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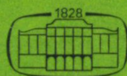

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Central Food Research Institute
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Phone: (36-1) 155-8244 ext. 169
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ACTA ALIMENTARIA

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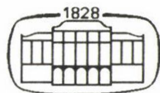

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CHANGES OF ANTIMICROBIAL ACTIVITY IN YOGHURT DURING STORAGE

A. KASALICA

Dairy Institute New Belgrade, Auto put 3. Yugoslavia

The ability of lactic acid bacteria to produce antimicrobial substances in yoghurt depending on the conditions of storage, time and temperature was examined. After completed incubations (44 °C; 2.40 h), yoghurts were stored at 5 °C, 20 °C and 30 °C for 18 h, 3 and 7 days respectively. Antimicrobial activities in yoghurt were examined, using the following test bacteria: *P. aeruginosa* ATCC 25853, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *Y. enterocolitica* 0.3 and 0.9. The differences in the established zones of inhibition ranged from non-significant ($P>0.05$) to highly significant ($P<0.001$) depending on the pathogenic bacteria tested. The lowest sensitivity to antimicrobial effects of yoghurt were exhibited by *S. aureus* ATCC 25923, and the highest by *Y. enterocolitica* 0.3 and 0.9. Antimicrobial substances did not disappear in yoghurts stored at different temperatures, but instead, they increased during storage depending on time and temperature.

Keywords: antimicrobial activity, yoghurt

Yoghurt possesses not only nutritive and dietetic values (RASIC & KURMAN, 1978; SHAHANI et al., 1979; DEETH & TAMIME, 1981; ALM, 1982; SCHAAFSMA et al., 1986, etc.), but also therapeutic ones (RASIC & KURMAN, 1978; PULUSANI & RAO, 1983; GOLDINI, 1989, etc.). Numbers of authors are devoting attention to the therapeutic characteristics of yoghurt, which has an inhibitory effect on harmful bacteria (SHAHANI et al., 1976; MITIC, 1979; KOTZ et al., 1988; MITIC et al., 1989, etc.). This characteristic is based not only on the lactic acid present in yoghurt, but also on the specific substances produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*.

The choice and selection of thermophilic lactic acid bacteria in yoghurt are based mostly on their ability to maintain acidity, aroma, consistency, to increase acidity at low temperatures, etc. (BOUILLANNE & DESMAZEAUD, 1980; HEMME et al., 1980; KASALICA, 1988, etc.). However, having in mind also the therapeutic characteristics of yoghurt, it is especially important to pay attention to strains which may also have the ability to produce antimicrobial substances (KASALICA, 1992).

The objectives were to examined the effect of temperature and time of storage on the preservation of antimicrobial substances in yoghurt.

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1. Materials and methods

1.1. Culture

In order to determine antimicrobial activities of associated compatible strains of lactobacilli and streptococci against pathogenic bacteria the following strains were used: *Lactobacillus delbrueckii* subsp. *bulgaricus* (ZL and SL) and *Streptococcus* subsp. *thermophilus* (OS). Strains were isolated from autochtonus material of yoghurt samples from different hilly-mountainous regions. The isolated strains were identified using 50 biochemical tests API 50CHL (API-system). Strains were maintained in 10% reconstructed skimmed autoclave milk (116 °C, 15 min) with added 0.3% yeast extract. The milk was inoculated with 1% culture and incubated at 37 °C for 18 h. After incubation, the cultures were maintained at 5 °C and before experiments they were subcultured at least twice.

The following test organisms were used to determine antimicrobial effect of yoghurt samples: *Yersinia enterocolitica* 0.3 and 0.9, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 25853. The cultures were regularly maintained on slants of Muller-Hinton agar (37 °C, 18 h), and stored at 5 °C.

1.2. Effect of temperature and time on the production of antimicrobial substances

The incubation of yoghurt (OS+ZL) and (OS+SL) with 2% inoculum was at 44 °C until milk coagulation. In addition to the usual temperature for storing yoghurt (5 °C), temperatures of 20 °C and 30 °C were also used.

Studies of the antimicrobial effects of yoghurt were carried out after 18 h, 3 and 7 days. The disc-diffusion method was used, and the degree of activity was evaluated according to the scale described by SHAHANI and co-workers (1976). The degree of produced acid (°SH), the pH values, and survival of lactic acid bacteria during storage were also determined.

1.3. Statistical analysis of results

The obtained results were analysed statistically using the "Microstat" statistical software package program by Ecosoft Inc. 1985 version.

Table 1

Effect of temperature and time of storage on antimicrobial effects of yoghurt

Type of yoghurt	Stored at (°C)	Zones of inhibition (mm)														
		E. coli ATCC 25922			*Y. enterocolitica* 09			*Y. enterocolitica* 03			*S. aureus* ATCC 25923			*F. aeruginosa* ATCC 27853		
		18 h	3 days	7 days	18 h	3 days	7 days	18 h	3 days	7 days	18 h	3 days	7 days	18 h	3 days	7 days
	5	–	–	–	–	17.5	17.0	–	18.0	18.5	–	–	–	–	–	–
Yoghurt	20	–	13.5	13.0	19.0	22.0	23.0	17.0	20.0	22.0	–	12.5	14.5	12.0	12.0	12.5
(OS+ZL)	30	13.5	18.0	15.0	20.0	23.5	26.0	17.0	23.0	25.5	–	17.5	18.5	12.5	16.5	16.5
	5	–	–	–	–	15.0	19.0	–	14.5	16.5	–	–	–	–	–	–
Yoghurt	20	–	12.5	14.5	12.0	20.0	22.0	17.0	19.0	20.0	–	12.0	13.5	–	11.5	13.5
(OS+SL)	30	–	15.5	18.0	16.0	22.0	26.0	17.0	23.5	25.5	–	15.5	18.0	11.5	12.5	16.0

Very strong inhibition 20–25 mm
Strong inhibition 15–18 mm
Medium inhibition 12–14 mm
Poor inhibition 9–12 mm
No inhibition (–)

2. Results

In the experiments yoghurts containing *L. bulgaricus* and *S. thermophilus* expressed antimicrobial activities against pathogenic bacteria were used. The effects of temperature and time of storage on the antimicrobial activity of yoghurt is presented in Table 1. The results show that after 18 h of storage at 5 °C there was no inhibition of the pathogenic bacteria tested. The yoghurts had an inhibitory effect after 3 and 7 days of storage at 5 °C against *Y. enterocolitica* 0.3 and 0.9, only. After 18 h of storage at 20 °C and 30 °C yoghurts exhibited antimicrobial activities against *Y. enterocolitica* 0.3 and 0.9, and yoghurts (OS+ZL) *P. aeruginosa* ATCC 27853, and also against *E. coli* ATCC 25922 (30 °C). At temperatures 20 °C and 30 °C after 3 and 7 days of storage, inhibition zones against the examined test organisms varied from 12.0 to 26.0 mm in yoghurt (OS+ZL) and from 11.5 to 26.0 mm in yoghurt (OS+SL), depending primarily on the sensitivity of the tested strains to the antimicrobial effects of yoghurt. The examined yoghurts showed different degree of inhibition to the same tested pathogenic microorganisms depending on the length and temperature of storage.

The differences between the microorganisms isolated from yoghurt (OS+ZL) and (OS+SL) in the antimicrobial activity expressed as zone of inhibition ranged from non-significant (P>0.05) to highly significant (P<0.001), depending on the pathogenic bacteria investigated (Tables 2 and 3).

Table 2

Statistical significance of differences between zones of inhibition (mm) of the tested microorganisms inhibited with yoghurt (OS+SL) incubated at 44 °C for 2.40 h with 2% inoculum

Tested microorganisms	Mean value	*Y. entero.* 03 1	*Y. entero.* 09 2	*P. aerug.* 3	*E. coli* 4	*S. aureus* 5
1 *Y. enterocolitica* 03	17.0	–	0.53^{NS}	4.65***	4.89***	4.97***
2 *Y. enterocolitica* 09	16.89	–	–	4.60***	4.84***	4.92***
3 *P. aeruginosa* ATCC 27853	7.22	–	–	–	0.24^{NS}	0.17^{NS}
4 *E. coli* ATCC 2592	6.72	–	–	–	–	0.08^{NS}
5 *S. aureus* ATCC 25923	6.55	–	–	–	–	–

n=90

^{NS} P>0.05 (not significant)

*** P<0.001 (highly significant)

Table 3

Statistical significance of differences between zones of inhibition (mm) of the tested microorganisms inhibited with yoghurt (OS+ZL) incubated at 44 °C for 2.40 h with 2% inoculum

Tested microorganisms	Mean value	*Y. entero.* 09 1	*Y. entero.* 03 2	*P. aerug.* 3	*E. coli* 4	*S. aureus* 5
1 *Y. enterocolitica* 09	18.67	–	0.34^{NS}	4.16***	4.59***	5.07***
2 *Y. enterocolitica* 03	17.89	–	–	3.82***	4.25***	4.73***
3 P. aeruginosa ATCC 27853	9.11	–	–	–	0.43^{NS}	0.92^{NS}
4 *E. coli* ATCC 25922	8.11	–	–	–	–	0.48^{NS}
5 *S. aureus* ATCC 25923	7.00	–	–	–	–	–

n=90

^{NS} P>0.05 (not significant)

*** P<0.001 (highly significant)

The most sensitive strains were *Y. enterocolitica* 03 and 09. This is also confirmed by the differences in the created zones of inhibition between *Y. enterocolitica* 03 and 09 and the other tested microorganisms, by the statistical analysis significant at P<0.001. The lowest sensitivity to the examined yoghurts was exhibited by *S. aureus* ATCC 25923.

During the experiment acid production (°SH), pH value and survival rate of lactic acid bacteria in the examined yoghurt samples were followed during storage at different temperatures (Tables 4, 5 and 6).

Table 4

Average values of titratable acidity (°SH), pH and number of lactic acid bacteria of yoghurt (CFU/ml) after 18 h of storage at temperatures of 5 °C, 20 °C and 30 °C

Type of yoghurt	Stored at (°C)	pH	°SH	CFU/ml
Yoghurt (OS+ZL)	5	4.93	32.00	$1.23*10^9$
	20	4.58	45.00	$2.75*10^9$
	30	3.87	67.20	$2.16*10^9$
Yoghurt (OS+SL)	5	5.11	28.00	$7.87*10^8$
	20	4.93	29.00	$1.25*10^9$
	30	4.21	56.00	$6.78*10^8$

Table 5

Average values of titratable acidity (°SH), pH and the number of lactic acidi bacteria of yoghurt (CFU/ml) after 3 days of storage at temperatures of 5 °C, 20 °C and 30 °C

Type of yoghurt	Stored at (°C)	pH	°SH	CFU/ml
	5	4.41	38.80	$2.65*10^9$
Yoghurt (OS+ZL)	20	3.70	83.60	$3.43*10^9$
	30	3.42	107.60	$3.92*10^9$
	5	4.97	30.80	$1.46*10^9$
Yoghurt (OS+SL)	20	3.73	74.80	$1.40*10^9$
	30	3.41	118.60	$1.03*10^9$

It is evident from the results that higher temperatures and longer storage of yoghurt led to higher acidity and lower pH values of the medium. Moreover, it can be said that the number of viable cells in yoghurt (OS+SL) increased during 3 and 7 days of storage in comparison to the 18 h storage. In yoghurt (OS+ZL) the number of viable cells increased after 7 days of storage.

Table 6

Average values of titratable acidity (°SH) pH and the number of lactic acid bacteria of yoghurt (CFU/ml) after 7 days of storage at temperatures of 5 °C, 20 °C and 30 °C

Type of yoghurt	Stored at (°C)	pH	°SH	CFU/ml
	5	4.23	42.00	$4.92*10^8$
Yoghurt (OS+ZL)	20	3.63	86.80	$6.63*10^8$
	30	3.40	118.00	$4.96*10^8$
	5	4.86	30.80	$1.31*10^9$
Yoghurt (OS+SL)	20	3.58	88.80	$1.70*10^9$
	30	3.34	138.40	$5.22*10^9$

3. Conclusions

The impression which immediately can be observed is that there was no inhibition of the pathogenic tested strains at 5 °C after 18 h of storage. This indicates that the incubation time of 2.40 h was insufficient for the production of antimicrobial substances, which is in agreement with our previous research (KASALICA, 1992) and with the data of other authors (HAINES & HARMON, 1973; SHAHANI et al., 1976; ANGELO et al., 1980, etc.). With higher temperatures and longer time of storage, zones of inhibition increased depending on the supplied test organisms. It seems that all

yoghurt cultures were biochemically active, to a lower (5 °C) or higher degree (20 °C and 30 °C) during storage. As a result of these activities, acidity increased and the pH values of the medium dropped in the examined samples during storage. However, the drop in pH value of yoghurt was only one of the factors, but not the decisive one, which affects the inhibition of pathogenic bacteria. The low correlation dependence ($r = -0.51$) obtained between the produced pH values of lactic acid bacteria and percent inhibition of pathogenic bacteria (KASALICA, 1992) are in favour of this conclusion. The higher activities of lactic acid bacteria in yoghurt at 30 °C compared to other temperatures of storage are a result of better development of these bacteria at this temperature. It can be concluded on this basis of the obtained results that storage at temperature of 30 °C against other temperatures (5 °C and 20 °C) was the optimal temperature for the production of antimicrobial substance during storage. However, this temperature is not suitable for storing yoghurt, since acidity increases at higher temperature, and the organoleptic characteristics of the product also change.

The results indicate that temperature and time of incubation are significant factors for the production of antimicrobial substances by specific lactic acid bacteria. Our results are in agreement with the findings of MITIC and co-workers (1989) who concluded on the basis of their research that antimicrobial activities of *L. helveticus* did not decrease after 5, 7 and 10 days of storage at 5 °C, 7 °C, 12 °C and 25 °C. Contrary to this, the results of RASIC and MITIC (1964) show that the antibiotic effect of yoghurt is the highest immediately after completed incubation, while storage at temperatures over 0 °C causes a decrease in the antibiotic activity. However, the degree of increase of antimicrobial activity in yoghurt during storage at different temperatures is also related to proteinase activities of yoghurt after completed incubation. Namely, on the basis of the latest data in literature (ZOURANI et al., 1992), antimicrobial activities of lactic acid bacteria in the yoghurt are connected with the produced peptides of low molecular weight.

The results of our research indicate from practical point of view the very important fact that antimicrobial activities of yoghurt are not lost during storage, but are in fact increased.

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STUDIES ON THE RECOVERY OF PUNGENCY-FREE COLOUR MATTER FROM INDIAN CAPSICUM EXTRACTS

K. V. BALAKRISHNAN[a,b] and J. VERGHESE[b,c]

[a] Department of Polymer Science & Rubber Technology. Cochin University of Science & Technology
Cochin 682 022. India

[b] C. U. Varkey Centre for Research & Quality Assurance. Synthite Industrial Chemicals Limited
Kolenchery 682 311. India

Colour concentrate devoid of pungency, derived from Indian capsicum oleoresin by eliminating pungent principles by fractionation using appropriate solvent systems, is a prized food colour. The present study examines specifically Tomato variety of capsicum spice with respect to yield and relative quality characteristics of the colour and pungency fractions recovered from its oleoresin by repeated extraction with aqueous methanol and acetone, and partitioning between hexane and aqueous methanol, acetone, ethanol and acetic acid.

It is seen that for fractionation of the oleoresin using aqueous methanol, increase of water content in the solvent from 0 to 30% enhances the yield of pigment fraction from 57.3 to 79.5%, with a corresponding decrease of colour value from 100 320 to 87 054 colour units (CU). The net colour recovery in the pigment phase increases from 72.1 to 86.80%. However, the yield of pungency fraction drops from 36.5 to 11.4%, with a progressive hike in capsaicin content from 5.68 to 16.06%. The net recovery of capsaicinoids in the pungency fraction decreases from 94.23 to 83.21%. With aqueous acetone containing 10 to 30% water, corresponding values are 51.4–77.9%, 98 868–87 648 CU, 63.74–85.63%, 40.3–11.9%, 5.10–15.84% and 93.42–85.67% respectively.

Backing by a second solvent such as hexane is necessary for sharp separation between colour and pungency phases when the solvent concentration drops below 70%. Values for the above factors for fractionation using aqueous methanol containing 20 to 50% water in presence of 10% hexane are 75.2–86.7%, 91 212–82 764 CU, 86.03–90.00%, 17.3–4.8%, 11.10–36.21% and 87.28–79.00% respectively. The corresponding values for acetone are 67.6–84.2%, 96 756–83 952 CU, 82.03–88.66%, 24.3–6.7%, 7.98–26.76% and 88.14–81.49%.

