wiley and Sons Ltd., pp. 66–70.

VÁRADI, M. & ADÁNYI, N. (1994): Application of biosensor with amperometric detection for determining ethanol. *Analyst*, *119*, 1843–1847.

VÁRADI, M., ADÁNYI, N., NAGY, G. & REZESSY-SZABÓ, J. (1993): Studying the bienzyme reaction with amperometric detection for measuring maltose. *Biosensors & Bioelectronics*, *8*, 339–345.

WATANABE, E., OGURA, T., TOYAMA, K., KARUBE, I., MATSUOKA, H. & SUZUKI, S. (1984): Determination of adenosine 5'-monophosphate in fish and shellfish using an enzyme sensor. *Enzyme microb. Technol.*, *6*, 207–211.

WISEMAN, A. (1985): *Handbook of enzyme biotechnology*. 2nd ed., Ellis Horwood Limited, pp. 299–309.

YAO, T. (1983): A chemically modified enzyme membrane electrode as an amperometric glucose sensor. *Analytica chim. Acta*, *148*, 27–33.

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THERMAL ABUSE DURING SLOW COOLING OF PALLETIZED CANS

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The influence of cooling rate after heat treatment of canned peeled tomatoes on some food parameters was studied. A pallet of 3168 not completely cooled cans was built up. During next cooling stage, a detailed thermal map was calculated, using a theoretical model compared with measured temperature data. A distribution of C-values associated to each can was obtained and food analyses with respect to C-value and storage time followed. Positive correlation of C-value with detinning rate, hydroxymethylfurfural formation and pectin fractionation was found. No influence was demonstrated on iron level, product colour and headspace gas composition.

Keywords: canned tomatoes, palletizing, slow cooling, thermal degradation

The final quality of a food product, besides raw material, depends on the proper carrying out of all processing steps. In general, storing of a foodstuff at high temperature is responsible for some heat damage that irreversibly modifies taste and nutrition properties.

Optimization of heat treatment for sterilization aims to reduce heat damage without increasing the risk of microbial spoilage. Based on the general principle that brief high temperature treatments are preferred to prolonged exposure at lower temperatures, the heating and cooling times should be as short as possible. All the factors that enhance heat transfer shorten heating and cooling times and reduce heat damage to the product.

In particular, ineffective cooling may cause the cans to leave the sterilizer hotter than guidelines recommend (ANON, 1988; LOPEZ, 1987); if immediately palletized, residence time at high temperature becomes very long. Although this

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occurrence is not recognized as usual practice in factories; deviations from recommendations are not rare, because of poor heat exchange, poor avaibility of water and shortening of cooling time to increase plant throughput.

The main negative consequences of very slow cooling are the increased thermal breakdown and the foodstuff-container interaction (NAGY et al., 1980; ROUSEFF & TING, 1985), besides the development of conditions that are potentially favourable to thermophilic bacterial growth.

In this study we considered the effects that conditions of this type may have on detinning rate in fruit and tomato products packaged in plain cans. This is of particular importance since the concentration of tin is one of the main quality indicators of the product and the progressive dissolution of tin during storage limits the shelf life of the product (in some countries the highest concentration allowed is 150 ppm). Therefore the storage time can be considerably reduced by accelerating factors. Many chemical factors are known to accelerate corrosion: acidity, sulphites (SAGUY et al., 1973), nitrates (JOHNSON, 1970; BIELIG & TREPTOW, 1983), oxygen (LOPEZ, 1965). LUH and co-workers (1964) studied the influence of slow cooling on tomato paste corrosivity and compared the effect of two constant cooling rate; he found a faster detinning associated with the slow cooling.

In order to determine the cooling course time of palletized hot peeled tomato cans and to establish the influence of variable cooling rate on some analytical parameters of the product, the following experiments have been carried out:

- processing and packaging of peeled tomatoes an industrial production line;
- palletization of not completely cooled cans and next cooling stage in air;
- measurement and calculation of the thermal history of each container and the relative thermal damage;
- splitting the cans in groups according to the thermal damage;
- determination of analytical parameters of the canned food.

1. Materials and methods

1.1. Product preparation

The test were carried out on 500 g (ø72.8 × 109 mm) cans of peeled tomatoes produced on an industrial line. Table 1 reports characteristics of product and container. Compared to the normal production cycle, cooling after heating treatment was carried out in two separate phases. The cans were immersed in cold water for only five minutes and immediately palletized. After that, cooling of the whole pallet was completed at room temperature without any thermal control.

Table 1

Characteristics of canned peeled tomatoes

Analytical parameters		Variation range
pH		4.35 – 4.45
Soluble solids (°Brix)		5.8 – 6.2
Net weight (g)		416 – 422
Internal vacuum (kPa)		10.1 – 16.0
Citric acid (g kg^{-1})		2.8 – 2.9
Can body tin weight (g m^{-2}) D11·2/2.8[a]	External	2.8 – 3.1
	Internal	10.4 – 11.3

[a] EURONORM 145 – 78

During the palletization, thermocouples (connected to recorder CMC 821 – Ellab) were inserted into 11 cans, placed in different parts of the pallet (Fig. 1). The temperature at the center of each can was continuously recorded until the pallet center reached the room temperature. The palletization temperature (i.e., the initial temperature of the cans during pallet formation) was 72 °C.

1.2. Calculation of the thermal distribution

Starting with the thermal data furnished by the thermocouples, the temperature distribution of the whole pallet was reconstructed to get more detailed

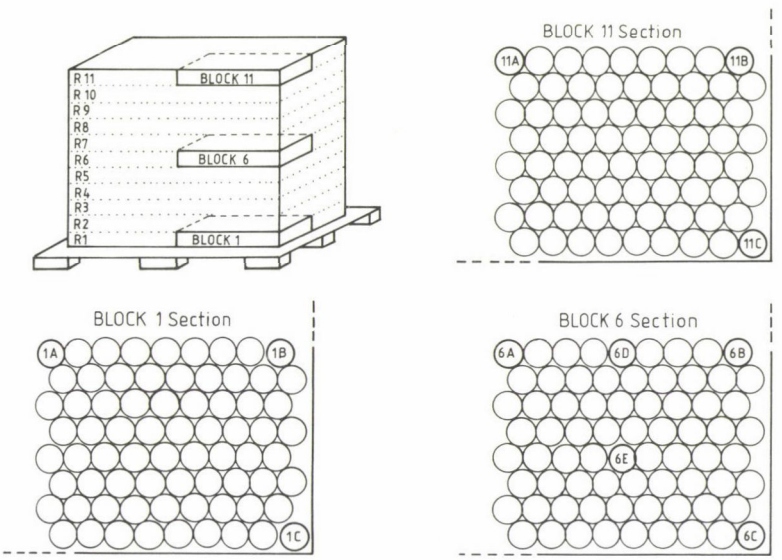

Fig. 1. Pallet and position of cans with thermocouples

information on the thermal history of the single cans. To do so a physical model based on some approximations was utilized: it was assumed that the pallet was a homogeneous body with a simple geometric shape (the irregular boundaries were approximated to planes) having constant thermophysical properties and that the internal heat transfer occurred for conduction only. In fact, convection heat and mass transfer might occur only in small regions (liquid phase convection inside the cans and air convection in the interstices between cans), where the temperature gradients are, on average, low and generate weak buoyancy forces. In a system where the conduction heat transfer is time dependent, the evolution of thermal distribution is regulated by the Fourier equation:

$$\frac{\partial^2 T^*}{\partial x^{*2}} + \frac{\partial^2 T^*}{\partial y^{*2}} + \frac{\partial^2 T^*}{\partial z^{*2}} = \frac{1}{\alpha} \cdot \frac{\partial T^*}{\partial t} \qquad (1)$$

where the temperature and space coordinates are dimensionless and physical parameters (ρ, C_p, k) are assumed constant with respect to position and temperature.