It is concluded that within a solvent system, variation in water content causes little deflection in the distribution pattern of red and yellow pigment groups in the colour fraction. Red pigment concentration in the pigment pool is the highest (61–62%) when the oleoresin is partitioned between aqueous acetone and hexane.

For fractionation using 50% aqueous acetic acid and hexane, the yield of colour matter is the highest (88.2%); however, the colour recovery in the pigment fraction (84.69%) and recovery of capsaicinoids (63.32%) are lower. On the other hand, performance of aqueous ethanol is almost comparable with that of aqueous methanol.

Keywords: Indian chilli oleoresin, fractionation, chilli colour devoid of pungency, yield and pigment distribution

[c] Author to whom correspondence should be addressed

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Capsicum pigments, mainly carotenoids, are among the highest tinctorial potency natural colourants (BAUERFEIND, 1975). Broadly, there are two types of capsicum: paprika, rich in colour with nil/faint pungency, and others with different levels of these qualities (ASTA, 1966, 1975, 1992). World's annual production of non-paprika varieties far outweighs paprika (GOVINDARAJAN, 1986; ASTA, 1995).

Paprika and its oleoresin are celebrated traditional food colourants (ASTA, 1966; BIACS et al., 1989). In view of the upsurge in the use of natural pigments, paprika by itself is not sufficient enough to meet the global demand for capsicum colour. For this reason, industry is compelled to exploit other varieties of the spice.

Depending on the type of capsicum, the oleoresin derived therefrom contains different levels of colour and pungent principles plus fixed oil, resins and waxes (GOVINDARAJAN, 1986). Resolution of capsicum oleoresin into colour and pungency parts has sparked numerous investigations. Earlier approaches were concerned with the enrichment of pungent principles through: (1) re-extraction of the oleoresin with aqueous ethanol, methanol, acetone, acetic acid or alkali, (2) partitioning between these solvents and hydrocarbon and (3) chromatography (SCOVILLE, 1912; DOTT, 1922; TICE, 1933; DICKEY & NITARDY, 1933; BERRY, 1935; BERRY & SAMWAYS, 1937; DODGE, 1941; B.P.C., 1949, 1968; LEASE & LEASE, 1956; HOLLA et al., 1957; SUZUKI et al., 1957; TODD, 1958; TANDON et al., 1964; NIPPON SHINYAKU CO. LTD., 1968; MATHEW et al., 1971a, 1971b; TIRIMANNA, 1972; GOVINDARAJAN & ANANTHAKRISHNA, 1973; DAMAYANTHY et al., 1980; NARAYANAN et al., 1980; PURSEGLOVE et al., 1987; GOVINDARAJAN, 1986). The chromatography route is only of academic interest. More recently, extraction with supercritical carbon dioxide has also been reported (MAYER, 1989). Currently, the upgradation of pungency level is largely engineered in industry by fractionation of the oleoresin using aqueous solvent system. Here, the colour and pungent principles are partitioned between the fixed oils of the oleoresin and the solvent system; the colour principles find a berth in the fixed oils and the pungent components in the solvent. Repeated extraction of the oleoresin with the chosen solvent system culminates in colour extract devoid of pungency. Thus, if the initial oleoresin is sufficiently rich in colour, this route assures the generation exclusively of the colour concentrate, the spin off being a concentrate containing pungent principles and residual pigments.

India cultivates only pungent types of capsicum-collectively termed chilli- and practically no paprika (KRISHNAMURTHY et al., 1970; GOVINDARAJAN, 1986; SPICES BOARD, 1995). Indian cultivar belongs to *Capsicum annuum* var. *annuum*, but each area has its characteristic horticultural variety of the cultivar (GOVINDARAJAN, 1985). Chilli constitutes a principal article of commerce, the total production amounting to 700 000–800 000 tons per annum (SPICES BOARD, 1995). The high colour, low pungent Byadgi chilli (*C. annuum* var. *annuum*) cultivated in Hubli district of Karnataka State

and Tomato chilli (*C. annuum* var. *annuum*) grown in Warangal, Khammam, East & West Godavari districts of Andhra Pradesh State can be regarded as the Indian counterparts of paprika (JOHN, 1993; SPICES BOARD, 1995). Of the oleoresins obtained from Indian chillies, those redeemed from these types have the highest tinctorial power, happily tempered with mild pungency. These oleoresins are, therefore, ideal substrates for the fabrication of chilli colour by liquid-liquid fractionation.

In the resolution of chilli oleoresin, the chosen solvent system dictates the yield, colour/capsaicin recovery and relative distribution of red and yellow pigment groups in the harvested colour and pungency concentrates, which are key parameters that determine product quality as well as process economy. Eventhough aqueous methanol or acetone or these solvents-cum-hydrocarbon media are frequently recommended for the fractionation, very little information is available on the variation of the above parameters in the two fractions with change in the composition of the solvent system. This commanding problem invited our attention. Our objectives were to examine the (1) yield, (2) colour strength, (3) pungency level and (4) relative distribution of red, yellow and total pigments, in the colour and pungency fractions obtained from a typical capsicum extract through fractionation using aqueous solvent systems of varying compositions and thus derive data that can form the basis for the selection of solvent for the optimisation of pigment recovery from the oleoresin.

1. Materials and methods

1.1. Materials

Capsicum oleoresin for the investigation (kindly donated by Synthite) was prepared from Tomato chilli. Tomato chilli pericarp with ca 5% residual seed content was dried to 4% moisture level, ground and extracted with ethylene dichloride. The extract was filtered and solvent removed under vacuum to yield the oleoresin. The properties of the product are given in Table 1. Pure capsanthin and lutein for HPLC were gifts from Laboratorios Bioquimex S.A. DE C.V., Mexico. Fractionation runs were carried out using laboratory reagent grade solvents. Analytical grade reagents were used for spectrophotometric estimations and chromatography grade for HPLC.

1.2. Methods

Capsicum oleoresin, 100 g, was stirred (magnet bar) with 400 ml solvent system for 1 h. The contents were transferred to a separating funnel and allowed to stand undisturbed. The pigment-rich layer was separated and treated further with 400 ml solvent system. This process was repeated until the pigment phase was practically free from pungency. The colour and pungency fractions were concentrated separately on

water bath under reduced pressure. Solvent-free pungency fraction was taken up in ethylene dichloride to remove water and desolventised.

Solvent systems consisting of methanol and acetone with (1) 0, 10, 20 and 30% water content and (2) 0, 10, 20, 30, 40 and 50% water content doped with 10% hexane were used for the fractionation.

Partitioning between hexane and aqueous acetic acid/ethanol was carried out as per procedure earlier reported (NIPPON SHINYAKU CO. LTD., 1968).

Colour values (CV) were determined by MSD-10 (MSD, 1959) and capsaicin content by spectrophotometric difference (JOINT COMMITTEE OF PHARM. SOC. FOR ANAL. CHEM., 1964) methods employing a Hitachi U-2000 Spectrophotometer. HPLC analyses of saponified samples (A.O.A.C., 1990) were conducted in a Hewlett-Packard Series 1050 High Pressure Liquid Chromatograph using Shimadzu LC Column, Shim-pack, CLC-SIL (M), 150 mm long × 4.6 mm ID; *n*-hexane – ethyl acetate – acetone (65:28:7) was used as mobile phase.

Colour and capsaicin recoveries were calculated from total colour and total capsaicin, obtained as quantity × colour value and quantity × capsaicin content, respectively. Pigment concentration was derived by dividing colour value by 1600 (BALAKRISHNAN et al., 1996).

Table 1

Oleoresin used for fractionation experiments

Property	$\bar{x}$	±s
Colour value (Colour units, CU)	79 728	162
Capsaicin content (%)	2.20	0.06
Pigment concentration (CV/1600) (g/kg)	49.83	0.10
Red pigments (HPLC) (%)	57.02	0.37
Yellow pigments (HPLC) (%)	42.98	0.37

$\bar{x}$ mean of 3 measurements
±s standard deviation of 3 measurements

2. Results

Quality characteristics of the oleoresin used for fractionation experiments are listed in Table 1. Extraction with aqueous methanol or acetone splits the oleoresin into colour and pungency-rich fractions. The colour and capsaicin level of these fractions with variation in the water content (Wc) of solvents are presented in Table 2.

Table 2

Fractionation of capsicum oleoresin with aqueous methanol and acetone

Solvent	Colour fraction						Pungency fraction									Overall		
	Quantity (g)		Colour value (CU)		Total colour (Units)	Colour recovery	Quantity (g)		Colour value (CU)		Total colour (Units)	Capsaicin content (%)		Total capsaicin (Units)	Colour recovery	Quantity recovery	Colour recovery	Capsaicin recovery
	$\bar{x}$	±s	$\bar{x}$	±s		%	$\bar{x}$	±s	$\bar{x}$	±s		$\bar{x}$	±s		(%)	(%)	(%)	(%)
Methanol (%)																		
100	57.3	1.77	100 320	1 971	5 748 336	72.10	36.5	1.36	39 336	1 145	1 435 764	5.68	0.39	207.32	18.00	93.8	90.10	94.23
90	67.2	1.68	96 360	2 045	6 475 392	81.21	25.5	1.15	24 948	1.434	636 174	7.95	0.47	203.49	7.98	92.7	89.19	92.49
80	75.1	2.03	90 948	1 763	6 830 194	85.66	15.2	0.79	22 440	1 075	341 088	12.61	0.78	191.67	4.27	90.3	89.93	87.12
70	79.5	2.25	87 054	2 334	6 920 793	86.80	11.4	0.83	21 054	946	240 015	16.06	0.94	183.08	3.01	90.9	89.81	83.21
Acetone (%)																		
100	Fully Soluble																	
90	51.4	2.18	98 868	1 824	5 081 815	63.74	40.3	1.76	50 292	1 585	2 026 767	5.10	0.45	205.53	25.42	91.7	89.16	93.42
80	67.4	1.89	95 964	1 268	6 467 973	81.12	23.8	1.02	25 014	1 282	595 333	8.07	0.72	192.06	7.46	91.2	88.58	87.30
70	77.9	2.41	87 648	2 261	6 827 779	85.63	11.9	0.75	21 450	1 004	255 255	15.84	1.12	188.49	3.20	89.80	88.83	85.67

$\bar{x}$ mean of 3 extractions (2 measurements for each extraction)

±s standard deviation of 3 extractions (2 measurements for each extraction)

As the Wc in methanol increases from 0 to 30%, the yield of colour fraction correspondingly uplifts from 57.3 to 79.5%, with synchronised decrease of colour value (CV) from 100 320 to 87 054 colour units. The net colour recovery calculated from total colour increases from 72.10% to 86.80%. Increase in Wc, therefore, favours the retrieval of pigments in the colour fraction. In this concentration range, the yield of pungency fraction drops from 36.5 to 11.4%, with a progressive hike in the capsaicin content from 5.68 to 16.06%. However, the net capsaicin recovery based on total capsaicin falls from 94.23% to 83.21%. Therefore, higher Wc in the solvent system leads to lower recovery of pungent principles.

Results of similar runs using acetone are also listed in Table 2. The oleoresin is fully soluble in pure acetone, but in presence of water it could be partitioned into the colour and pungency-rich fractions. With increase in the Wc of acetone from 10 to 30%, the yield of colour fraction elevates from 51.4 to 77.9% and the recovery of total colour from 63.74 to 85.63%. At low Wcs, the yield and colour recovery are less compared to methanol, but the difference narrows down with increase in Wc. The performance of both the solvents is almost comparable at 70% concentration. As with methanol, the CV of the colour fraction progressively falls with increase in the Wc. The recovery of capsaicin is also comparable.

With increase in Wc, the density difference between the solvent system and oleoresin decreases, leading to difficulty in layer separation. Below 70% concentration, clear separation cannot be achieved. Better partitioning is effected by the addition of *n*-hexane to the system. In Table 3 are assembled the results obtained on fractionation of oleoresin capsicum incorporating 10% *n*-hexane to acetone and methanol at varying Wcs'.

When methanol at 0% and 10% Wcs are doped with hexane, separation between the layers is not satisfactory; a minimum of 20% water is the key to clear separation. With increase in Wc from 20 to 50%, the yield of colour fraction increases and the CV correspondingly reduces, with a net hike in colour recovery from 86.03 to 90.00%. It may be noted that upto 70% methanol, the addition of hexane has little influence on the colour recovery. The presence of hexane, however, facilitated the use of still lower methanol concentrations by effecting better separation between colour and pungency phases. Yield of colour fraction and recovery of total colour improve as the solvent concentration reduces from 70 to 50%; on the other hand, a further decrease in the recovery of capsaicin is noticed.

With hexane in acetone, the oleoresin is fully soluble in the absence of water. The minimum requisite is 20% water for adequate partitioning. As the Wc increases from 20 to 50%, the yield of colour fraction rises from 67.6 to 84.2% and the colour recovery from 82.03 to 88.66%.

Table 3

Fractionation of capsicum oleoresin with aqueous methanol and acetone in presence of hexane

Solvent	Colour fraction: Quantity (g) $\bar{x}$	Colour fraction: Quantity (g) ±s	Colour fraction: Colour value (CU) $\bar{x}$	Colour fraction: Colour value (CU) ±s	Colour fraction: Total colour (Units)	Colour fraction: Colour recovery (%)	Pungency fraction: Quantity (g) $\bar{x}$	Pungency fraction: Quantity (g) ±s	Pungency fraction: Colour value (CU) $\bar{x}$	Pungency fraction: Colour value (CU) ±s	Pungency fraction: Total colour (Units)	Pungency fraction: Capsaicin content (%) $\bar{x}$	Pungency fraction: Capsaicin content (%) ±s	Pungency fraction: Total capsaicin (Units)	Pungency fraction: Colour recovery (%)	Overall: Quantity recovery (%)	Overall: Colour recovery (%)	Overall: Capsaicin recovery (%)
Methanol (%)																		
100	No clear separation																	
90	No clear separation																	
80	75.2	1.54	91 212	1 582	6 859 142	86.03	17.3	1.10	20 460	1 312	353 958	11.10	0.55	192.03	4.44	92.5	90.47	87.28
70	79.1	1.79	88 044	1 834	6 964 280	87.35	11.7	0.75	19 536	1.520	228 571	15.83	0.86	185.21	2.86	90.8	90.21	84.18
60	84.1	2.27	84 480	2 248	7 104 768	89.11	8.2	0.57	17 424	1 126	142 876	22.09	0.81	181.13	1.79	92.3	90.90	82.33
50	86.7	2.54	82 764	2 013	7 175 639	90.00	4.8	0.34	9 570	820	45 936	36.21	1.31	173.80	0.57	91.5	90.57	79.00
Acetone (%)																		
100	Fully soluble																	
90	No clear separation																	
80	67.6	1.82	96 756	1 682	6 540 705	82.03	24.3	1.25	26 862	2 224	652 746	7.98	0.42	193.91	8.18	91.7	90.21	88.14
70	77.1	2.20	88 242	1 454	6 803 458	85.33	13.5	0.91	24 024	1 745	324 324	14.13	0.84	190.75	4.06	90.6	89.39	86.70
60	81.0	2.57	85 932	2 112	6 960 492	87.30	10.8	0.72	21 714	1 222	234 511	17.13	0.77	185.00	2.94	91.8	90.24	84.09
50	84.2	2.18	83 952	2 342	7 068 758	88.66	6.7	0.57	18 480	1 019	123 816	26.76	1.22	179.29	1.55	90.9	90.21	81.49

$\bar{x}$ mean of 3 extractions (2 measurements for each extraction)

±s standard deviation of 3 extractions (2 measurements for each extraction)

Table 4

HPLC concentration of red and yellow pigment groups in the colour and pungency fractions from capsicum oleoresin

Solvent	Conc (%)	Colour fraction (%)			Pungency fraction (%)		
		Red, $\bar{x}$	Yellow, $\bar{x}$	$\pm s$	Red, $\bar{x}$	Yellow, $\bar{x}$	$\pm s$
Methanol	100	58.83	41.17	1.21	40.17	59.83	0.95
	90	58.20	41.80	0.90	29.40	70.60	1.11
	80	57.28	42.72	1.46	26.79	73.21	0.81
	70	58.25	41.75	0.75	22.69	77.31	1.97
Acetone	90	59.15	40.85	2.14	46.79	53.21	1.23
	80	58.77	41.23	1.04	43.62	56.38	0.84
	70	58.03	41.97	0.89	36.50	63.50	1.56
Methanol + Hexane	80	58.06	41.94	0.85	40.65	59.35	0.76
	70	58.45	41.55	1.32	38.82	61.18	1.08
	60	58.26	41.74	0.73	39.05	60.95	1.75
	50	58.59	41.41	1.68	39.45	60.55	2.19
Acetone + Hexane	80	61.40	38.60	1.06	36.06	63.94	1.66
	70	61.28	38.72	1.25	35.24	64.76	0.92
	60	61.43	38.57	0.94	33.91	66.09	1.77
	50	62.15	37.85	1.44	34.12	65.88	2.38

$\bar{x}$ mean of 3 extractions (2 measurements for each extraction)

$\pm s$ standard deviation of 3 extractions (2 measurements for each extraction)

The HPLC concentration of red and yellow pigments in the colour and pungency fractions are presented in Table 4. An immediate observation is that within a solvent system, distribution of these pigment groups in the colour fraction remains more or less steady irrespective of the water content. The pungency fraction derived in the presence of hexane also follows the same pattern. On the other hand, in the absence of hexane, the red pigment concentration in the pungency fraction drops with increase in Wc and that of yellow pigments elevates correspondingly. Aqueous acetone in presence of hexane yields colour concentrate with the highest level of red pigments.

In Table 5 are assembled the recovery of red, yellow and total pigments in the colour and pungency fractions. These figures are derived from the results presented in Tables 1–4. Bulk of the pigments, both red and yellow, are concentrated in the colour fraction. For all the four solvent systems, the recovery of red pigments in the colour fraction progressively increases with Wc. Aqueous acetone-hexane system ranks first in the recovery of total red pigments from the oleoresin.

Table 5

Recovery and distribution of red and yellow pigment groups in the colour and pungency fractions from capsicum oleoresin
(Calculated from the mean values of the results presented in Tables 1, 2, 3 and 4)

Solvent	Conc (%)	Red pigments (mg) in			Recovery of red pigments (%) in			Yellow pigments (mg) in			Recovery of yellow pigments (%) in		
		Colour fraction	Pungency fraction	Total	Colour fraction	Pungency fraction	Total	Colour fraction	Pungency fraction	Total	Colour fraction	Pungency fraction	Total
Methanol	100	2 113	360	2 473	74.37	12.67	87.04	1 479	537	2 016	69.04	25.07	94.11
	90	2 355	117	2 472	82.89	4.12	87.01	1 691	281	1 972	78.94	13.12	92.06
	80	2 445	57	2 502	86.06	2.00	88.06	1 823	156	1 979	85.10	7.28	92.38
	70	2 519	34	2 553	88.66	1.19	89.85	1 805	116	1 921	84.26	5.41	89.67
Acetone	90	1 878	592	2 470	66.10	20.83	86.93	1 297	674	1 971	60.55	31.46	92.01
	80	2 375	162	2 537	83.59	5.70	89.29	1 666	210	1 876	77.77	9.80	87.57
	70	2 476	58	2 534	87.15	2.04	89.19	1 791	101	1 892	83.61	4.71	88.32
Methanol + Hexane	80	2 488	90	2 578	87.57	3.16	90.73	1 798	131	1 929	83.94	6.11	90.05
	70	2 543	55	2 598	89.51	1.93	91.44	1 808	87	1 895	84.40	4.06	88.46
	60	2 587	35	2 622	91.05	1.23	92.28	1 853	54	1 907	86.50	2.52	89.02
	50	2 627	11	2 638	92.46	0.38	92.84	1 856	17	1 873	86.65	0.79	87.44
Acetone + Hexane	80	2 509	147	2 656	88.31	5.17	97.46	1 578	261	1 839	73.66	12.18	85.84
	70	2 606	71	2 677	91.72	2.50	96.33	1 646	131	1 777	76.84	6.11	82.95
	60	2 672	49	2 721	94.05	1.72	97.43	1 677	97	1 774	78.29	4.52	82.82
	50	2 745	26	2 771	96.62	0.91	98.41	1 672	51	1 723	78.05	2.38	80.43

Table 6

Fractionation of capsicum oleoresin using aqueous acetic acid and ethanol in the presence of hexane

	Aq. acetic acid				Aq. ethanol			
	Colour fraction		Pungency fraction		Colour fraction		Pungency fraction	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Quantity (g)	88.2	2.31	3.6	0.54	78.5	1.95	12.7	1.02
Colour value (Colour units)	76 560	1 720	4 620	330	87 912	1 314	23 100	1 022
Red pigment conc. (HPLC) (%)	55.73	1.86	31.42	2.11	57.68	1.27	34.01	1.44
Yellow pigment conc. (HPLC) (%)	44.27	1.86	68.58	2.11	42.32	1.27	65.99	1.44
Capsaicin content (%)	–	–	38.7	1.76	–	–	15.60	0.61
Total colour (Units)	6 752 592		16 632		6 901 092		293 370	
Colour recovery (%)	84.69		0.21		86.55		3.68	
Total pigment conc. (g/kg)	47.85		2.88		53.87		14.43	
Red pigments (mg)	2 352		3		2 439		62	
Yellow pigments (mg)	1 868		7		1 789		121	
Recovery of red pigments (%)	82.78		0.10		85.85		2.18	
Recovery of yellow pigments (%)	87.20		0.32		83.52		5.65	
Total capsaicin (Units)	–		139.32		–		198.12	
Recovery of capsaicin (%)	–		63.32		–		90.05	

$\bar{x}$ mean of 3 extractions (2 measurements for each run)

±s standard deviation of 3 extractions (2 measurements for each run)

The results on the fractionation of chilli oleoresin by partitioning between hexane and 50% acetic acid are presented in Table 6. Lower part of the Table displays recoveries calculated from the mean of experimental values. A striking observation is that in this case, the yield of colour matter is maximum and pungency fraction is minimum. However, the total recovery of colour from the oleoresin is 4–5% less than the previous experiments; almost all the total colour is contributed by the pigment fraction. The pigment phase retains traces of acetic acid and its complete removal requires higher temperature and longer desolventisation time or additional process steps, leading to colour loss. Recovery of red pigment pool and its concentration in the colour fraction are also lower. The acetic acid layer contains all the capsaicinoids, the recovery of which from acid is difficult. About two-third of the capsaicinoids present in the starting material could only be recovered.

Table 6 also displays the results of similar runs using hexane and 80% ethanol. The recoveries are fairly comparable with runs using aqueous methanol.

3. Conclusions

Fractionation with methanol or acetone upto 30% water content can be conveniently used for the retrieval of pungency-free colour concentrate from Indian chilli oleoresin. Lower concentrations can be employed in conjunction with *n*-hexane for better recovery of colour matter and red pigments. Yield of colour matter and pigment recovery increase with the water content of the solvent system. However, the recovery of heat principles exhibit a gradual fall as the water content is increased.

Concentration of red pigments in colour fraction is an important quality criterion of capsicum concentrates. Fractionation with aqueous acetone in the presence of hexane yields a colour concentrate rich in red pigments.

In the solvent systems considered above, variation in water content has little influence on the distribution pattern of red and yellow pigments in the colour fraction. As regards pungency fraction, gradual fall in the concentration of red pigments with increase in water content is noticed when derived in the absence of hexane.

Yield of colour fraction is the highest when the oleoresin is partitioned between hexane and aqueous acetic acid. However, recovery of total colour and red pigments in the colour fraction are lower.

Partitioning between hexane and aqueous ethanol yields results almost in line with that of aqueous methanol.

*

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ISOLATION AND PARTIAL CHARACTERIZATION OF A COTTON ROOT LIPASE

N.S. PRIOLO[a], M.C. ARRIBÉRE and N.O. CAFFINI

Laboratorio de Investigación de Proteínas Vegetales (LIPROVE), Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata. Casilla de Correo 711, 1900 La Plata. Argentina.

Crude extracts of cotton seedling roots (*Gossypium hirsutum* L. *cv. Guazuncho2*) exhibit lipolytic activity on cottonseed oil. When the crude extract is centrifuged for 30 min at 4 °C and 11 000 g lipolytic activity is recovered from the pellet by adding 20 mmol Tris-HCl buffer (pH 8.5) containing 1% Triton X-100, 1 mmol DTT and 0.5 mmol EDTA. After centrifugation for 30 min at 50 000 g and for 1 h at 100.000 g the enzyme is recovered in the supernatant. The lipase shows maximum activity at pH 8.0–8.5 and at 55 °C–65 °C and is stimulated by calcium. HPLC analysis of the remaining triacylglycerols (TAG) after the action of the lipase on cottonseed oil reveals higher affinity for TAG containing oleic acid. Fractionation of the soluble enzyme preparation by gel chromatography (Sephacryl S-300) shows the presence of two active fractions.

Keywords: lipase, cotton, *Gossypium hirsutum*

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) represent about 3% of the enzymes used in different kinds of industrial processes (HUANG et al., 1988). A usual application of lipases is the production of fatty acids and glycerol from triacylglycerols, replacing the traditional chemical hydrolysis, which requires high pressure and temperature. The use of lipases diminishes energetic costs and prevents the production of polymers and coloured compounds, avoiding further purification of the reaction products (DANDIK et al., 1993).

The introduction of enzymatic techniques in organic medium has considerably increased the scope of lipase-catalyzed reactions. In an aqueous medium lipases principally favour the hydrolysis of triacylglycerols and other esters, but in a prevailing organic medium they catalyze wide range of esterifications and transesterifications, giving products of high commercial value (MUKHERJEE, 1990; NCUBE et al., 1993).

Although most lipase preparations used in food and nonfood industries are of fungal or bacterial origin, those coming from plants show higher specificity and this property makes them a very interesting product in lipid biotechnology (HUANG et al., 1988). Unfortunately, only a few plant lipases have been purified to homogeneity (LIN

[a] To whom correspondence should be addressed

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& HUANG, 1984; MAESHIMA & BEEVERS, 1985; HAMMER & MURPHY, 1993; NCUBE et al., 1993). On the other hand, most bibliography is referred to seed lipases and very little information has been provided on lipases located in other plant organs like roots (HEIMAN-MATILE & PILET, 1977; AKHTAR et al., 1981).

As far as we know, the only reference on cotton lipases is a short paper (OLCOTT & FONTAINE, 1941) informing that lipolytic activity had been detected in non-purified enzymic preparations from whole seedlings. In the present paper we report the presence of an alkaline lipase in primary roots of cotton seedlings (*Gossypium hirsutum* L. cv *Guazuncho2*), which has been isolated and partially characterized.

1. Materials and methods

1.1. Plant material

Cottonseeds (*Gossypium hirsutum* L. cv. *Guazuncho2*) were provided by Sáenz Peña Experimental Station of the National Institute of Agricultural Technology (INTA Sáenz Peña, Chaco Province, Argentina). The seeds were soaked in running top water for 24 h at room temperature (20 °C) and then allowed to germinate on moist paper towels at 33 °C in darkness. The end of the imbibition period was designated day zero of germination.

1.2. Preparation and fractionation of extracts

Primary roots from 1–2 days seedlings were carefully removed from the rest of the seedling, washed with distilled water and homogenized for 1 min (Omnimixer, Sorval) with 0.1 mol Tris-HCl buffer (pH 8.5). The crude extract was obtained by filtering the homogenate through a piece of nylon cloth (pore size: 0.20 mm^2) and centrifuging the filtrate at 11 000 g for 30 min at 4 °C. The supernatant and the pellet resuspended in 0.1 mol Tris-HCl buffer (pH 8.5) were checked for lipolytic activity.

1.3. Isolation of the lipase

The pellet obtained after centrifugation at 11 000 g was extracted with gentle agitation for 3 h at 4 °C with 20 mmol Tris-HCl buffer (pH 8.5) containing 1 mmol dithiotreytol (DTT), 0.5 mmol EDTA and 1% (v/v) Triton X-100. The resulting suspension was centrifuged at 50 000 g for 30 min at 4 °C and the supernatant was centrifuged again at 100 000 g for 1 h at 4 °C (Fraction S-100). The protein content was determined according to BRADFORD (1976).

1.4. Determination of the lipolytic activity

Lipase activity was assayed by a colorimetric method based on the estimation of the absorbance at 715 nm of the isooctane soluble copper complexes formed by the fatty acids released during lipolysis (KWON & RHEE, 1986). The assay mixture contained 5 cm^3 of isooctane with 10% (v/v) of cottonseed oil, 0.2 cm^3 of 0.15 mol calcium chloride and 0.5 cm^3 of sample. The mixture was stirred for 20 min at 37 °C, then the reaction was stopped by adding 0.5 cm^3 of 6 mol HCl and the stirring was continued for one more min. Three cm^3 of isooctane was withdrawn, mixed with 0.6 cm^3 of 5% (w/v) copper acetate solution (pH 6.1) and stirred for 90 s in a vortex (Kartell). The absorbance of the solution was measured at 715 nm and the results expressed as µmol of fatty acid (equivalent to oleic acid) per min per mg of proteins. The reactions were carried out in a specially designed equipment to perform multiple thermostatized reactions in a two-phase system (CAFFINI et al., 1990).

In the fractions obtained by gel-filtration lipolytic activity was determined on 1–^{14}C triolein (Dupont). The sample (0.2 cm^3) was incubated with 0.01 cm^3 of 0.8 mol calcium chloride and 0.01 cm^3 of the substrate for 30 min at 55 °C, emulsifying the mixture by sonication; the reaction was stopped by inmersion of the test tube in boiling water. To each tube 3 cm^3 of chloroform-methanol (2:1) was added, the chloroformic phase was evaporated and the residue redissolved in 0.01 cm^3 of the same mixture. The reaction products were identified by HPTLC (Silicagel 60 Merck) using hexane-ethyl ether-acetic acid (80:20:1.5) as mobil phase and quantified by the use of a scanning radioactivity counter (Berthold, type 2D), argon as counting gas and an Autochron XY recorder. The areas under the peaks, proportional to the quantity of radioactivity, were calculated by triangulation of the tangent that cuts the baseline at both sides of the peak and expressed as percentage of the total radioactivity (GONZÁLEZ BARÓ, 1991).

1.5. Effect of calcium on lipolytic activity

The effect of different concentrations of calcium chloride (5–50 mmol) was assayed on the lipolytic activity of fraction S-100. Determinations were made at 37 °C and pH 8.5.

1.6. Effect of pH on lipolytic activity

The following buffer systems (STOLL & BLANCHARD, 1990) were used to determine the effect of pH on lipolytic activity: citrate–phosphate (pH 2.6–7.0), boric acid–sodium borate (pH 7.6–9.2) and glycine–sodium hydroxide (pH 8.6–10.6). The enzyme activity was assayed for 20 min at 37 °C and the reaction mixture contained 40 mmol calcium chloride (pH values were measured in the reaction mixture).

1.7. Effect of temperature on lipolytic activity

The effect of temperature on lipolytic activity was carried out by determining the enzyme activity for 20 min at pH 8.5 in a reaction mixture containing 40 mmol calcium chloride within the range of 37 °C to 65 °C.

1.8. Thermal stability

The samples were incubated at 37 °C, 45 °C, 50 °C, and 55 °C for 0, 10, 20, 30, 40, 50, and 60 min. The residual lipolytic activity was determined at 55 °C for 20 min in a reaction mixture containing 40 mmol calcium chloride.

1.9. Variation of triacylglycerol content during lipolysis

Cottonseed oil residual TAG composition after 20 min of lipolysis (pH 8.5, 55 °C) was determined by HPLC, carried out in a Konik chromatograph using a Bio-Sil C-18 HL 90-5 S column (250×4.6 mm, particle size 5 μm, Bio-Rad). TGA detection was carried out at 210 nm with a UVIS 204 UV-detector. The chromatograms were recorded and integrated with a Spectra Physics 4600 integrator.

Three cm^3 of the isooctane phase proceeding from the reaction mixture was evaporated under nitrogen stream and then redissolved in chloroform (ca. 5 mg of TAG per cm^3) and injected into the chromatograph. The samples were eluted with ethanol-acetonitrile (7:3) with a flow rate of 1.3 cm^3 min^{-1}. Tentative identification of TAG were based on comparing retention times of standards (Sigma) and elution orders of cottonseed oil TAG obtained from the literature (BALESDENT et al., 1989; BLAND et al., 1991).

1.10. Gel-chromatography

Fraction S-100 was partially purified by gel-chromatography on a Sephacryl S-300 column (Pharmacia K 15/30) equilibrated and eluted with 0.1 mol Tris-HCl buffer, pH 8.5, containing 1% (v/v) reduced Triton X-100 (Sigma) at a flow rate of 0.2 cm^3 min^{-1}. Two cm^3 fractions were collected and each fraction was assayed for protein content both for measuring the absorbance at 280 nm as well as by the BRADFORD (1976) method. The lipolytic activity of each fraction was determined on 1–^{14}C triolein as indicated above.