On the boundary surfaces there are natural convection conditions represented by:

on the bottom horizontal plane $z^* = 0$: $\frac{\partial T^*}{\partial z^*} = Bi_0 \cdot T^*$

on the top horizontal plane $z^* = 1$: $\frac{\partial T^*}{\partial z^*} = -Bi_1 \cdot T^*$

on the 2 vertical planes $x^* = 0$ and $y^* = 0$: $\frac{\partial T^*}{\partial x^*} = \frac{\partial T^*}{\partial y^*} = Bi_2 \cdot T^*$

on the 2 vertical planes $x^* = 1$ and $y^* = 1$: $\frac{\partial T^*}{\partial x^*} = \frac{\partial T^*}{\partial y^*} = -Bi_2 \cdot T^*$

which are valid at any time. The dimensionless parameter Bi (Biot number = $h \cdot L/k$) depends on the superficial convection coefficient which takes the same value on the four vertical surfaces (h_2) and different values on the two horizontal surfaces (h_0 and h_1). The h_0 coefficient (related to the bottom horizontal plane) incorporates the effect of the wooden base which further slows heat exchange. The Biot parameters also include the size of pallet L, which is given by three components L_x = 1.35 m, L_y = 1.05 m and L_z = 1.25 m; moreover, k is the mean thermal conductivity of the bulk of the pallet and it is assumed identical on the 3 directions, i.e. $k_x = k_y = k_z$.

The exact solution of this system comes from separation of the variables after decomposition of eq (1) with respect to x*, y*, and z* and was derived from CARSLAW and JAEGER (1959).

A feature of this type of solution is that the irregularity of thermal gradients corresponding to the structural discontinuities of the pallet, are mediated by a regular trend without flexi as a consequence of the assumption of thermophysical uniformity.

The values of the physical constants in the T(x, y, z, t) function (i.e., the convection coefficients and the thermal diffusivity) were determined from the measured temperature data, from the literature (CHOI & OKOS, 1986), and from empirical correlations of Nusselt and Rayleigh numbers established for heat exchange between a fluid and flat surface (INCROPERA & DEWITT, 1990).

1.3. Calculation of thermal damage

The thermal damage measures the degradation of a food product due to maintenance at high temperature. This is quantified by the disappearance of nutrients and/or flavour which are most sensitive to heat.

In general, the most significant thermal degradation reactions are assumed to follow a first order kinetics (THIJSSEN & KOCHEN, 1980) so that the concentration reduction of a component may be determined by the equation:

$$\log \frac{c}{c_0} = -\frac{k_r}{2.3} \cdot \int_{t_0}^{t} 10^{\frac{T-T_r}{z}} dt \qquad (2)$$

where k_r is the kinetic constant of the reaction at the reference temperature T_r. Through this expression an evaluation of the thermolabile residual concentration is possible, knowing the thermal history an kinetic parameters. In agreement with MANSFIELD (1962), many investigators define the integral of the earlier equation as C value or cook value (T_r = 100 °C):

$$\text{C-value} = \int_{t_0}^{t} 10^{\frac{T-T_r}{z}} dt \qquad (3)$$

In this study the C-value has been used as a measure of the product thermal change during cooling: T(t) is the mean temperature of the can (approximated with its center temperature) during cooling and $(t_f - t_0)$ is the cooling time. A z value set to 31 °C was used according to what MNKENI an BEVERIDGE (1982) measured in temperature range 100 – 140 °C. Data of z at lower temperatures were unavailable.

So the thermal abuse is expressed as equivalent hours of storage at 100 °C and refers only to the cooling phase since the contribution of heating phase is the same for all cans. A negligible thermal damage (C = O h) was given to cans quickly cooled in running water.

1.4. Analytical methods

The soluble solids, expressed as Brix degrees, were determined by measurement of refractive index at 20 °C.

The pH was determined by pH meter, Crison model micro TT2050.

The pectic substances were determined according to the procedure of DIETZ and ROUSE (1953); the pectic substances were differentiated as pectin (water soluble, WS), calcium pectate (sodium exametaphosphate soluble pectin, ES), and insoluble pectin (NaOH soluble, NS). All the pectin substances were precipitated by ethanol and the separated precipitate was dissolved in water, in sodium exametaphosphate and in alkali solution. The pectins were hydrolyzed by the alkali to galacturonic acid and its concentration was determined in each solution by the reaction with carbazide in the presence of sulfuric acid. The red colour produced was measured at 525 nm by spectrophotometer. The hydroxymethylfurfural (HMF) content was determined according to International Federation of Fruit Juice Producers (IFFJP, 1985). Luminance (L* value), redness (a* value) and yellowness (b* value) were measured using a tristimulus Hunter colorimeter model D25A. Standard plate No. C20-2105 with Hunter L* value of 25.8, a* value of 28.6 and b* value of 12.9 was used as a reference. The Hunter colour values were measured after homogenization of sample. The vacuum was measured by Budenberg gauge (Cheshire U.K.). The content of Sn and Fe in product was determined by atomic absorption after sample mineralization performed with sulfo-nitric mixture according to the MUACV (1961).

2. Results and discussion

2.1. Temperature of the pallet and thermal damage during cooling

The analytical solution $T = f(x, y, z, t)$ was used to calculate the thermal history of the packaged cans after determining the thermal diffusivity α and the convection coefficients h_0, h_1 and h_2.

The thermophysical constants have been found minimizing the sum of squares (SS) of deviations of calculated temperatures (T^c) from the measured temperatures (T^m) in 11 cans at 2 different times (t1 = 2 and t2 = 6 days of cooling) and the following expression was used to compute the sum of squares:

$$SS = \sum_{i=1}^{i=11} \left(T^{m}_{i,t1} - T^{c}(x_i, y_i, z_i, t_1) \right)^2 + \sum_{i=1}^{i=11} \left(T^{m}_{i,t2} - T^{c}(x_i, y_i, z_i, t_2) \right)^2 \quad (4)$$

where x_i, y_i and z_i are the positional coordinates of the i-th can and t1 = 2 days and t2 = 6 days. So the minimum value has been found (SS = 23.1) after varying the coefficients α, Bi_0, Bi_1, Bi_2. Table 2 shows the values of the coefficients which minimizes SS. This means that, by taking on such coefficients, the T = f(x, y, z, t) function reproduces the can temperature with a mean deviation of 1 °C. The same table also reports the theoretical values of the coefficients found either directly from the literature or through the empirical correlations. The deviation from theoretical values is probably due to the fact that the real boundary area is greater than the assumed one and that the air between cans reduces the overall conduction inside the pallet.

The thermal curves for some cans of the pallet are reported in Fig. 2. The inner cans cool very slowly and reach external temperature after the first 11 days: at the beginning of the cooling the heat exchange is almost zero and the temperature remains constant for about two days. On the boundary instead, the external temperature is reached in a shorter time: in the slowest case (can 11A) the cooling occurs in half the time (5–6 days). However, the cooling rate remains low if it is compared to the isolated cans left outside the pallet (marked EXT in Fig. 2). At the bottom, the cans (see can 1A in Fig. 2) are in contact with the wood base, so the slow heat transfer puts the cooling rate between the fastest and the slowest ones.