2. Results and discussion

Crude extracts of cotton seedling roots (*Gossypium hirsutum* L. *cv. Guazuncho2*) exhibited lipolytic activity on cottonseed oil. Enzyme activity was at its maximum at day 1 of the germination period, but was almost negligible after day 4; lipolytic activity was nil in ungerminated seeds.

When crude extracts were centrifuged for 30 min at 4 °C and 11 000 g lipolytic activity was recovered from the pellet by adding 20 mmol Tris-HCl buffer (pH 8.5) containing 1% Triton X-100, 1 mmol DTT, and 0.5 mmol EDTA. Similar attempts made with sodium deoxycolate and sodium chloride instead of Triton X–100 gave negative results. After centrifugation for 30 min at 50 000 g followed by 1 h at 100 000 g the enzyme was recovered in the supernatant (soluble form).

When assayed on cottonseed oil the lipase showed maximum activity at pH 8.0–8.5 (Fig. 1), like most of seed lipases up to date studied, whose actvities are higher at alkaline pH (ARRIBÉRE et al., 1994). Lipolytic activity was notably stimulated by calcium, as it was earlier reported in the case of melon seed lipase (AKHTAR et al., 1981); maximum activity was reached when calcium concentration was raised to 40 mmol (Fig. 2). The presence of calcium in the reaction mixture is essential for detecting lipolytic activity, as Triton X-100 strongly depresses the action of the lipase up to one third of the initial values (data not shown).

The lipolytic activity of the enzyme is notably stimulated by temperature (Fig. 3), reaching maximum values between 50 °C and 60 °C after 20 min reaction. Nevertheless, the lipase is not very thermostable (Fig. 4): even when after 30 min of heating at 37 °C–50 °C the residual activity was 80–85% of that exhibited at time zero, when the enzyme was pre-heated for 10 min at 55 °C activity fell to 20% of the initial value, and reduced only to 6–7% after 20 min at the same temperature. Though great effort is made all over the world to find thermostable lipase preparations for industrial applications, when a lipase is used in food processing it is necessary to eliminate the enzyme from the elaborated foodstuffs and then thermoinstability could be a valuable property, as the enzyme can be easily inactivated by heating at not very high temperature (PRIOLO et al., 1991).

The TAG composition of cottonseed oil (time zero) and the modifications produced after 20 min of lipolysis at 55 °C (pH 8.5) is shown in Fig. 5. The relative area of each TAG from the chromatogram is expressed as percentage of total area in Table 1: as it can be seen, dilinoleoyl-oleoyl-glycerol (LLO), linoleoyl-oleoyl-palmitoyl-glycerol (LOP) and specially linoleoyl-dioleolyl-glycerol (LOO) decreased after 20 min of lipase action, which would indicate a relatively strong preference of the enzyme for TAG with higher oleic acid content.

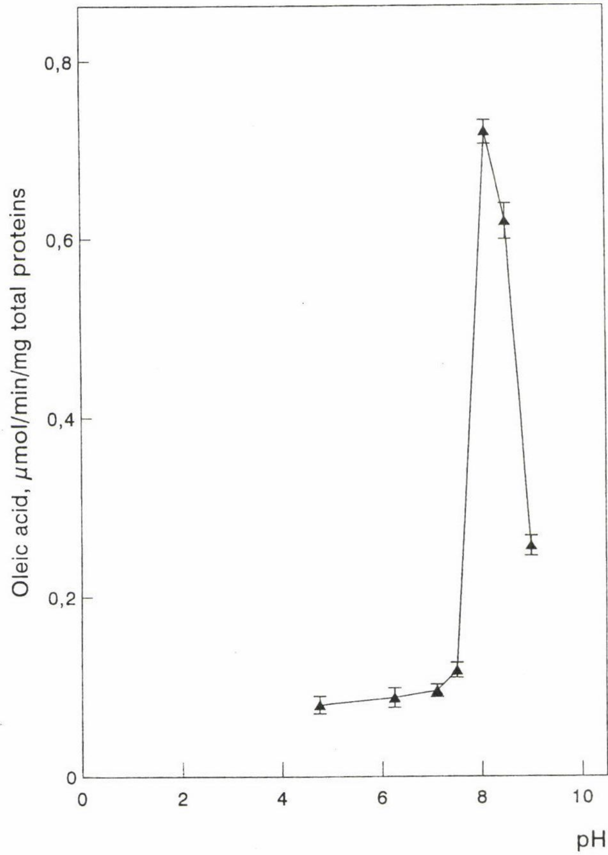

Fig. 1. Lipolytic activity on dependence of pH. Data are the average of at least four determinations. Bars indicate standard deviation

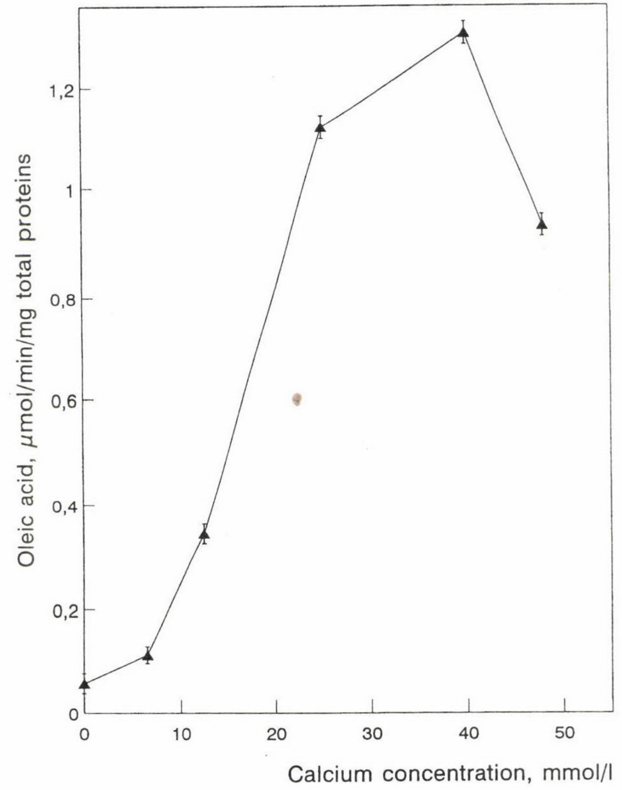

Fig. 2. Effect of calcium concentration on lipolytic activity. Data are the average of at least four determinations. Bars indicate standard deviation

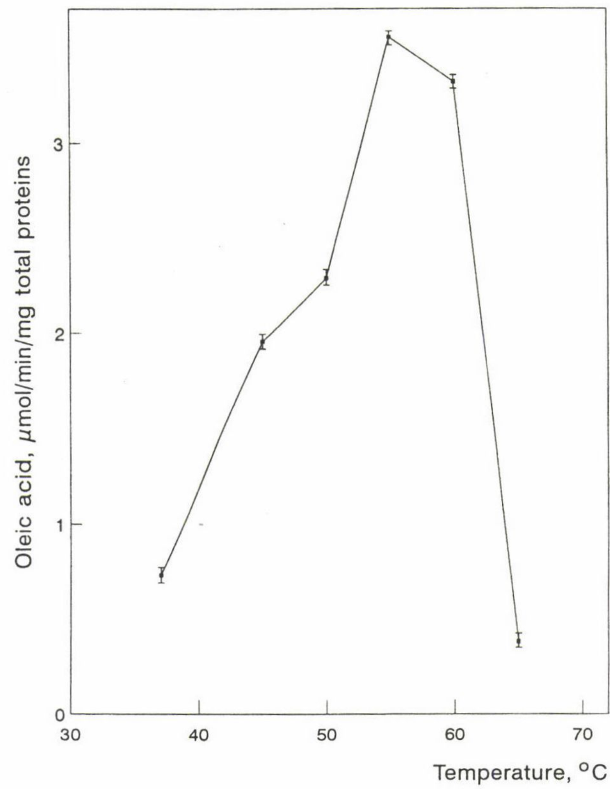

Fig. 3. Effect of temperature on lipolytic activity (20 min reaction). Data are the average of at least four determinations. Bars indicate standard deviation

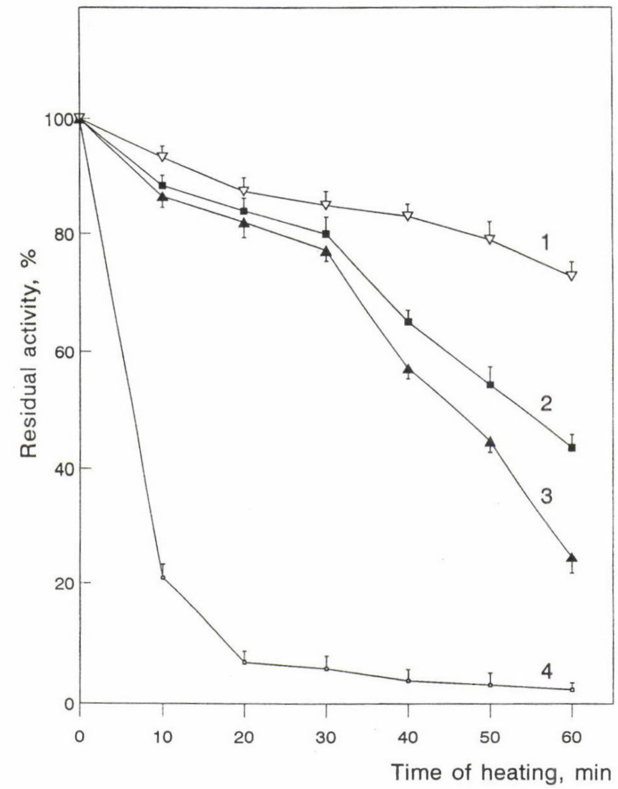

Fig. 4. Thermal stability of the cotton root lipase. Data are the average of at least four determinations. Bars indicate standard deviation.
1: 37 °C; 2: 45 °C; 3: 50 °C; 4: 55 °C

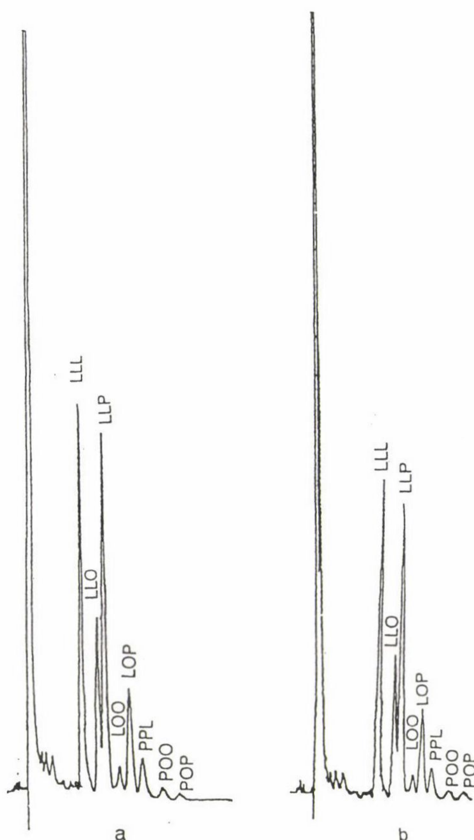

Fig. 5. HPLC of TAG of cottonseed oil (a) before and (b) after 20 min of lipolysis at 55 °C and pH 8.5. LLL: trilinoleoyl-glycerol, LLO: dilinoleoyl-oleoyl-glycerol, LLP: dilinoleoyl-palmitoyl-glycerol, LOO: linoleoyl-dioleolyl-glycerol, LOP: linoleoyl-oleoyl-palmitoyl-glycerol, PPL: dipalmitoyl-linoleoyl-glycerol

Table 1.

TAG composition of cottonseed oil before (time zero) and after 20 min of lipolysis at 55 °C and pH 8.5. Values consigned are the relative areas of TAG (Fig. 5). LLL: trilinoleoyl-glycerol, LLO: dilinoleoyl-oleoyl-glycerol, LLP: dilinoleoyl-palmitoyl-glycerol, LOO: linoleoyl-dioleolyl-glycerol, LOP: linoleoyl-oleoyl-palmitoyl-glycerol, PPL: dipalmitoyl-linoleoyl-glycerol.

TAG	LLL	LLO	LLP	LOO	LOP	PPL
Time zero	31.3	15.1	35.6	2.4	11.8	3.7
20 min	32.1	15.0	36.1	1.7	11.3	3.7
% variation	+2.5	–0.6	+1.4	–29.0	–4.2	–

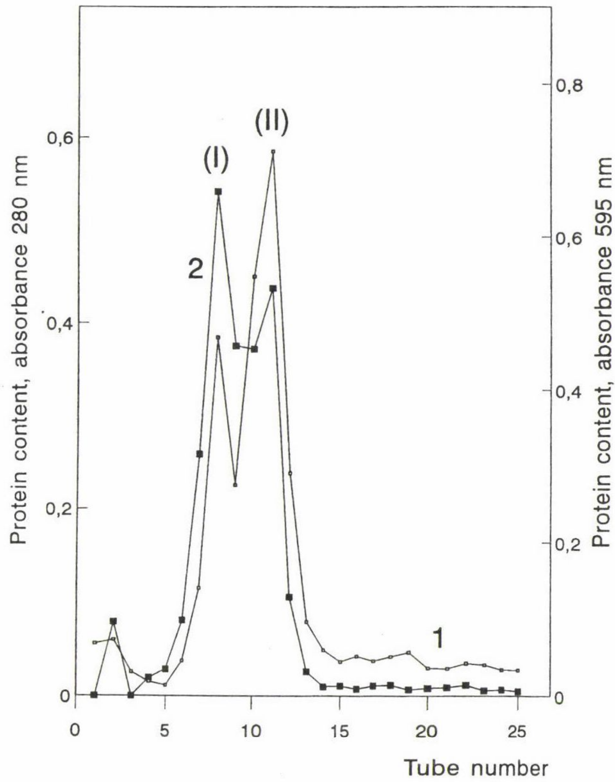

Fig. 6. Gel-filtration (Sephacryl S-300) of the S-100 fraction. 1: A 280 nm; 2: A 595 nm (Bradford)

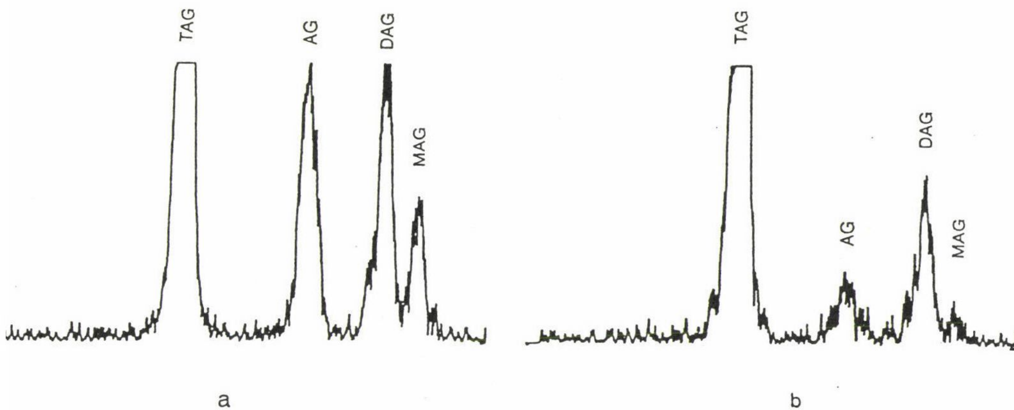

Fig. 7. HPTLC radiograms of the lipolysis products obtained by the action of fractions I (a) and II (b) on 1–C^{14} triolein. FFA: free fatty acids, MAG: monoacylglicerides, DAG: diacylglicerides, TAG : triacylglicerides

When the S-100 fraction was chromatographed by gel-filtration (Fig. 6) the presence of two active fractions (I and II) with close molecular weights were revealed. Fraction I seemed to be more active than Fraction II (the former hydrolyzed 53% of 1–^{14}C triolein and the latter only 34.4% of the radioactive substrate in the same conditions). Additionally, the HPTLC analysis of the reaction products (Fig. 7) revealed a different lipolytic behavior of both fractions, as the ratio diacylglycerol/monoacylglycerol of Fraction I (2:1) is lower than that of showed by Fraction II (4.5:1).