Table 2

Thermophysical parameters of pallet

Parameters	Empirical	Theoretical data from literature
Pallet overall thermal diffusivity α	1.37×10^{-7} m^2 s^{-1}	[a] 1.53×10^{-7} m^2 s^{-1}
Biot numbers		
$Bi_0 = (h_0 L_z/k)$	7	[b] 2.7
$Bi_1 = (h_1 L_z/k)$	18	[b] 12
$Bi_2 = (h_2 L_x/k \approx h_2 L_y/k)$	18	[b] 9.3

[a] approximated by tomato juice thermal diffusivity (CHOI & OKOS, 1986)

[b] calculated through Nusselt number (Nu), obtained from relationships between dimensionless numbers

(natural convention of fluids on plane surfaces) (INCROPERA & DEWITT, 1990)

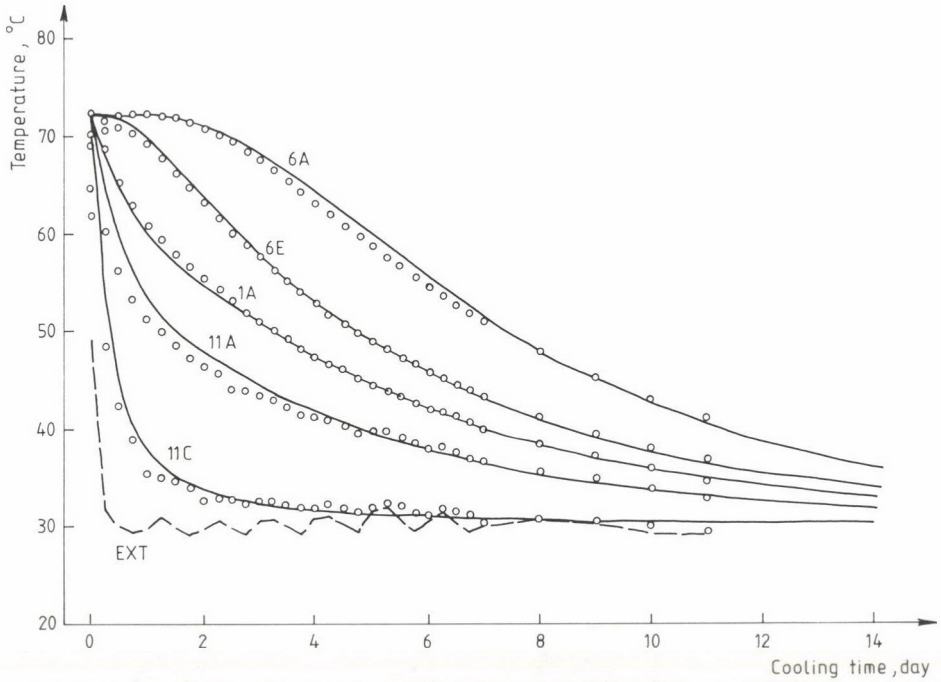

Fig. 2. Measured (○) and calculated (-) temperatures of 5 cans during pallet cooling. Curves are related to the positions in pallet by the same marks of Fig. 1. The measured temperature of a can cooled outside pallet showed by the curve marked EXT

Based on the thermal history of each can, the associated heat damage was calculated. This takes on values between a minimum C-value of 2–3 h for the cans at the side to a maximum C-value of 15 h for the center cans. Distribution of the heat damage (Fig. 3) shows that about 19% of the cans undergo a high heat damage (C > 10 h) and these make up the hot core of the pallet, while the great majority of the cans features low heat damage values.

2.2. Effect of cooling rate on the product

2.2.1. Levels of tin and iron. Figure 4 reports trends of tin levels in the product in relationship to heat damage at various storage times.

It was found that the cooling rate had a major effect on tin concentrations, since at each storage time, tin levels were higher in the cans with the higher heat damage.

The relationship is always linear with correlation coefficients greater than 0.80 in most cases.

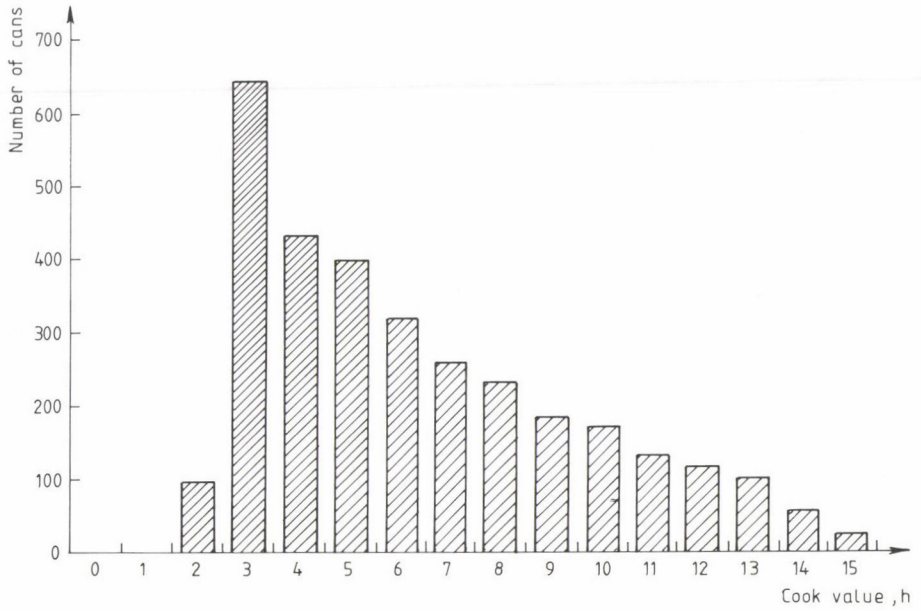

Fig. 3. Frequency distribution of palletizated cans with respect to the thermal damage

At the beginning of cooling (t = 0) the tin dissolved in a correctly cooled can (C = 0) is about 40 ppm, while the cans from the center core of the pallet (C > 11 h) contain tin levels that exceed 100 ppm.

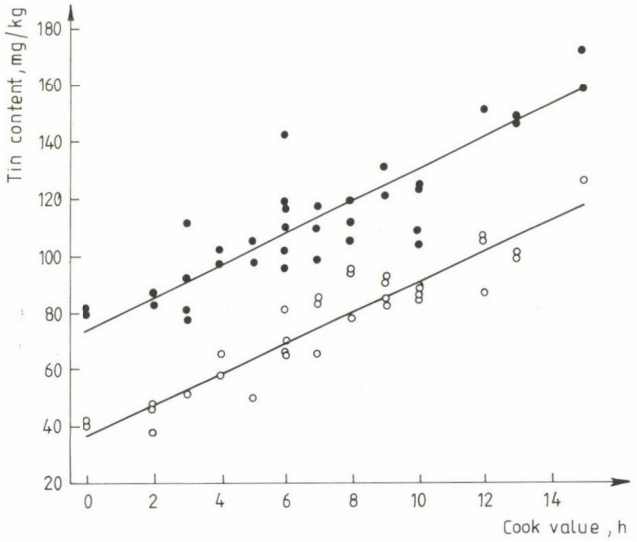

Fig. 4. Detinning vs. C-value at 2 storage times. ○: cans at the beginning of storage. Statistical regression: $y = 36.8 + 5.4x$, $R^2 = 0.88$; ●: cans after 1-year storage. Statistical regression: $y = 74.3 + 5.6x$, $R^2 = 0.73$

Adding the storage contribution to the heat damage, the cans from the center of the pallet have tin levels over 150 ppm at the end of a year.