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ENZYMATIC HYDROLYSIS OF PROTEINS FROM CHICKEN HEADS USING ALCALASE AND ESPERASE

M. FIK and K. SURÓWKA

Department of Refrigeration and Food Industry Engineering, Agricultural University, Podluzna Street 3, 30–239 Kraków. Poland

A possible application of Alcalase® from *Bacillus licheniformis* and Esperase® from *Bacillus lentus* to obtain protein hydrolysates from heads of broiler chickens was investigated. Enzymic hydrolysis was conducted under varying conditions of pH (7–10), temperature (45–75 °C) and time (0.5–6.5 h), and with varying additions of water (0–1 kg) and enzymes (0.5–4.5 g) per kg of the comminuted raw material. After optimization of proteolysis it was found that proteins of the studied raw material are hydrolysed at an optimum rate, by both enzymes, at pH 8.0 to 8.5 and at 65 °C. The additions of 0.75 kg water and 2 g Alcalase or 2.5 g Esperase per kg of the heads were found to be optimal. It was shown, moreover, that excessively prolonged hydrolysis time causes undesirable organoleptic changes in the hydrolysates, and so optimal time was chosen as 3 to 3.5 h. Under the established optimal conditions the dry hydrolysate yield amounted to about 69 and 76 g per kg of the raw material, and nitrogen recovery reached 37 and 41% for hydrolysis with Alcalase and Esperase, respectively.

Keywords: poultry by-products, protein hydrolysates, Alcalase®, Esperase®

The proteins of by-products from processed animals are of high biological value and can be incorporated into the human nutrition (LAWRIE & LEDWARD, 1988). In recent years, protein recovery from raw materials of this kind has been carried out mostly by biological methods, especially by enzymic hydrolysis (FIK, 1979; WEBSTER et al., 1982; FIK & SURÓWKA, 1986; SURÓWKA & FIK, 1992; SURÓWKA & FIK, 1994; SURÓWKA & FIK, 1995). The hydrolysis process leads, through peptide bonds breaking, to degradation of protein molecules. Higher degree of hydrolysis causes better solubility of protein hydrolysates and improves the biological properties which make these products more attractive as food additives. However, if the degree of hydrolysis is too high, sometimes only above 20%, worsening of certain functional properties may occur, together with significant changes of organoleptic features, in particular taste and colour, eventually leading to a bitter taste of the product (BLENDFORD, 1994; QUAGLIA & ORBAN, 1990).

The proper course of enzymic hydrolysis, its intensity and yield depend, in a great measure, on the kind of protease used. Each enzyme has its own specificity and its activity depends on a number of parameters such as time, temperature and pH, as well

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as the type and degree of substrate denaturation. Due to the rapid progress of biotechnology and the introduction of various new enzyme preparations, such as Esperase®, it becomes reasonable to study their activity under various technological conditions. Hence, the aim of the present study was an attempt to optimize and compare the rates of hydrolysis of proteins from poultry heads using Alcalase® and Esperase. The raw material was chosen in view of its possible use in obtaining food products.

1. Materials and methods

The heads of two months old broiler chickens collected directly from the production line of a local poultry packing plant were used as a raw material in this study. They were packed in polyethylene bags and blast frozen to –20 °C, and stored at this temperature throughout the period of analysis. Portions of the raw material assigned for assays were defrosted at about 4 °C for 14–20 h, then minced twice using a strainer with holes of 3 mm diameter, and thoroughly mixed.

Enzymic hydrolysis of proteins from the studied material was conducted using Alcalase 2.4 l from *Bacillus licheniformis* and Esperase 7.5 l from *Bacillus lentus* supplied by Novo-Nordisk a/s, Bagsvaerd, Denmark, with the respective activities 2.4 and 2.2 Anson Units per gramme. A portion of 70 g of the comminuted raw material was mixed with water and brought to a desired pH by adding 4 M NaOH solution. Then a proper amount of enzyme was added, the reaction mixture was mixed again and the samples were incubated at a specific temperature in a water bath with agitation, for a specific time. During incubation a constant pH was maintained with 4 M NaOH. After the hydrolysis was completed, the non-digested residue was separated by centrifugation (3000×g, 10 min) and the obtained hydrolysate was assigned for chemical assays.

While establishing optimum pH, the proteolysis was conducted for 1 h at 60 °C with enzyme addition of 2.5 g kg^{-1} and water addition of 1 kg kg^{-1} with respect to the mass of the minced raw material, varying pH at 0.5 intervals from 7 to 10. During the optimization of water and enzyme additions, the conditions of hydrolysis were as follows: pH 8, temperature 60 °C, time 2 h, water added in a ratio of 0.25, 0.50, 0.75 and 1 kg kg^{-1}, enzyme added to the raw material in 1 g intervals from 0.5 to 4.5 g kg^{-1}. In order to optimize temperature and time, the optimal water addition (0.75 kg kg^{-1}) and optimal enzyme addition (2 g kg^{-1} Alcalase and 2.5 g kg^{-1} Esperase) were applied and the mixtures were hydrolysed at 45, 50, 55, 60, 65, 70 and 75 °C for 0.5, 2.0, 3.5, 5.0 and 6.5 h. Triplicate measurements were performed for each set of parameters applied.

In each stage of optimization the total nitrogen content in the hydrolysates was determined by Kjeldahl method (BUDSLAWSKI & DRABENT, 1972), and while establishing the optimal temperature and time, also the degree of hydrolysis (DH) was

determined according to ADLER-NISSEN (1979), involving the reaction of trinitrobenzenesulfonic acid (TNBS) with free amino groups.

The relationships between water and enzyme additions, as well as time and temperature and the amount of the released total nitrogen and the degree of hydrolysis were presented as contour plots made using a statistical package CSS Statistica (Stat Soft, Tulsa, OK, USA). The same programme was employed to fit, for each of the response variables (determinants of protein hydrolysis intensity), a second order polynomial equation of the following form:

$$Z_i = b_0 + b_1 X_j + b_2 Y_k + b_3 X_j^2 + b_4 Y_k^2 + b_5 X_j Y_k$$

where Z_i is the estimated response (total nitrogen or the degree of hydrolysis), b coefficients are the equation parameters (b_0 – constant; b_1 and b_2 – parameters for linear terms; b_3 and b_4 – parameters for quadratic terms and b_5 – for the interaction terms), X_j is the water addition or time and Y_k is the enzyme addition or temperature.

For each of the response variables, the variance was partitioned into linear, quadratic and interaction components in order to assess the adequacy of the second order polynomial function and the relative significance of those components, which was assessed using a t-test.

2. Results and discussion

The dependence of the reaction rate upon the concentration of the hydrogen ions is an important element of the kinetics of enzymic processes. According to KARPIAK (1986), a direct effect of pH on the rate of these reactions is connected with the interaction of hydrogen ions with the enzyme's active site and ionization of the functional groups. Various groups of the active site reveal different ionization ranges, and the reaction of substrate binding by the enzyme runs with maximum yield only at a specific pH.

Figure 1 illustrates the influence of pH on the rate of proteolysis of broiler chicken heads protein with Alcalase and Esperase measured by total nitrogen release. It can be noticed that both enzymes hydrolyzed proteins of the studied raw material at an optimum rate at pH values between 8.0 and 8.5, although Alcalase revealed greater activity than Esperase. Below and above this optimum, a gradual decrease in activity of both enzymes was observed, indicated by a decrease in the amounts of released nitrogen-containing substances. However, above pH 9.5, an increase of the total nitrogen content in the hydrolysates was again observed, which was probably due to an increase in the solubility of proteins with increasing pH.

The optimal pH for Alcalase and Esperase action on a raw protein of poultry heads, as established in the present study, are essentially close to those given by the supplier as optimum for hydrolysis of hemoglobin. Also, many authors (ADLER-NISSEN, 1978; MARQUEZ-MORENO & FERNANDEZ-CUADRADO, 1993; OLSEN, 1983; WEBSTER et al., 1982) reported successful hydrolysis of plant and animal proteins with Alcalase at pH 8.0–8.5.

Data obtained in the current study were used to develop the polynominal models describing the effect of independent variables (water and enzyme additions and time and temperature) on protein hydrolysis intensity measured by total nitrogen and degree of hydrolysis. Estimated parameters for these equations and their statistical significance are presented in Table 1.

Contour plots showing the effects of water and enzyme additions on the rate of protein hydrolysis are shown in Fig. 2. Greater amounts of water, by making easier the access of the enzyme to the substrate and dilution of the hydrolysis products, contributed in a significant way to the increase in total nitrogen yield. This increase has predominantly linear effect ($p \leq 0.01$), both for proteolysis with Alcalase and Esperase (Table 1). It has been shown, moreover, that the effect is more pronounced with water addition in the range 0 to 0.75 kg kg^{-1} than in the range 0.75 to 1 kg kg^{-1}.

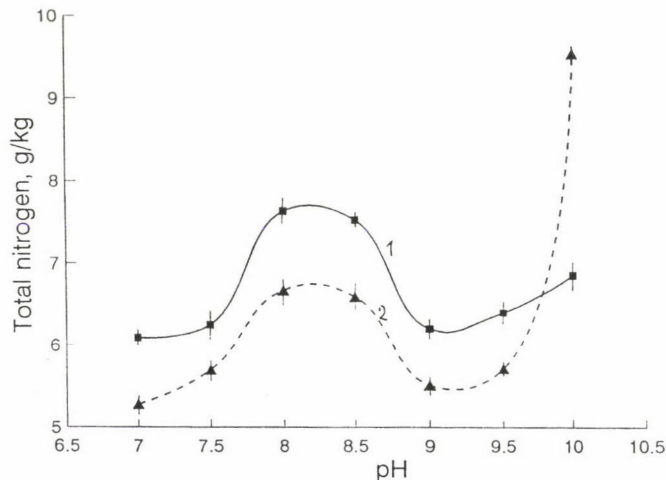

Fig. 1. Influence of pH on the content of total nitrogen in hydrolysates obtained from 1 kg of chicken heads. Conditions of hydrolysis: T 60 °C, time 1 h, enzyme addition 2.5 g kg^{-1}, water addition 1 kg kg^{-1}. 1:hydrolysis with Alcalase, 2: hydrolysis with Esperase.

Table 1

Parameters of the second order polynomial equations developed for protein hydrolysis

Enzyme	Dependent variable	Independent variables		Equation parameters					
	Z_i	X_j	Y_k	b_0	b_1	b_2	b_3	b_4	b_5
Alcalase	Total N (g/kg)	Water addition (kg/kg)	Enzyme addition (g/kg)	1.972*	6.249**	0.617*	–2.269	0.105*	0.033*
Esperase	Total N (g/kg)	Water addition (kg/kg)	Enzyme addition (g/kg)	4.870***	3.078**	–0.239	–0.939	0.208***	0.197*
Alcalase	Total N (g/kg)	Time (h)	Temp. (°C)	–37.28*	1.244*	1.311**	–0.212***	–0.010*	0.012
Esperase	Total N (g/kg)	Time (h)	Temp. (°C)	–80.77***	0.298*	2.650***	–0.022	–0.020***	0.008
Alcalase	DH (%)	Time (h)	Temp. (°C)	–101.4*	–0.593	3.838*	–0.075	–0.031*	0.031*
Esperase	DH (%)	Time (h)	Temp. (°C)	–96.70*	–1.190*	3.355*	0.027**	–0.026	0.048

*, **, ***, significant at 5%, 1% and 0.1% probability level, respectively

Consequently, H_2O addition in a proportion 3:4 was selected as optimal for the hydrolysis process. This choice was partly due to the technological and economical considerations, associated with the necessity of subsequent drying of the product. Optimal H_2O addition adopted here is in agreement with those established earlier for proteolysis of the same raw material with the use of Neutrase® (SURÓWKA & FIK, 1992) and pepsin (SURÓWKA & FIK, 1994).

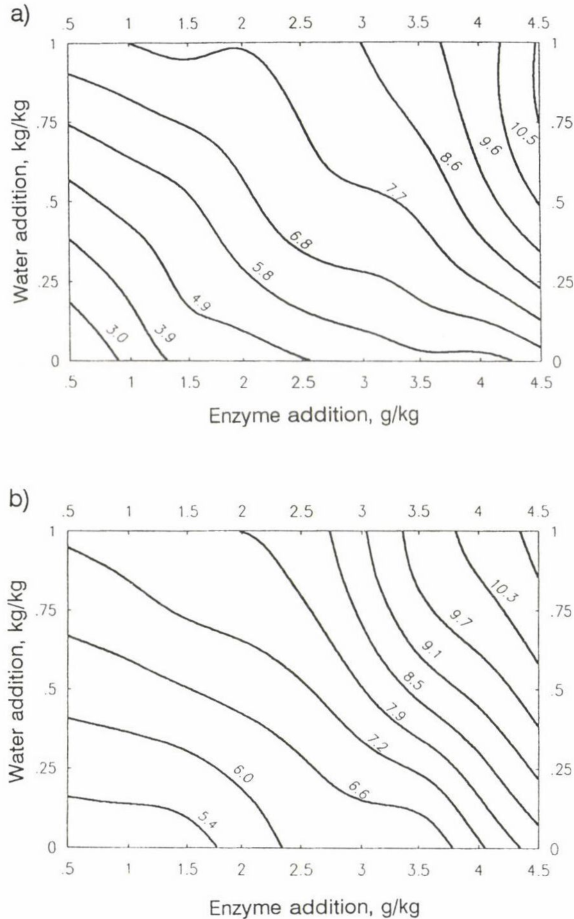

Fig. 2. Contour plots showing the effects of water addition ($kg\ kg^{-1}$) and enzyme addition ($g\ kg^{-1}$) on the content of total nitrogen ($g\ kg^{-1}$) in hydrolysates obtained with Alcalase (a) and Esperase (b) from 1 kg of chicken heads. Conditions of hydrolysis: pH 8, T 60 °C, time 2 h.

Greater concentrations of enzymes also increased the amount of nitrogen released (Fig. 2). For hydrolysis with Esperase a very significant ($p \leq 0.001$) quadratic relationship was observed whereas with Alcalase the increase had equally linear and quadratic character, but the significance level was in this case smaller ($p \leq 0.05$) (Table 1). Taking enzyme activity into account and trying to reconcile technological and economical considerations, optimal additions of enzymes were chosen as 2 g kg^{-1} for Alcalase and 2.5 g kg^{-1} for Esperase, with respect to the comminuted raw material. These values of enzyme concentration ensure similar hydrolysis rate of poultry heads proteins with 0.75 kg kg^{-1} water addition as 4.5 g kg^{-1} concentration of these enzymes without addition of H_2O. Similar additions of proteases were used by SALES and co-workers (1991) and WEBSTER and co-workers (1982) for hydrolysis of proteins of animal by-products.

Statistical analysis revealed moreover interaction ($p \leq 0.05$) between enzyme and water additions, which means that the magnitude of the observed growth of nitrogen release with increasing enzyme additions, is also affected by the H_2O level (Table 1).

The next stage of optimization of proteolysis concerned the choice of the appropriate temperature and time of the process at pH 8.0 and using the previously determined optimal water and enzyme additions. The effect of these parameters on nitrogen release from 1 kg of the raw material is presented in Fig. 3. It was found that the intensity of hydrolysis measured by the total nitrogen released to the hydrolysates grows with the increasing temperature and time, and reaches maximum at 65 °C and the duration of the process of 4.5 h and 6 h for Alcalase and Esperase, respectively. Further growth of temperature above 65 °C led to a gradual decline of enzyme activity, and consequently to a diminished rate of proteolysis. Optimum temperature for hydrolysis of proteins from broiler chicken heads using Alcalase and Esperase was, therefore, chosen as 65 °C, which is higher in comparison with the results of some authors (ADLER-NISSEN, 1978; MA et al., 1983; WEBSTER et al., 1982), who successfully conducted proteolysis with Alcalase at 50 °C. This discrepancy may be due to the specificity of the hydrolysed substrate, which has a stabilizing effect upon the enzyme. With the enzymic preparations used in the present research, it was shown in Table 1 that temperature had a positive linear effect on nitrogen recovery from the raw material, as well as a negative second-order effect. This relationship was very significant for Esperase ($p \leq 0.001$) and slightly less significant for Alcalase ($p \leq 0.01$).

The duration of hydrolysis has also a considerable influence upon its yield. Statistical analysis revealed that this parameter has a linear effect ($p \leq 0.05$) on the total nitrogen increments for both enzymic preparations employed, however, with Alcalase, also a highly significant ($p \leq 0.001$) negative second-order relationship was observed (Table 1), which, with longer hydrolysis may have a limiting effect upon its yield. On the other hand, optimum time of proteolysis should also ensure a desirable quality of

the final product. It has been noticed in this study that excessive prolongation of this parameter causes undesirable changes in colour, taste and smell of the poultry heads hydrolysate. The time of hydrolysis of this material should therefore not exceed 3 or 3.5 h for Alcalase and Esperase, respectively. Such conditions will ensure not only a desirable organoleptic quality of the final product, but also its relatively high yield. Energy saving and lowering of the overall costs are further advantages.

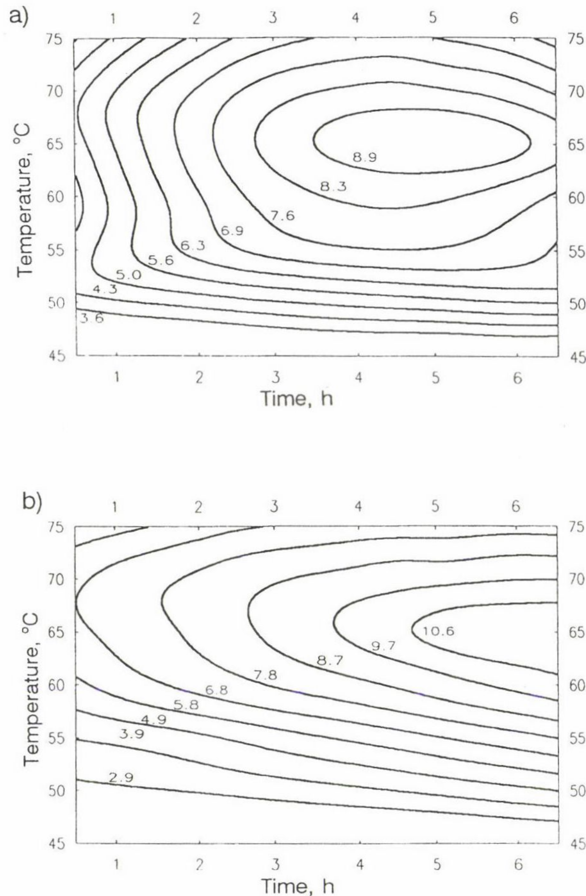

Fig. 3. Contour plots showing the effects of temperature (°C) and time (h) on the content of total nitrogen (g kg^{-1}) in hydrolysates obtained with Alcalase (a) and Esperase (b) from 1 kg of chicken heads. Conditions of hydrolysis: pH 8, enzyme additive 2 g kg^{-1} and 2.5 g kg^{-1} for Alcalase and Esperase respectively, water additive 0.75 kg kg^{-1}.

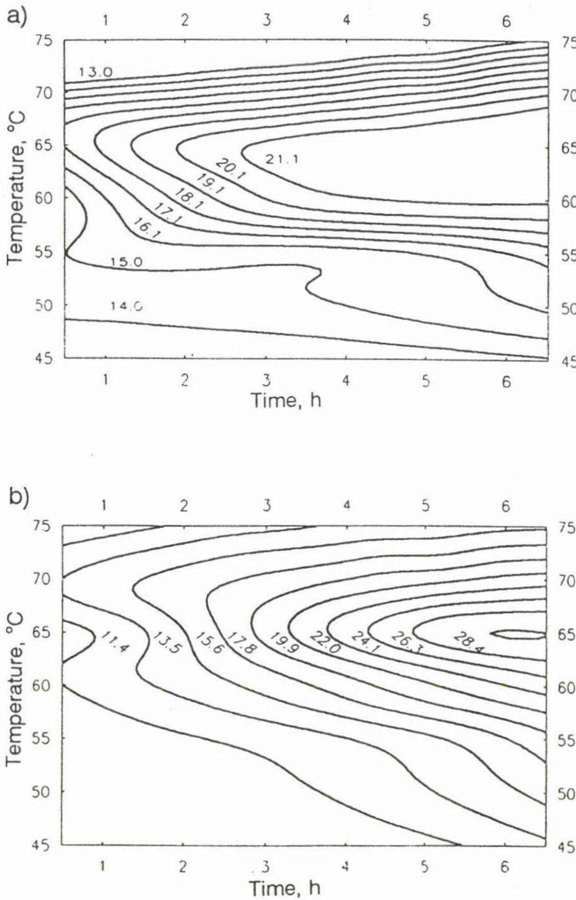

Fig. 4. Contour plots showing the effects of temperature (°C) and time (h) on the degree of hydrolysis (%) in hydrolysates obtained with Alcalase (a) and Esperase (b). Conditions of hydrolysis: pH 8, enzyme additive 2 g kg^{-1} and 2.5 g kg^{-1} for Alcalase and Esperase respectively, water additive 0.75 kg kg^{-1}.

For a more complete characterization of the intensity of proteolysis of poultry heads proteins a relationship was analysed between the temperature and time and the degree of hydrolysis (Fig. 4), which is defined as the number of peptide bonds cleaved, expressed as a percentage of the total number of such bonds in the hydrolysate. It was observed that raising temperature up to about 65 °C produces an increase in the degree of hydrolysis (DH) for each given value of time, irrespectively of the enzymic preparation used. On the other hand, at temperatures above 65 °C, a decrease in DH was observed, due to the thermal inactivation of the enzymes. With Esperase

hydrolysis, the relationship between temperature and the degree of hydrolysis is basically linear ($p \leq 0.05$), whereas with Alcalase it is a resultant of a positive first-order effect and a negative second-order effect at a significance level $p \leq 0.01$ (Table 1). The degree of hydrolysis increased also with increasing hydrolysis time. For proteolysis with Esperase, the positive quadratic effect of time on DH was found to be dominant ($p \leq 0.01$) over the negative linear effect, whereas with Alcalase, only the interaction between time and temperature was found significant ($p \leq 0.05$) (Table 1). Such interaction was not observed in Esperase hydrolysis. It was shown, moreover, that there is a very significant ($p \leq 0.001$) correlation between the amount of nitrogen released from the studied material and the degree of hydrolysis, with high values of the correlation coefficients r, which were equal to 0.81 and 0.90 for Alcalase and Esperase proteolysis, respectively.

Under the established optimal conditions (pH 8.0, temperature 65 °C, time 3.0–3.5 h, water addition 0.75 kg kg^{-1} and enzyme addition 2–2.5 g kg^{-1}) the dry hydrolysate yield amounted to 69 and 76 g kg^{-1} of the raw material, and the percentage of nitrogen recovery reached 37 and 41% for Alcalase and Esperase, respectively. These results are close to the data obtained for protein hydrolysis from the same raw material using Neutrase (SURÓWKA & FIK, 1992), but in the latter case optimal time of proteolysis was approximately twice as long.

3. Conclusions

Alcalase from *Bacillus licheniformis* and Esperase from *Bacillus lentus* reveal a relatively high hydrolytic activity with respect to proteins of poultry heads and can be successfuly used for protein recovery from this raw material for food use.

Both enzyme preparations employed in this study for chicken heads proteins hydrolysis require essentially similar conditions for their optimal activity (pH 8.0, temperature 65 °C, time 3.0–3.5 h, water addition 0.75 kg kg^{-1} and enzyme addition 2–2.5 g kg^{-1}).

Excessive prolongation of hydrolysis (above 3.5 h) partly increases the yield, particularly when using Esperase, but at the same time causes worsening of the organoleptic quality of the obtained hydrolysate.

Hydrolysis with the use of Esperase offers, under optimal conditions, somewhat greater yield of dry hydrolysate and nitrogen recovery, in comparison with the proteolysis with Alcalase.

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Short communication

APPLICATION OF THE DEFT AND MEM TECHNIQUES AS RAPID METHODS FOR SCREENING MYCOLOGICAL QUALITY OF SPICES

G. KISKÓ[a], H. STEGEMAN[b] and J. FARKAS[a]

[a] Department of Refrigeration and Livestock Products Technology, University of Horticulture and Food Industry, H–1118 Budapest, Ménesi út 45. Hungary
[b] Department of Microbiology and Biotechnology, RIKILT-DLO, P.O. Box 230, 6700 AE Wageningen. The Nederlands

Microbial quality of spice paprika pods before industrial processing into paprika powder depends very much on the conditions of harvest, after-ripening and storage prior to processing. Mechanical damage of fresh pods and bulk storage increase drastically the microbial load, particularly mouldiness. The processing technology leads to partial inactivation of viable mould without inactivating toxic metabolites eventually present. Although methods for the most important mycotoxins have been worked out (e.g. HPLC in combination with solid phase extraction procedures for aflatoxins, ochratoxin and some trichotecenes) these are too expensive and cumbersome for routine analysis. Thus, rapid screening methods for paprika powder would be useful for detecting both viable and dead fungi to indicate poor pre-processing handling and storage practices. Therefore, the applicability of the direct epifluorescence filter technique (DEFT) and the microcolony epifluorescence microscopy (MEM) were investigated for this purpose. Diluted suspensions of paprika powders were filtered through Nucleopore polycarbonate membrane filters, stained on the membrane either by Acridin Orange or Congo Red, then counted in non-fluorescent immersion oil on a slide and examined by an epifluorescence microscope at 1000X magnification. Due to a more distinct differentiation of mould parts and paprika particles, Congo Red proved to be a more suitable fluorescent stain. Highly contaminated samples could be quickly estimated by this DEFT method, however, more systematic work is required for quantitative classification. For rapid and selective enumeration of viable mould propagula in paprika powder, aliquots of paprika suspensions deposited on the filter membrane were incubated on the surface of a mould selective agar (RBCA) for 24 h at 30 °C prior to staining and epifluoroscence microscopy at 20X magnification detecting the developed mould microcolonies. Linear correlation ($R=0.96$) was found between the traditional log plate counts of moulds and the log MEM counts. The detection limit was 10^3 $CFUg^{-1}$. The time needed for enumeration of viable fungi was reduced to less than 2 days. DEFT-counting of bacterial contamination seemed to be applicable as a screening method for detecting decontaminated or very old samples of various spices.

Keywords: DEFT, spices, MEM, mould count

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Microbial contamination of spices originates from the microflora of soil, postharvest contamination from dust and insanitary harvesting and processing techniques. The most frequently used means for reducing viable cell counts in spices has been fumigation with ethylene oxide. Because of the mutagenicity and carcinogenicity of ethylene oxide, its use is being increasingly restricted (FARKAS & ANDRÁSSY, 1988). Radiation decontamination is a viable alternative in the case of moulds, radiation has been shown to be more efficient than fumigation (TOOFANIAN & STEGEMAN, 1988; SZABAD & KISS, 1979; FARKAS, 1985). A current problem with the use of irradiation is the need for a specific method for identifying foods that have been irradiated.

In general a method which can assess quickly the level of microbiological contamination would be beneficial. In particular, detection of mould content is important because of economic losses due to moulding spoilage and the public health hazard (many mould species are able to produce mycotoxins). Furthermore, in order to facilitate proper uses of decontamination technologies, testing methods for both dead and viable microorganisms in processed products are needed which can provide an index of good pre-processing practices, indicating whether the processed products have been prepared from good quality materials or not. Testing for mould biomasses is of particular significance in this context because processing technologies lead to inactivation of viable mould without inactivating toxic metabolites eventually present.

Microcolony epifluorescence microscopy (MEM) was described for rapid selective enumeration of bacteria in foods by RODRIGUES and KROLL (1988). The direct epifluorescence filter technique (DEFT method) was developed by PETTIPHER (1983). The method was adapted for detection of irradiated spices by evaluating the large differences between DEFT count and total viable cell count in decontaminated spices (SJÖBERG et al., 1990). PETTIPHER and co-workers (1985) applied the DEFT method to detect moulds in tomato concentrates as an alternative to the Howard Mould Count. Other methods which estimate the chitin or ergosterol content of a sample as a measure for the content of fungal biomass have also been suggested (JARVIS et al., 1984;. HARRIS & KELL, 1985). These chemical methods would not be dependent on the conditions of sample homogenisation which influence the fragmentation of hyphae.

The aim of our present studies was to evaluate the application potential of the MEM and DEFT methods for estimating mycological quality of spices.

1. Materials and methods

1.1. Samples of spices

Samples of caraway and onion powder were obtained from RIKILT-DLO (Wageningen), black pepper and paprika from KÉKI (Budapest). The samples of these spices were irradiated and fumigated.

1.2. Aerobic plate count (APC)

One ml aliquots of the desired dilution were placed in duplicate into sterile Petri dishes. Standard Plate Count Agar (OXOID) was used with 2,3,5 triphenyltetrazolium-chloride (TTC) (0.5% w/v in molten agar) to aid the counting. Plates were incubated at 30 °C for 2–4 days.

1.3. Preparations of DEFT slides

Method I: Samples were prepared as described by SJÖBERG and co-workers (1990). The number of fluorescent microorganisms were counted at a magnification of 1000X, and the number of microorganisms per g was calculated by multiplying the average number of organisms counted per microscope field by the microscope factor MF. (MF = filtering area of membrane (mm)/(microscope field area (mm) × sample volume (ml)).

Method II: The preparation of samples was the same as described above, except for the prefiltration. The samples were prefiltered through glass-wool and Acro-disk (1.2 μm pore size), respectively. Depending on the concentration of suspension, 0.5–3.0 ml of the prefiltered suspension were filtered through a Nuclepore polycarbonate membrane filter (as above). After filtration the membrane was stained with 1 ml acridin orange solution (0.0057 g AO in phosphate solution: 4.4 g KH_2PO_4 in 100 ml distilled water, pH 6.2) for 2 min. The vacuum was maintained, the filter was rinsed with phosphate solution (10 ml), and a rapid rinse with 2.5 ml iso-propanol was performed. Further steps were done as above.

1.4. DEFT measurement for enumeration of mould content of paprika powder

To samples of 1 g 99 ml of a dilution medium (0.1% peptone, 0.9% NaCl) were added, then homogenized for 2 min in a Stomacher. 0.5–1 ml of the diluted stomachered suspension was filtered through a Nuclepore polycarbonate membrane filter (0.6 μm pore size, 25 mm diameter) under vacuum. After filtration the membrane was overlayered with 2 ml of congo red solution (50 μmol in distilled water). After staining (contact time 0.5–30 min) the vacuum was re-applied in order to filter the stain. The filter was washed with different amount of ethanol or ethanol and

isopropanol. The stained membrane was airdried and mounted in non-fluorescent immersion oil on a slide beneath a cover slip and examined by epifluorescent microscope.

1.5. Application of microcolony epifluoroscence microscopy (MEM) for enumeration of mould content in paprika powder

One ml of the diluted sample was filtered through a Nuclepore polycarbonate membrane filter (0.6 μm) under vacuum. The membrane was removed from the filter funnel and incubated on the surface of a mould-selective agar (Rose-Bengal Chloramfenicol Agar, OXOID CM 549) for 24 h at 30 °C. Before incubation a few drops of sterile distilled water was placed on the agar surface to avoid drying of the membranes. After 24 h the membrane was remounted in the filter tower and stained with congo red for 5 min. Further steps were done as described above in DEFT procedure.

1.6. Irradiation and fumigation

Paprika samples were irradiated at cobalt-60 panoramic radiation source at ambient temperature in the Isotope Institute Ltd. (Budapest) in 1994. Other samples were irradiated at the 40 kCi cobalt-60 research source of the Pilot Plant for Food Irradiation in Wageningen in 1985. The samples were fumigated with ethylene oxide at a concentration of 1500 g m^{-3} during eight hours at ambient temperature in 1985.

2. Results and discussion

In microscopic preparations, debris may make the counting of microorganisms difficult or impossible. For removal of food debris single glass-wool and Acro-disk filter (1.2 μm pore size), respectively were used. The use of these prefilters led to an improvement in the quality and visibility of the preparations. It was important that the handling should affect the APC of the samples as little as possible. Filtration through the glass-wool (a cheap filter) affected the APC much less than filtration through an Acro-disk (1.2 μm pore size) and gave almost the same results as the unprefiltered (only stomachered) samples (Table 1).

Although the visibility of preparations prefiltered with Acro-disk was much better, it was not possible to use it for preparation of spice samples because of the significant reduction of APC.

Table 1

The aerob plate count of some spices (unprefiltered = stomachered and prefiltered with glass-wool or Acro-disk (1.2 μm pore size))

Prefilter	Material	Quality	Irradiation dose (kGy)	log APC (count/g)
unprefiltered	black pepper	whole	0	7.9
glass-wool	black-pepper	whole	0	7.3
Acro-disk	balck-pepper	whole	0	5.3
unprefiltered	onion	powder	0	4.8
glass-wool	onion	powder	0	4.7
Acro-disk	onion	powder	0	3.4
unprefiltered	paprika	powder	2	5.2
glass-wool	paprika	powder	2	4.5
Acro-disk	paprika	powder	2	3.2
unprefiltered	paprika	powder	5	4.5
glass-wool	paprika	powder	5	4.4
Acro-disk	paprika	powder	5	4.3

It is to be noted that in case of non-stomachered whole black pepper satisfactory preparations could be made without prefiltration.