Storage affects tin levels by shifting them higher, regardless to the thermal influence (regression lines with very similar slopes): the difference between cans measured at the beginning of storage remains constant in time. Theoretically, a can with greater tin loss should continue to lose tin faster than a can with less tin going into solution because of the greater discontinuity of the tin layer. The scarce incidence of this effect is probably due to the fact that the residual tin layer is anyway still thick, even in the more detinned cans (where the residual coating weight does not fall below 8.5 g m^{-2}). The increase in tin levels in relation to time (Fig. 5), heat damage being constant, is characterized by correlation coefficients that are rather low in most cases. The wide range of data could derive from the environment thermal fluctuations, because storage occurred without temperature control.

In regards to iron corrosion, the protective action that tin layer plays on steel does not seem to be influenced by heat damage, since the iron level was always below 10 ppm.

2.2.2. Pectin fractions and HMF. Figure 6 reports the influence of thermal damage on pectin substances in the canned peeled tomato studied after extraction of

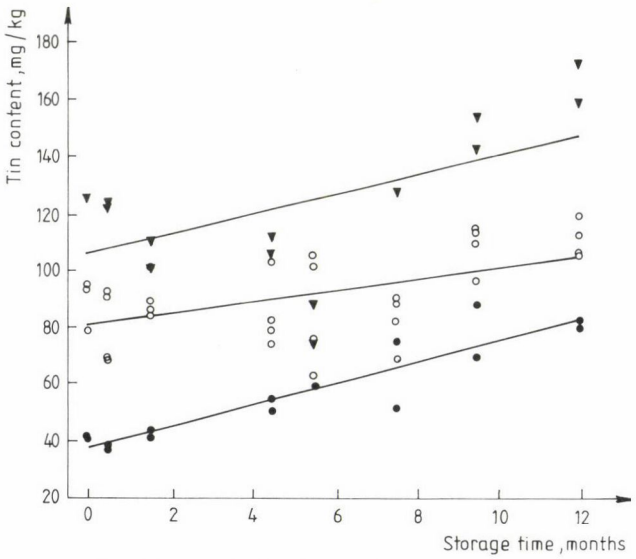

Fig. 5. Detinning vs. storage time in cans with different thermal damage. ●: cans immediately cooled (C = O h). Statistical regression: $y = 37.8 + 3.7x$, $R^2 = 0.87$; ∇: cans from center of pallet (C = 15 h). Statistical regression: $y = 106.0 + 3.4x$, $R^2 = 0.32$; ○: cans from intermediate positions (C = 8 h). Statistical regression: $y = 80.8 + 2.0x$, $R^2 = 0.88$

the alcohol insoluble residue by water, followed by sodium exametaphosphate and finally diluted by NaOH (0.1 mol l^{-1}) extractions. The functional extractions of pectic substances give three different fractions (Fig. 6): the water-soluble fraction (WA), the fraction extracted by sodium exametaphosphate (ES) and the NaOH soluble fraction (NS).

As seen in Fig. 6, the water soluble fraction (WA) considerably increases with cooking. An analogous finding, but less pronounced, was seen for the sodium exametaphosphate fraction soluble. Conversely, the NaOH soluble fraction shows a considerable diminution.

In terms of percentage we observed that the hydrosoluble fraction increases by about 160%, while the sodium exametaphosphate soluble fraction increases by about 19% and the NaOH soluble fraction decreases of about 50% and, on the whole, the total pectic fractions (Fig. 6) increase by about 47%. These variations may be taken to mean that there is a partial solubilization of the insoluble protopectic fractions with continued thermal treatment. In fact, added to the decreased content in insoluble pectic fractions, there is an increase in hydrosoluble pectic fractions (Fig. 6). Nevertheless, the quantity of the more soluble pectic fractions (WA and ES) was found greater than the contribution due to the partial solubilization of the more

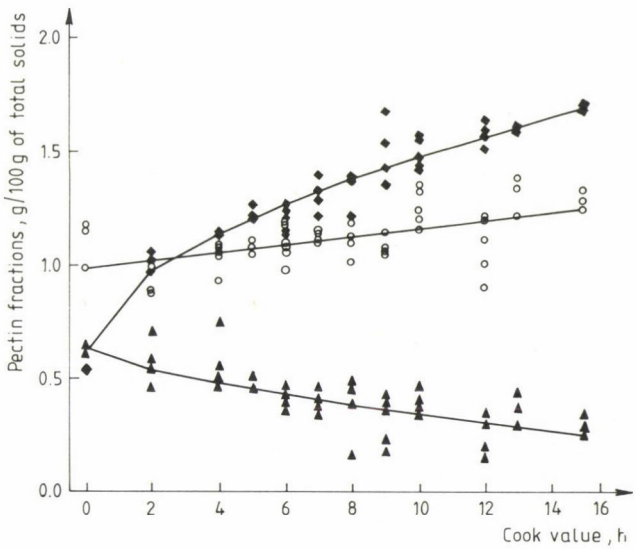

Fig. 6. Fractionation of pectin vs. C–value. ◆: water soluble pectin. Statistical regression: $y = 0.58 + 0.28x^{0.5}$, $R^2 = 0.92$; ○: exametaphosphate soluble pectin. Statistical regression: $y = 0.98 + 0.02x$, $R^2 = 0.29$; Δ: NaOH soluble pectin. Statistical regression: $y = 0.67 - 0.11x^{0.5}$, $R^2 = 0.44$

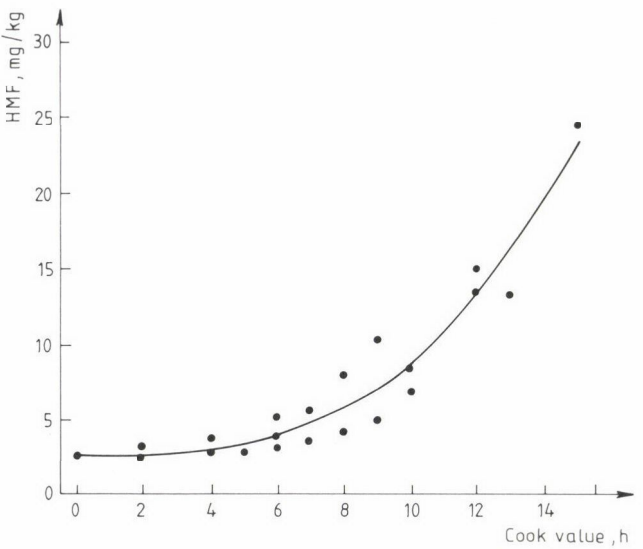

Fig. 7. Hydroxymethylfurfural vs. C-value. ●: Hydroxymethylfurfural. Statistical regression: $y = 2.64 + 0.006x^3$, $R^2 = 0.93$