When the decontaminated spice samples were examined, the DEFT count (green and orange fluorescing cells) was much higher than the APC count (Fig. 1).

When the unirradiated spice samples were examined, a smaller difference between DEFT and APC count was noticed. The DEFT counts of the non-irradiated, irradiated and fumigated spice samples were of the same order. APC reflects the number of viable microorganisms in the sample at the time of determination capable of growth under the culturing conditions used. The DEFT count can be used to estimate the total cell number of microorganisms, both viable and non-viable. The difference between the DEFT count and the APC count indicates the number of organisms rendered non-viable. Large (more than 3–4 log cycles) differences between DEFT and APC may point for a decontamination treatment, although it could not differentiate between methods of decontamination. The large differences between DEFT and APC values also in the non-decontaminated samples of caraway and onion powder were obviously due to the long storage of these dry samples before investigation resulting in loss of viability of the vegetative cells.

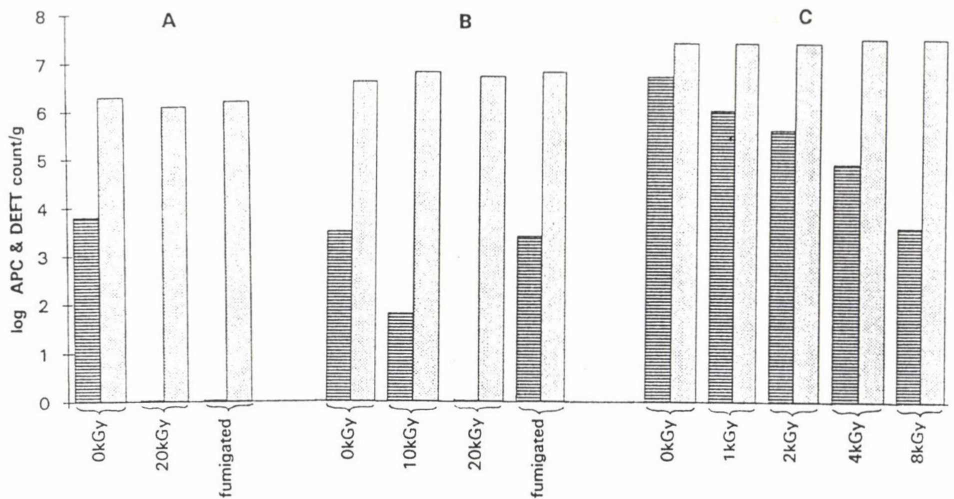

Fig. 1. Differences between DEFT (▢) and APC (▤) count in various spices. A: caraway, B: onion powder by DEFT method II, C paprika by DEFT method I

Another advantage of the method is that once membranes are mounted in immersion oil, preparations are stable and they could be stored at 5 °C for a period up to 2 months (WIRTANEN et al., 1993) and so can be used as references.

The DEFT method as described by PETTIPHER and co-workers (1985) for detection of moulds in tomato concentrates uses acridin orange to stain the mould mycelium. This method could be applied with difficulty for detection moulds in paprika powder because of the similar staining of the mould propagules and plant fragments (both fluoresce in green or orange). However, in the preparation of highly contaminated samples one can distinguish between them.

For staining mould, congo red was used by MATSUOKA and co-workers (1995). They reported that congo red strongly bound to chitin chains. This staining technique was carried out with dry mould biomass and different samples of paprika powder. Influence of contact time, concentration of the fluorochrome and type of washing with alcohol were examined. The visibility and quality of dry mould biomass preparations were very good. The hyphae of moulds fluoresced in red. The visibility of the mould in paprika preparations was independent of contact time and concentration of the dye. Recognition of hyphae was spoiled in less contaminated samples by the staining of the plant fragments, but distinction could be easily done in highly contaminated samples (Fig. 2). It was not significantly improved by increasing the amount of washing alcohol or changing the washing fluid.

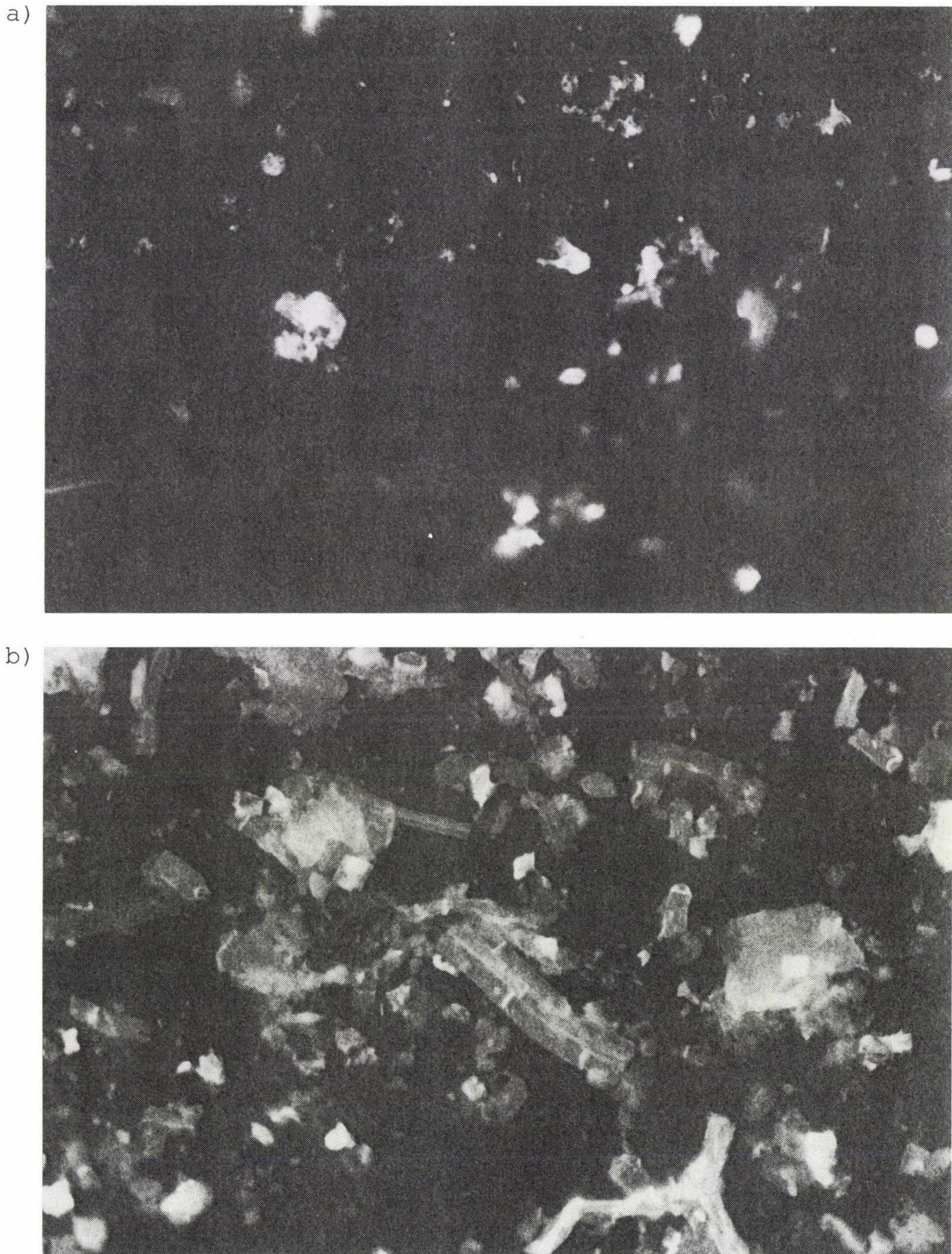

Fig. 2. View of a 10^2 CFU g^{-1} (a) and a 10^6 CFU g^{-1} (b) mouldy paprika sample stained with congo red. Magnification 1000X

The mould count was always higher (up to 2 log units) using this method than the count of CFU-s. This can be explained by saying that biomass is not equivalent with the viable mould count. It is a problem that using low dilution of the samples the visibiliby of the moulds is very poor because of the high amount of plant fragments. Removing these particles is not possible since using e.g. prefiltration or centrifugation moulds would also be removed from the sample suspension.

In microcolony epifluorescence microscopy the membranes were stained after incubation on agar medium and were also examined by epifluorescent microscope. Debris and mycelia on the filter surface fluoresce in yellow, green and mainly red or light red. Quality of view in stained preparation was very good.

Mould micro-colonies could be distinguished well. At 20X magnification branching of mycelia sometimes filled the whole view of microscope field, but smaller micro-colonies could easily be recognised (Fig. 3). The required dilution of samples to make suitably visible preparations for detection mould count in paprika powder was third decimal dilution level otherwise preparation of the samples would be crowded by too much debris. Consequently, with this method mould contamination only at level of 3 or higher log CFU g^{-1} can be detected.

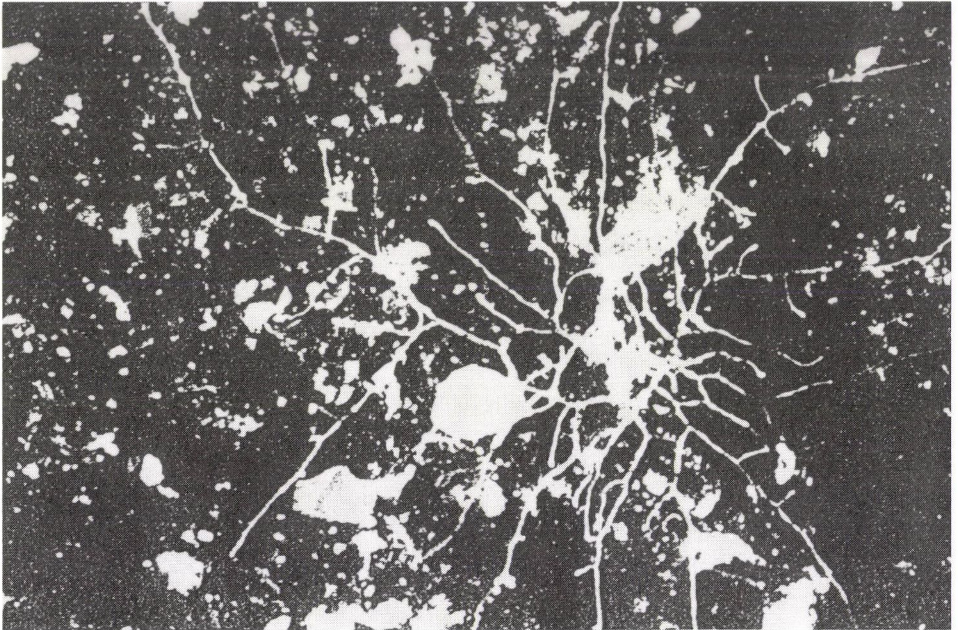

Fig. 3. View of a growing mould hypha on the stained membrane. Magnification 20X

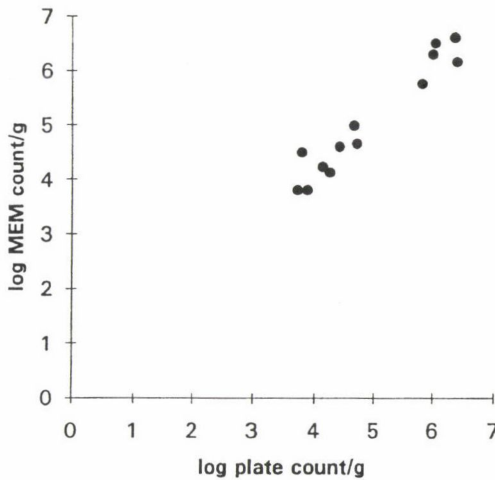

Fig. 4. Relationship between traditional plate counts and MEM counts of moulds in paprika powder

There was a good correlation between log plate count and log MEM count (R=0.96) (Fig. 4). In some cases the comparison was not possible because of overgrowing the plates by *Rhizopus/Mucor* groups. MEM determination of the number of moulds could be carried out within 25 h instead of 4–5 days incubation requirement for regular mould counting.

3. Conclusion

The DEFT count can be used for determination of total cell number giving a picture of the total microbiological contamination of spices and in this way offers information on the prehistory of the product. The method is suitable for screening to assess decontamination of the samples, although it can not distinguish between irradiation and fumigation treatment. DEFT preparations are stable and they can be stored so can be used later for comparison.

The DEFT was less suitable for direct enumeration of moulds in spices because of the problems of separating mycelium from samples and unreliable staining. Microcolony epifluorescence microscopy enables to determine viable mould count within 25 h instead of 4–5 days of traditional plate counting.

*

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ABSTRACTS
of paper presented at the
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30–31 May 1996
Budapest, Hungary

DIMENSIONS OF FOOD QUALITY AND SAFETY

P. A. BIACS

Central Food Research Institute, H–1022 Budapest, Herman Ottó út 15. Hungary

Qualitative and quantitative data of food production and consumption were different in the period of 1945–1995 years. Until 1960 the lack of food and malnutrition of the population in the European countries were dominant. After this period intensification of agricultural production in most of the countries brought success, until 1980 were established the conditions of preservation, storage and manufacture of food.

Most decisive steps were done in the years of 1980–1990 where food items rich in protein and in energy went to commerce. Our period after 1990 seems to be the decade of the micro-nutrients, where food and drink enriched with vitamins and essential metals are manufactured and licensed from nutrition aspects.

The marketability of food as trade product is equally based on safety aspects described by institutional control requirements and on conformity of existing standards determined by the competition on the market. Safety and quality are functioning together in a strong coexistence, however tendencies are visible to subordinate each other both in theory and in practice. The safety concern of consumers is a regulatory task of the state and effective inspection is requested by control institutions of public health, plant protection, animal health or consumer's protection. More intensive

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involvement of interest-organisations is expected now for strengthening market-oriented economy, whereas associations of food manufacturers and other employee representatives and federations should request supply of raw and processed food free of chemical residues, toxins and radioactive contamination. The chemical, biological and physical contamination level should not exceed the accepted daily intake limitation. Unavoidable contact with packaging material, technofunctional use of additives are new sources of contamination, but up-to-date food processing technology can reduce this harmful effect. The demand of consumer for organic grown bio-food could not be neglected today with respect to environmentally sound production and restricted regional application in Hungary.

The transformation of food regulation and its administration should not be delayed for long, where special aspects of European harmonisation must be respected. Privatisation and accreditation of food control laboratories seem to be an important milestone of the road to EU.

THE TRACE ELEMENT CONTENT OF THE FOODS AND THE HUMAN HEALTH

I. PAIS

University of Horticulture and Food Science, H–1118 Budapest, Villányi út 35. Hungary

From the 88 permanent elements composing the Earth, 76 elements may be regarded as physiologically important constituents of our planet. As the Fig. 1 shows, we have enough knowledge on the 11 macroelements and the 12 essential trace elements. The great liability of the science is that our knowledge on 53 trace elements, which compose the lower part of the iceberg, is far from satisfying.

This last fact means a great danger to human health, because these elements are or may be the member of the general food chain, and they can have a fatal effect to human health being in deficiency, or in superflous quantity. One of our important task is to know more and more from their additional physiological role using the interdisciplinary scientific cooperation.

Very important additional factors of the food-research are the speciation-analysis and the search for free-radical processes in order to understand the mechanism of some peroxidative diseases.

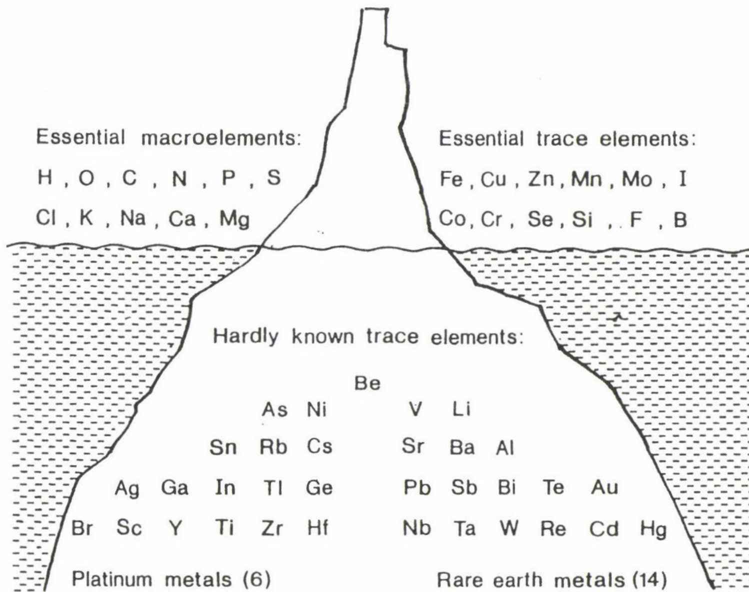

Fig. 1.