insoluble fraction. Therefore, it can be hypothesized that with increased cooking there is a progressive release of pectic fractions trapped in the polysaccharide matrix of the cell wall. The influence from increased cooking on pectic fractions may therefore be seen as a mechanism by which there is at first a partial solubilization of the more insoluble protopectin pectic fractions bound to the cell wall, followed by a release of the more insoluble pectic fractions that were trapped and/or covered by the protopectins. These findings are in agreement with KEIJBETS and PILNIK (1974) who studied potato sloughing during heat treatment. Keijbets and Pilnik reported that with increasing temperature there is not only a notable solubilization of pectins but that this process was accompanied by an increase of unsaturated galacturonic acid oligomers and this indicates β-elimination as a mechanism for cell wall disintegration. Finally, Fig. 7 depicts the level of HMF associated with the intensity of heat treatment of the product. The data confirm what has already been qualitatively reported by numerous authors (TRIFIRO', 1961; PARKINSON & BARKER, 1957) where the higher the storage temperature the sooner and greater darkening occurs. It is known that nonenzymatic darkening, commonly called Maillard reaction, occurs when proteins or amino acids react with reducing sugars. The first stage of this reaction is the formation of the well-known Amadori compound (1-amino-1-deoxy-2-*-D-fructopyranose) which is then transformed into 5-dihydroxymethyl-2-

furaldehyde, better known as HMF (HODGE, 1967). Besides the heat treatment of the product, the formation of HMF depends on the composition of the product itself and in particular on pH, sugar, amino acids and total solid contents. HMF levels are not enough to shed light on the product's heat damage. To do so the kinetics of HMF formation based on those earlier parameters would have to be known. So far there are no kinetic data available in the literature regarding HMF. The findings in Fig. 7 show that the dependence of HMF on cooking is well enough described by the simple exponential law. The increasing of HMF concentration with thermal damage may be helpful in finding the detinning causes when high tin concentrations are associated with high HMF concentration.

2.2.2. Other parameters. In regards to heat damage and storage time, other analytic parameters of the food product have been studied: pH, food colour (L, a, b, a/b), soluble solids, the internal vacuum and the headspace gas composition. None of these had significant variations ($P > 0.05$) with respect to the two variation sources.

3. Conclusion

Thermal analysis of pallet during cooling time provided data which are quite close to experimental data: either the temperature data at any time and the thermophysical parameters (convective coefficients and thermal diffusivity) are similar to the theoretical ones. So the initial assumption of purely conductive heat transfer defines a simple and sufficiently accurate model that permits to calculate the cooling times of palletized cans also when the initial temperature is less than 70 °C. In that case calculations should be found accurate at greater extension, because the convective contribution to the inner heat transfer is less important.

Many days are required by cans to be cooled if they are palletized when they are still hot. This results in a significantly higher detinning rate which is able to reduce the shelf life of foods in plain cans.

Application of the mathematical model of cooling pallet and the extrapolation of analytical data show that the shelf life reduction would increase with the high temperature of palletization.

Furthermore, it should be observed that, in the food factories, cooling can be much slower (compared to experimental conditions), because the pallet is wrapped in a thick plastic film during storage. The heat dispersion is thus further blocked, lengthening the cooling time and increasing the proportion of heat damaged cans.

Symbols

T^*: dimensionless temperature $(T - T_a)/(T_p - T_a)$
T_a: air temperature (~30 °C)
T_p: palletization temperature (72 °C)
T: mean temperature of can (Eqns 2, 3)
T_r: reference temperature
T^c: calculated temperature
T^m: measured temperature
x^*: dimensionless coordinate x/L_x
y^*: dimensionless coordinate y/L_y
z^*: dimensionless coordinate z/L_z
L: size in Biot number
t: time (h)
α: thermal diffusivity ($m^2\ s^{-1}$)
k: thermal conductivity (W/m·°C)
ρ: density ($kg\ m^{-3}$)
c_p: specific heat inside can at constant pressure (J/Kg·°C)
h: convection coefficient in air (W/m^2·°C)
Bi: Biot number
k_r: kinetic constant of thermolabile degradation at $T = T_r$
c: thermolabile concentration at time t
c_0: thermolabile concentration at time $t = 0$
C: cook value (h)
z: temperature coefficient of degradation (°C)

References

ANON (1988): *Canned foods*. (5th ed.) Food Processors Institute Washington, D.C., pp. 27–32.

BIELIG, H. J. & TREPTOW, H. (1983): Heavy metal content of fruit juices and the influence of the packaging material. – in: *Proc. 8th. Int. Congress on Canned Foods – Thessoloniki*. pp. 1–6.

CARSLAW, H. S. & JAEGER, J. C. (1959): *Conduction of heat in solids*. (2nd ed.) Oxford Univ. Press, pp. 353–386.

CHOI, Y. & OKOS, M. R. (1986): Thermal properties of liquid foods. – in: OKOS, M. R. (Ed.), *Physical and chemical properties of food*. American Society of Agricultural Engineers, St. Joseph, Michigan, 35–77.

DIETZ, J. H. & ROUSE, A. H. (1953): A rapid method for estimating pectic substances in citrus juices. *Fd Res., 18*, 169–173.

HODGE, J. E. (1967): Origin of flavor in foods-non enzymatic browning reactions. – in: SCHULTZ, H. W., DAY, E. A. & LIBBEY, L. M. (Eds), *The chemistry and physiology of flavors*. AVI, Westport, pp. 74–97.

IFFJP (1985): Methods Analysen-Analyses. International Federation of Fruit Juice Producers, Fruit Union, Zurig, Suisse, pp. 1–4.

INCROPERA, F. P. & DEWITT, D. P. (1990): *Fundamentals of heat and mass transfer*. (3rd ed.), J. Wiley & Sons, pp. 541 – 555.

JOHNSON, H. T. (1970): The corrosive action of nitrates in canned soft drinks measured by polarization techniques. *Am. Soft. Drink J.*, *125*, 83 – 84.

KEIJBETS, M. J. H. & PILNIK, W. (1974): Some problems in the analysis of pectin in potato tuber tissue. *Potato Res.*, *17*, 169 – 177.

LOPEZ, A. (1965): Processing factors affecting can corrosion in canned apple sauce. *Fd Technol.*, *19*, 221 – 226.

LOPEZ, A. (1987): *A complete course in canning*. (12th ed.) The Canning Trade Inc. Baltimore, Maryland, pp. 127 – 142.

LUH, B. S., CHICHESTER, C. O., HENRY, CO. & LEONARD, S. J. (1964): Factors influencing storage stability of canned tomato paste. *Fd Technol.*, *18*, 159 – 162.

MANSFIELD, T. (1962): High-temperature-short-time sterilization. – in: *Proc. of 1st Int. Congress on Food Science & Technology*, London, Vol. 4, pp. 311 – 316.

MNKENI, A. P. & BEVERIDGE, T. (1982): Thermal destruction of pteroylglutamic acid in buffer and model systems. *J. Fd Sci.*, *47*, 2038 – 2044.

MUACV (1961): Metodi ufficiali di analisi delle conserve vegetali. Ministero dell'agricoltura e delle foreste. Instituto poligrafico dello stato. Roma, pp. 3 – 127.

NAGY, S., ROUSEFF, R. & TING, S. V. (1980): Effects of temperature and storage on the iron and tin content of commercially canned single-strength orange juice. *J. agric. Fd Chem.*, *28*, 1166 – 1169.

PARKINSON, T. L. & BARKER, C. J. (1957): Isolation and identification of hydroxymethylfurfural from stored canned plum syrup. *J. Sci. Fd Agric.*, *8*, 639 – 645.

ROUSEFF, R. & TING, S. V. (1985): Effects of pH, storage time and temperature on the tin content of single strength canned grapefruit juice. *J. Fd Sci.*, *50*, 333 – 339.

SAGUY, I., MANNHEIM, C. H. & PASSY, N. (1973): The role of sulphur dioxide and nitrate on detinning of canned grape fruit juice. *J. Fd Technol.*, *8*, 147 – 149.

THIJSSEN, H. A. C. & KOCHEN, L. H. P. J. M. (1980): Calculation of optimum sterilization conditions for packed conduction-type foods. *J. Fd Sci.*, *45*, 1267 – 1272.

TRIFIRO', E. (1961): Sulla formazione e sul significato dell'ossimetilfurfurolo nei concentrati di pomodoro. *Ind. Conserve*, *36*, 314 – 318.

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SHORT COMMUNICATION

INVESTIGATION ON THE PRESENCE OF TOXIGENIC FUNGI AND AFLATOXINS IN RAW MILK

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All of the raw milk samples, obtained from the collecting stations, were contaminated with fungi. Their total viable counts per cm^3 varied from 5 to 4×10^4. The number of colony forming units was between 1000 and 5000 per cm^3 in 28% of the samples.

The isolated fungal strains were classified into 19 genera (*Absidia*, *Acremonium*, *Aspergillus*, *Cladosporium*, *Eurotium*, *Fusarium*, *Geotrichum*, *Gliocladium*, *Monilia*, *Mucor*, *Penicillium*, *Phoma*, *Scopulariopsis*, *Scytalidium*, *Stemphilium*, *Trichoderma*, *Ulocladium* and *Verticillium*) and 39 species. Genera *Aspergillus* and *Penicillium* were presented with the largest number (6) of different species. These moulds were isolated from 39 and 28% of raw milk samples, respectively.

High incidence of toxigenic fungi was observed. They were found to be contaminants of 91% of milk samples. Toxigenic species belonged to the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Eurotium*, *Fusarium*, *Geotrichum*, *Penicillium* and *Trichoderma*. The most common fungal species were *Geotrichum candidum* and *Penicillium aurantiogriseum*, isolated from 62 and 23% of milk samples, respectively.

Aflatoxin AB1 and AM1 were not found in milk.

Keywords: raw milk, toxigenic fungi, aflatoxins

The composition of milk makes it an optimum medium for the growth of many fungi. Contamination level of raw milk with fungi and other microorganisms depends on various factors, such as health and hygienic conditions of dairy cattle, hygienic quality of milk-handling equipment, fungal distribution in air and climate conditions.

ŠKRINJAR and co-workers (1986) reported that total viable counts of moulds in raw milk obtained from collecting stations varied from 1×10^3 per cm^3 in winter, to 1×10^6 in fall.

Many fungal species isolated from raw milk are toxigenic. This fact particulary refers to *Aspergillus* and *Penicillium* species, which are wide-spread fungi in the

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environment. The number of fungi in milk is reduced by pasteurisation, but some of them survive this process. For that reason they can contaminate milk products and produce mycotoxins under certain conditions (direct contamination).

Milk and milk products may also be contaminated with mycotoxins, including aflatoxins, by indirect contamination. Aflatoxin B1 (AB1) and M1 (AM1) may occur in milk after the consumption of AM1-contaminated feeds by the lactating animals. AB1 is metabolized by animals to AM1. The excreted amounts of AM1 as a percentage of AB1 average 1 to 2% (VAN EGMOND, 1991).

CARBONEL and SUNDREAU (1981) found that 79% of milk samples investigated in January-March 1981 in France, contained AM1 (0.05 – 0.5 $\mu g\ kg^{-1}$). SREBRNIK (1982) reported that about 10% of milk samples tested in winter 1982 – 83 in Belgium was contaminated with AM1 at concentrations from 0.02 to 0.08 $\mu g\ kg^{-1}$. One year later, AM1 was found in 52% of commercial blended milk (0.02 – 0.5 $\mu g\ kg^{-1}$) (SREBRNIK, 1986). Investigating the contamination of milk (13 samples) with AM1, BORGSTRÖM (1983) established the presence of AM1 in all samples examined. In Sweden, 90% of milk samples was contaminated with AM1 in the period of January – March and 44% of them in April – June 1986, as MÖLLER (1987) reported. Four raw milk samples, collected at farm gate in winter 1985 in Vojvodina, were contaminated with AB1, but at very low concentrations (traces) (ŠKRINJAR et al., 1986).

Having in mind the high frequency of fungal species in environment, the purpose of this study was to investigate the presence of fungi, with special attention to toxigenic species, and aflatoxins in raw milk.

1. Materials and methods

The presence of fungi, with special attention to toxigenic species, and that of aflatoxin B1 (AB1) and M1 (AM1) was investigated in 47 raw milk samples. The milk samples were obtained from the collecting stations in Vojvodina, in the period of October – February 1992 and December – May 1993.

1.1. Mycological analyses

The dilution plate technique was used for the isolation of fungi and for determination of their total viable counts per cm^3. Sabouraud dextrose agar (SDA) with streptomycin (0.01 – 0.02%) was used as isolation medium. Incubation was done at 25 °C for 7 days. After the incubation, colonies with different morphological characteristics were inoculated on appropriate media.

Identification of species was performed according to RAPER and THOM (1949), RAPER an FENNELL (1965), ELLIS (1971), PIDOPLIČKO and MILKO (1971), SAMSON

and co-workers (1976), NELSON and co-workers (1983), PITT and HOCKING (1985), FASSATIOVA (1986) and SAMSON and VAN REENEN-HOEKSTRA (1988).

1.2. Aflatoxin analysis

Qualitative and quantitative determination of AB1 and AM1 was carried out using a TLC method according to A.O.A.C. (1990).

2. Results and discussion

2.1. Mycological analyses

All the raw milk samples were contaminated with fungi at various degree. The total viable counts per cm^3 varied from 5 to 4×10^4. About 17% of milk samples were contaminated with a low number of fungi (< 100 per cm^3). Further, 13% of samples contained between 100 and 500 fungi per cm^3, 23% from 500 to 1000, 28% from 1000 to 5000, 4% between 5000 and 10 000 and about 15% of them more than 10000 fungi per cm^3.

Isolated fungal strains were classified into 19 genera and 39 species as follows: *Absidia corymbifera* (Cohn) Sacc. & Trotter, *Acremonium charticola* (Lindau) W. Gams, *A. strictum* W. Gams, *Alternaria alternata* (Fr.) Keissler, *Aspergillus caespitosus* Raper and Thom, *A. flavus* Link, *A. fumigatus* Fres. *A. niger* van Tieghem, *A. ochraceus* Wilhelm, *A. versicolor* (Vuill.) Tiraboschi, *Cladosporium cladosporioides* (Fres.) de Vries, *C. herbarum* (Pers.) Link, *C. macrocarpum* Preuss, *Eurotium herbariorum* (Wiggers) Link, *Fusarium graminearum* Schwabe, *F. moniliforme* Sheldon, *F. oxysporum* Schlecht. emend. Snyd. & Hans., *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans., *F. sporotrichoides* Sherb., *Geotrichum candidum* Link, *Geotrichum* sp., *Gliocladium roseum* (Link) Bainier, *Monilia* sp., *Mucor ciricinelloides* v. Tiegh., *M. dimorphosporus* Lendn., *M. hiemalis* Wehmer, *Penicillium aurantiogriseum* Dierckx, *P. claviforme* Bain., *P. hordei* Stolk, *P. nigricans* (Bainier) Thom, *P. raistrickii* Smith, *P. roqueforti* Thom, *Phoma pomorum* Thüm, *Scopulariopsis brevicaulis* (Sacc.) Bainier, *Scytalidium lignicola* Pesante, *Stemphilium* state of *Pleospora herbarum* (Pers. ex Fr.) Rabenh., *Trichoderma harzianum* Rifai, *T. viride* Pers., *Ulocladium botrytis* Preuss and *Verticillium tenerum* Nees.

Genera *Aspergillus* and *Penicillium* were presented with the largest number of different species (6), genus *Fusarium* with 5, and 11 genera (*Absidia*, *Alternaria*, *Eurotium*, *Gliocladium*, *Monilia*, *Phoma*, *Scopulariopsis*, *Scytalidium*, *Stemphilium*, *Ulocladium* and *Verticillium*) only with one species.

Aspergillus and *Penicillium* spp. were found to be the most frequent in milk mycopopulations. They were isolated from 39% and 28% of milk samples tested, respectively (Fig. 1). About 19% of milk samples were contaminated with *Fusarium* species and 11% with *Mucor* spp.

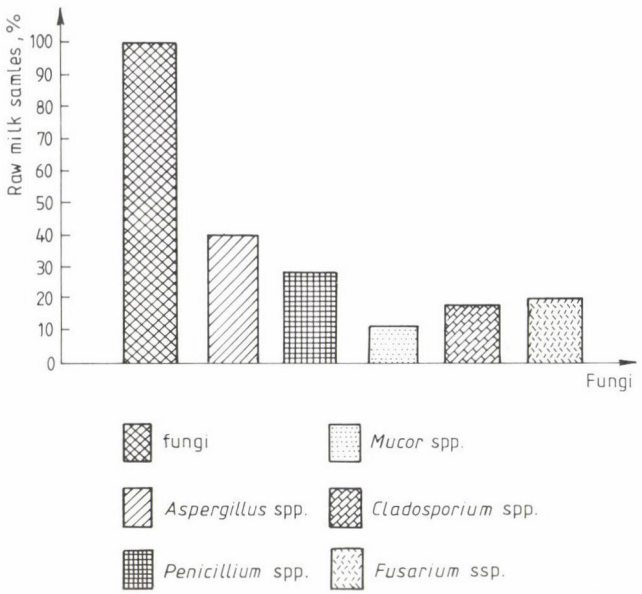

Fig. 1. Frequency of some fungal genera in raw milk samples

It is necessary to point out the frequency of toxigenic species in raw milk samples. These species belonged to the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Eurotium*, *Fusarium*, *Geotrichum*, *Penicillium* and *Trichoderma*. They were found to be contaminants of 91% of milk samples.

Of the 39 species isolated in these experiments, 20 are known as potential producers of various toxic metabolites (SMITH & HACKING, 1983; MARASAS et al., 1984; FRISVAD, 1988). These species and some of their toxins are presented in Table 1.

All *Fusarium* species (100%), isolated from milk and five (83%) *Aspergillus* and *Penicillium* species are toxigenic according to the literature data.

The most common fungal species was *Geotrichum candidum*, which was isolated from 62% of raw milk samples (Fig. 2). According to FRISVAD (1988) this fungus is a producer of unknown toxins. ŠKRINJAR and co-workers (1986) observed in their earlier experiments a high incidence of *G. candidum* in raw milk, pasteurised milk and Edam cheese during ripening. This fungus can cause rancidity on butter and other edible fats. Under certain conditions it may be pathogenic for humans and animals (FASSATIOVA, 1986).

Table 1

Some toxic metabolites produced by fungal species isolated from raw milk

Species	Toxin
Alternaria alternata	Alternariols, altertoxins, tenazoic acid
Aspergillus flavus	Aflatoxins, aspergillic acid, cyclopiazonic acid
A. fumigatus	Fumigaclavines, fumitoxins, fumitremorgins, gliotoxin, verruculogen
A. niger	Aflatoxins, malformins
A. ochraceus	Ochratoxin A, penicillic acid
A. versicolor	Nidulotoxin, sterigmatocystin
Cladosporium herbarum	Cladosporic acid
Eurotium herbariorum	Sterigmatocystin
Fusarium graminearum	Deoxynivalenol, diacetoxyscirpenol, HT-2 toxin, nivalenol, T-2 toxin, zearalenone
F. moniliforme	Deoxynivalenol, diacetoxyscirpenol, furanoterpenoids, fusaric acid, gibberellins, moniliformin, T-2 toxin, zearalenone
F. oxysporum	Diacetoxyscirpenol, diacetylnivalenol, furanoterpenoids, fusarenon-X, fusaric acid, moniliformin, neosolaniol, T-2 toxin, zearalenone
F. solani	Furanoterpenoids, fusarenon-X, HT-2 toxin, neosolaniol, T-2 toxin, zearalenone
F. sporotrichoides	Deoxynivalenol, diacetoxyscirpenol, diacetylnivalenol, fusarenon-X, HT-2 toxin, neosolaniol, nivalenol, T-2 toxin, T-2 tetraol, zearalenone
Geotrichum candidum	Unknown toxins
Penicillium aurantiogriseum	Cyclopiazonic acid, ochratoxin A, penicillic acid, penitrem A, xanthomegnin, viomellein
P. claviforme	Citrinin, patulin
P. hordei	Roquefortine C, terrestic acid
P. raistrickii	Griseofulvin, penicillic acid, terrein
P. roqueforti	Mycophenolic acid, patulin, penicillic acid, roquefortine
Trichoderma viride	Trichodermin

The next most frequent species was *P. aurantiogriseum*, isolated from about 23% of raw milk samples. One can notice from Table 1, this fungus produces various toxic metabolites. Its ability to produce ochratoxin A (OA) is of special importance. According to some authors (HULT et al., 1982; PETKOVA-BOCHAROVA & CASTEGNARO, 1985) the occurrence of Balkan endemic nephropathy (BEN) and urinary system tumours (UST) are associated with OA presence. In our climatic conditions *P. aurantiogriseum* was often found as a dominant fungal species in feeds (SKRINJAR et al., 1992), milk and milk products (SKRINJAR, 1985) and meat products (SKRINJAR & DANEV, 1994).

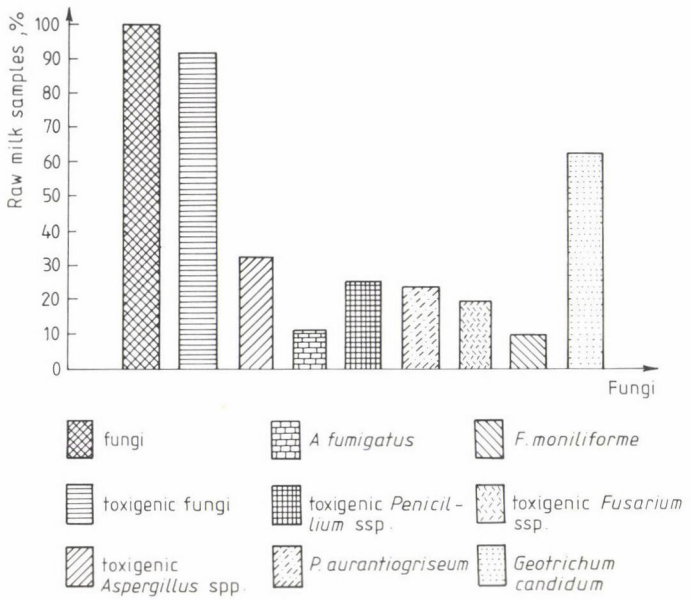

Fig. 2. Frequency of toxigenic fungi in raw milk samples

Among *Aspergillus* species, *A. fumigatus* was the most common. It was isolated from 11% of samples. *A. flavus*, *A. ochraceus* and *A. versicolor* were found to contaminate 6% of milk samples.

F. moniliforme was the most frequent *Fusarium* species. About 9% of milk samples was contaminated with it. *F. moniliforme* was on of the most prevalent fungal species in dairy cattle feeds in Vojvodina throughout the three-year investigations (ŠKRINJAR et al., 1995). It was found to produce various toxic compounds (Table 1) inducing in some areas neurotoxicoses of pigs and poultry (FASSATIOVA, 1986). In this work, other *Fusarium* species, *F. oxysporum*, *F. graminearum*, *F. solani* and *F. sporotrichoides*, were isolated from 6 and 2% of raw milk samples, respectively.

2.2. Aflatoxin analysis

None of 47 raw milk samples was contaminated with AB1 and AM1.

3. Conclusions

All of the raw milk samples tested (47), obtained from the collecting stations in Vojvodina in period of October – February 1992 and December – May 1993, were contaminated with moulds at various degree.

The total viable counts of moulds ranged between 5 and 4×10^4 per cm^3.

The high incidence of mould contamination of milk was probably the result of high fungal distribution in air or/and low hygienic quality of milk-handling equipment at that time.

Fungal strains isolated from raw milk belonged to 19 genera and 39 species. *Aspergillus* and *Penicillium* were presented with the largest number (6) of different species.

The presence of toxigenic moulds in milk was of great significance. It is known that spores of some toxigenic species can survive milk pasteurisation process and than they can be found in pasteurised milk and its products. Under certain conditions they can germinate and produce toxic metabolites. This is of special importance in cheesemaking. In these experiments, about 91% of milk samples were contaminated with toxigenic fungi. Out of the 39 fungal species isolated 20 were toxigenic according to the reference (FRISVAD, 1988).

Geotrichum candidum and *Penicillium aurantiogriseum* were found to be the most frequent fungi. They were isolated from 62 and 23% of raw milk samples, respectively.

Having in mind that toxigenic fungi are widely distributed and also their thermal resistance, permanent mycological and mycotoxicological control of raw milk is necessary as well as the improvement of the hygienic conditions during milking.

AB1 and AM1, which can contaminate raw milk by indirect way, were not found in milk samples tested.

References

A.O.A.C. (1990): Official Methods of Analysis of the Association of Official Analytical Chemist. Natural poisons. SCOTT, P. M. (Ed.), pp. 1184 – 1213.

BORGSTRÖM, S. (1983): Investigation of aflatoxin M1 in milk and butter and organochloropesticides in milk. Swedish Dairy Association, Internal report SMRC-ö 107/a-2-1983.

CARBONEL, F. & SUDREAU, P. (1981): Annual Report from Direction Generale de la Consommation, de la Concurrence et des Prix. Service de la Repression des Fraudes, Paris, France, pp. 66 – 68.

VAN EGMOND, H. P. (1991): Mycotoxins in dairy products. *Prehrambano-tehnol. biotehnol. rev.*, *29*, 71 – 77.

ELLIS, M. B. (1971): *Dematiaceous hyphomycetes*. Commonwealth Mycological Institute, Kew & Surrey, England, p. 608.

FASSATIOVA, O. (1986): *Moulds and filamentous fungi in technical microbiology*. Elsevier, Amsterdam-Oxford-New York-Tokyo, p. 233.

FRISVAD, J. C. (1988): *Fungal species and their production of mycotoxins*. – in: SAMSON, R. A. & VAN REENEN-HOEKSTRA, E. S. *Introduction to food-borne fungi*. Centraalbureau voor Schimmelcultures, Baarn-Delft, The Netherlands, pp. 239 – 249.

HULT, K., PLEŠTINA, R., HABAZIN-NOVAK, V., RADIĆ, B. & ČEOVIĆ, S. (1982): Ochratoxin A in human blood and Balkan endemic nephropathy. *Arch. Toxicol.*, *51*, 313 – 321.

MARASAS, W. F. O., NELSON, P. E. & TOUSSOUN, T. A. (1984): *Toxigenic Fusarium species, identity and mycotoxicology*. The Pennsylvania State University Press, University Park & London, pp. xix + 328.

MÖLLER, A. T. (1987): Pers. comm.

NELSON, P. E., TOUSSOUN, T. A. & MARASAS, W. F. O. (1983): *Fusarium species. An illustrated manual for identification*. The Pennsylvania State University Press, University Park and London, pp. ix + 193.

PETKOVA-BOCHAROVA, T. & CASTEGNARO, M. (1985): Ochratoxin A contamination of cereals in an area of high incidence of Balkan endemic nephropathy in Bulgaria. *Fd Addit. Contaminants*, *2*, 267 – 270.

PIDOPLIČKO, N. M. & MILKO, A. A. (1971): *Atlas mukoraljnih gribov*. Izdateljstvo "Naukova dumka", Kiel, p. 187.

PITT, J. I. & HOCKING, A. D. (1985): *Fungi and food spoilage*. Academic Press Australia, pp. xi + 413.

RAPER, K. B. & FENNELL, D. I. (1965): *The genus Aspergillus*. Williams & Wilkins Co., Baltimore, p. 686.

RAPER, K. B. & THOM, C. (1949): *A manual of the Penicillia*. Williams & Wilkins Co., Baltimore, pp. xi + 875.

SAMSON, R. A. & VAN REENEN-HOEKSTRA E. S. (1988): *Introduction to food-borne fungi*. Centraalbureau voor Schimmelcultures, Baarn-Delft, The Netherlands, p. 299.

SAMSON, R. A., STOLK, A. C. & HADLOK, R. (1976): *Revision of the subsection Fasciculata of Penicillium and some allied species*. Studies in Mycology, No. 11, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, p. 47.

ŠKRINJAR, M. (1985): *Penicillium verrucosum* Dierckx var. *Cyclopium* (Westling) Samson, Stolk & Hadlok: Appearance in Edam cheese, feasibility of ochratoxin A production and toxicity. *Prehrambeno-tehnološka revija*, *23*, 35 – 38.

ŠKRINJAR, M. & DANEV, M. (1994): Occurrence of ochratoxin A – producing moulds and ochratoxin A in dry sausage. Paper presented at the 40th International Congress of Meat Science and Technology, Hagues, The Netherlands.

ŠKRINJAR, M., ŽAKULA, R. & STOJANOVIĆ, E. (1986): Moulds and mycotoxins in milk and semi-hard cheese. *Mljekarstvo*, *36*, 35 – 38.

ŠKRINJAR, M., STUBBLEFIELD, R. D. & VUJIČIĆ, I. F. (1992): Ochratoxigenic moulds and ochratoxin A in forages and grain feeds. *Acta vet. Hung.*, *40*, 185 – 190.

ŠKRINJAR, M., STUBBLEFIELD, R. D., STOJANOVIĆ, E. & DIMIĆ, G. (1995): Occurrence of *Fusarium* species and zearalenone in dairy cattle feeds in Vojvodina. *Acta vet. Hung.*, (in press).

SMITH, J. E. & HACKING, A. (1983): Fungal toxicity. – in: SMITH, J. E., BERRY, D. R. & KRISTIANSEN, B. (Eds) *The filamentous fungi*. Edward Arnold (Publishers) Ltd., London, p. 238.

SREBRNIK, S. (1982): Annual report Institute of Hygiene and Epidemiology. Ministry of Health, Brussels, p. 75.

SREBRNIK, S. (1986): Pers. comm.

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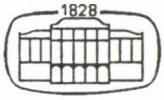

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