Nowadays one of the greatest problem is the supplementation of foods or our daily diet with additional trace elements. The author is convinced – in spite of that these processes may have dangerous consequences, too – we can find the way of thoughtful trace-element addition: the methods to ensure a well-balanced trace-element supplementation. The "optimum-supplementation" can ensure the physiological conditions for a nutrition, and at the same time avoid the deficiency and toxicity state, as well.

STUDY OF THE MECHANISM OF PLANT LECTIN TOXICITY

É. GELENCSÉR

Central Food Research Institute, H–1111 Budapest, Budafoki út 59. Hungary

The inclusion of raw legumes in the diet of humans and monogastric animals has long been known to cause toxicity of varying severity. Most of these effects are caused by seed lectins.

Depending on their carbohydrate specificity, lectins recognize and bind to epithelial membrane receptors of brush border cells for both endogenous and exogenous luminal factors. Soya bean agglutinin, SBA (Glycine max) induces hyperplastic growth of the small intestine and also has other deleterious effects on digestion and absorption. Furthermore, the presence of functionally active trypsin inhibitors may reduce the digestibility of proteins including SBA, in the diet. Gut-associated or humoral immune responses to dietary antigens including antinutrients can either reinforce or suppress toxic reactions to soya and other legume components. However the precise mechanism of lectin toxicity is not fully understood.

The main tasks of our work were to establish whether these antinutrients were stable under normal feeding practices and capable of interfering with the proper functioning of the digestive system. Thus, it was shown that although the potentially harmful consequences of exposing rats to antinutrient-containing diets could be minimised by periodically switching their diet between SBA-containing and SBA-free, control diets, the nutritional performance of rats on the SBA-diets was inferior to that of rats pair-fed isonitrogenous and isocaloric control diets. As SBA, but not the trypsin inhibitors, was shown to have survived the passage through the gut and bound to the intestinal epithelium to the same extent as on acute exposure suggests that SBA may indeed be the primary reason for the poor nutritional value of soya diets. As SBA recognises and binds to N-acetylgalactosamine (galactose terminals of epithelial membrane glycans, some of which are receptors of endogenous or exogenous growth) metabolic factors for brush border cells, by interacting with these receptors, SBA can modify the structure and metabolism of the rat gut, interfere with the digestion and absorption of nutrients modulate hormone and immune functions of the digestive tract and induce intestinal growth and malabsorption of dietary components leading to nutrient deficiency. The edocytosed and transcytosed immunoreactive compounds induce local and humoral immunoresponses to luminal SBALB antigens.

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This work is a part of the EU COST 98 Action. The financial support of Ministry of Agriculture (Hungary) and OTKA (Hungarian Academy of Science) and SOAFD (Rowett Research Institute, Aberdeen, Scotland) is greatly appreciated.

OXIDATION OF CHOLESTEROLS DURING IRRADIATION TREATMENT AND FROZEN STORAGE OF MECHANICALLY SEPARATED MINCED TURKEY MEAT

V. K. LEBOVICS[a], Ö. GAÁL[a], K. KORÁNY[b], L. SOMOGYI[c] and J. FARKAS[c]

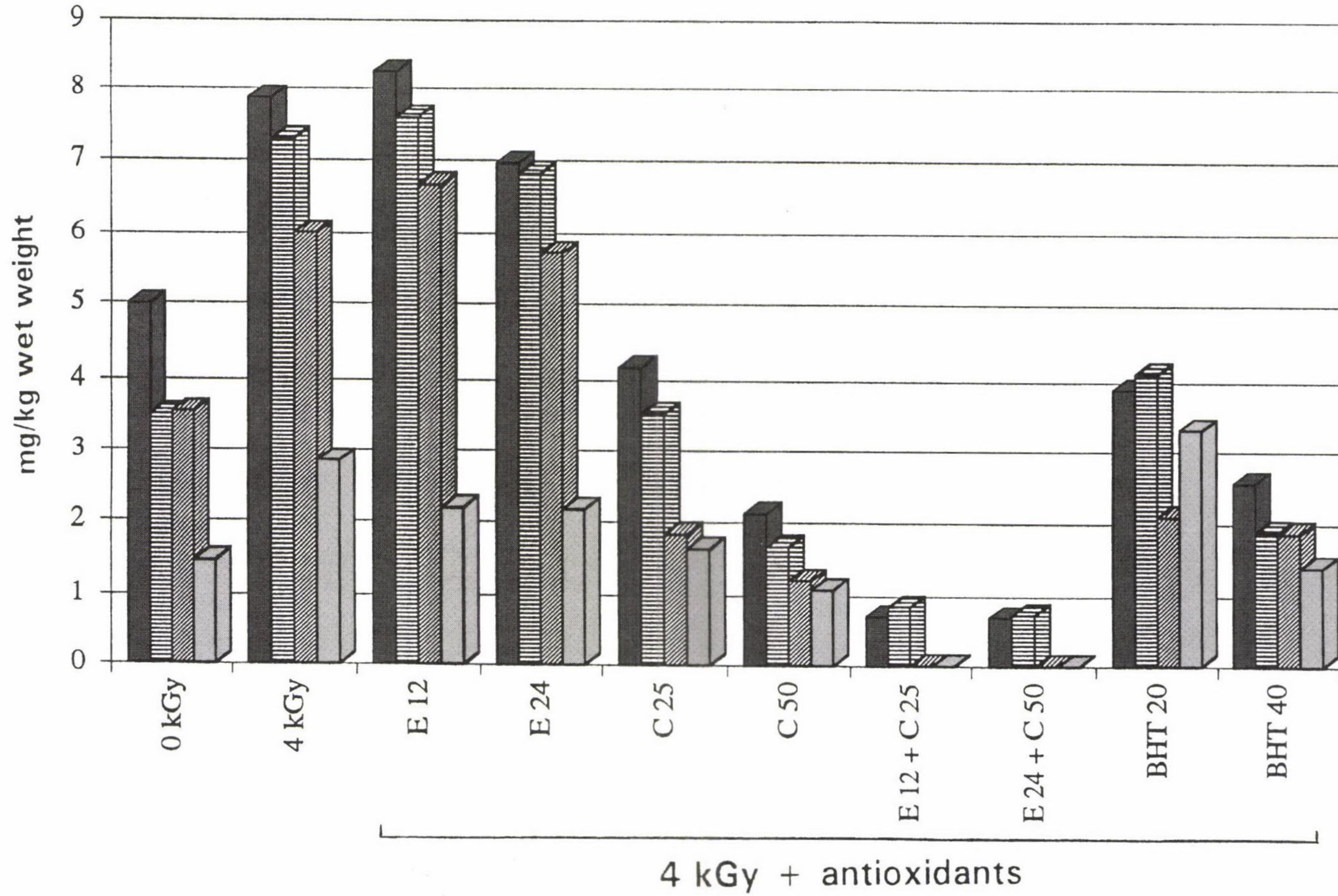
[a] National Institute of Food Hygiene and Nutrition, H–1097 Budapest, Gyáli út 3/a. Hungary
[b] University of Horticulture and Food Industry, Department of Refrigeration and Livestock Products Technology, H–1118 Budapest, Ménesi út 45. Hungary
[c] University of Horticulture and Food Industry, Department of Food Chemistry and Nutrition, H–1118 Budapest, Somlói út 14–16. Hungary

Mechanically separated poultry meat is an important raw material in the industrial meat processing, which is mainly used in Bologna type sausages. During deboning and mincing the increased oxygen uptake could initiate the lipid oxidation due to the relatively high lipid content of the product. Radiation treatment has some application potential for elimination of non-spore forming pathogenic bacteria but it could also accelerate some free radical processes such as lipid-oxidation.

The purpose of this work was the study the effects of various antioxidants to prevent cholesterol oxidation caused by irradiation and storage of mechanically separated turkey meat.

In model experiments the back-bones meat of turkies were used. The samples were treated with gamma irradiation dosage level of 4 kGy, wrapped in air-packed Multiseven-80 laminated PE-PA foil packages at –18 °C in the presence of the following antioxidants: 12 and 24 mg α-tocopherol /E 12, E 24/; 20 and 40 mg BHT BHT 20, BHT 40/; 25 and 50 mg ascorbic acid /C 25, C 50/; 12 mg and α-tocopherol + 25 mg ascorbic acid /E 12 + C 25/; 24 mg α-tocopherol + 50 mg ascorbic acid /E 24 + C 50/ in 100–100 g minced meat. Measurements were also performed after 4 months storage at –18 °C with non-irradiated and irradiated samples. The non-saponifiable lipid fraction extracted from the meat contained cholesterol and also its oxidation products were separated by TLC. The fractionated components scraped from the silicagel were measured spectrophotometrically based on enzymatic method. The peroxide value of the extracted total lipids were also determined.

The results showed that 4 kGy dose level without addition of antioxidants initiated the oxidation of cholesterol. 7-hydroxycholesterol (α and β isomers), 7-ketocholesterol, cholesterol-5α, 6α-epoxide were formed. The quantity of these compounds considerably increased during 4 months storage at –18 °C. α-tocopherol was practically uneffective in suppression of cholesterol oxidation. BHT and ascorbic acid decreased the formation of predominant 7-hydroxycholesterol isomers by 50–70%



cholesterol content of the product: 368 mg/kg

Fig. 1. Formation of cholesterol oxides in mechanically deboned minced turkey meat during 4 kGy irradiation and frozen storage in the presence of antioxidants (4 months storage, –18 °C). ⊠: 7 alpha; ⊟: 7 beta; ▨: 7 keto; □: epoxide

and the combination of α-tocopherol and ascorbic acid by 80–90%. The antioxidant combinations used during gamma-irradiation and storage proved to be so effective that 7-ketocholesterol and cholesterol-5α, 6α-epoxide were not formed in detectable quantity. Changes in the levels of oxidized cholesterol derivatives during irradiation and storage are shown in Fig. 1. The tendency of increase of peroxide values (PV) ond oxidized cholesterol levels were similar. The PV of the untreated samples at the beginning of storage were 1.5 and after 4 months 11 meq peroxide/kg respectively. The PV of the irradiated minced turkey samples without presence of antioxidants were 1.8 meq peroxide/kg at the beginning, raised to 31.5 meq peroxide/kg during 4 month.

*

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FOOD PROTEIN ALLERGENS

GY. HAJÓS,[a] E. SZERDAHELYI,[b] É. GELENCSÉR[a] and M. POLGÁR[b]

[a] Central Food Research Institute, H–1022 Budapest, Herman O. út 15. Hungary
[b] Madarász Children Hospital, H–1188 Budapest, Madarász V. u. 22/24. Hungary

Food allergy has been known for about two and a half thousand years. More than four hundred years before Christ Hippocrates recorded the observation that "milk could cause special illnesses". Four hundred years later Lucretius stated, that "one man's food may be another man's poison".

Food allergy is an adverse reaction to a food or food component (mainly a protein) involving reactions of the body's immune system.

Patients suffering from food allergy have to avoid the allergenic components, they need hypoallergenic foods. Proteins of several foods have been identified as common allergens such as: milk, legumes, eggs, cereals, seafood etc. However, many patients suffering from a given food protein allergy, are often sensitive also to other food proteins.

The identification of food allergens is mainly based on clinical observations before and after administration of an allergen-free diet to patients suffering from a given allergy. Because of its absence in human milk β-lactoglobulin is considered to be one of the major allergenic proteins of cow's milk. Matsuda and Nakamura summarize the major food allergens along with their structural and immunological properties. According to their collection, potent allergens in cow's milk area: α_{S1}-casein (23 KDa), β-lactoglobulin (18 KDa) and Maillard adducts (amino-carbonyl reaction products between lactose and protein amino groups). The egg white's potent allergens are:

ovalbumin (43 KDa) and ovomucoid (28 KDa). Soybean has also several potential allergenic proteins such as glicinin (320–360 KDa), 2S-globulin (a mixture of low molecular weight proteins including trypsin inhibitors), and a 32 KDa allergen. There is a 16 KDa allergen in rice (belonging to the amylase/trypsin inhibitor family).

It is also concluded that no common universal structural characteristic of the allergenic molecules has been found so far.

The allergenic cross-reactivity of some food proteins were investigated in vitro by immuno-electrophoretic methods using sera of cow's milk allergy patients.

On the basis of our results, goat- and sheep milk and the products of sheep and goat milk showed strong cross-reactivity with sera of patients suffering from cow's milk allergy. Among the meat samples investigated, beef and pork samples showed moderate cross-reactivity, the samples originated from lamb and poultry can be recommended for cow's milk allergy patients.

SIMULTANEOUS HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF POSSIBLE NITRITE AND FORMALDEHYDE CONTAMINATION IN FOODS

A. Csiba, M. Szentgyörgyi, S. Juhász, M. Csikor and Gy. Lombai

Veterinary and Food Control Station, H–1135 Budapest, Lehel u. 43–47. Hungary

Hydralazine, used as a depressor of hypertension as well, reacts with nitrite to give tetrazolo (5,1–a) phtalazine and with aliphatic aldehydes to give triazolo (3,4–α) phtalazine (Tri–P) under acidic conditions. Hydralazin used in excess is suitable for simultaneous determination of the adduct(s) of nitrite and/or aldehyde.

Nitrite and aldehyde derivatives were chromatographied directly in reverse phase system. Mixture of acetonitrile – 0.05 mol l^{-1} KH_2PO_4 (30:70) served as mobile phase, executing the detection fluorimetrically at 237 nm excitation, 370 nm emission. The samples were prepared by Carrez I and II solutions. The hydralazine reaction was carried out under acidic condition (0.1 mol l^{-1} HCl) in boiling water bath for 15 min. The method was calibrated and applied for the determination of nitrite and formaldehyde concentration of some food basic materials and products.

The detection limits are 0.1 ng/g for sodium nitrite and 0.5 ng/g for formaldehyde. The perceptible limit for the above mentioned compounds are 3.0 ng/g and 5.0 ng/g, respectively. The recovery is 95±2% in a range of 20–200 ng/g concentration. The daily repeatability at the determination of 10.0 µg/l sodium nitrite and/or formaldehyde can be followed only by 0.20% standard error.

SAPROPHYTIC YEASTS IN HUMAN INFECTIONS, PATHOGENIC YEASTS IN FOODS – AN EMERGING POTENTIAL RISK

T. DEÁK

Department of Microbiology and Biotechnology, University of Horticulture and Food Science, H–1118 Budapest, Somlói út 14–16. Hungary

Yeasts are usually considered as harmless saprophytic microorganisms some species of which have become domesticated for long and are now indispensible for the production of bread, wine and beer. These are of great economic importance. Over 600 species of yeasts have been described so far and only a few have been shown to cause disease in humans such as *Candida albicans*, *Cryptococcus neoformans*, *Trichosporon beigelii*, *Geotrichum capitatum* and *Malassezia furfur*.

In the past decades the spectrum of yeasts isolated from clinical cases increased dramatically. Many yeast species previously thought innocuous can accompany underlying diseases and turn opportunistic pathogen capable of damaging the human body. Many of the emerging yeast pathogens attack the bloodstream causing fungemia, occasionally, however, cause disease at sites in the body other than blood such as mucous membranes, gastrointestinal tract and vagina. The increase of yeast infections can be ascribed to many factors such as the use of immunosuppressive therapeuticals, antibacterial and antifungal antibiotics or long-term intravenous catheterization.

Most yeasts isolated from clinical materials are identified as members of the anamorphic genus *Candida*. However, *Candida* species may correspond to various teleomorphic forms in diverse taxonomic genera among them *Pichia*, *Kluyveromyces*, *Issatchenkia*, *Clavispora*, *Metschnikowia*, and even *Saccharomyces*.

From epidemiological point of view, it is striking that most emerging yeast pathogens normally occur in the environment and as common food-borne species. Many are known as frequent cause of food spoilage. In turn, genuine pathogenic yeasts have been isolated from various foods, mainly from products treated or handled under inferior hygienic conditions.

The emerging and increasing importance of yeasts in human diseases should not be interpreted that once innocuous species changed to be pathogenic. The diverse physiological properties of yeasts and their common occurrence in the environment and in our foods accompanying with certain predisposing factors may open the door for them to behave as opportunistic pathogens. Physicians, clinical and food microbiologists should be aware of the potential risk these yeasts may pose.

FOOD-BORNE DISEASES AND CONCLUSIONS

G. BIRÓ[a], J. SIMON[b] and GY. BIRÓ[b]

[a] University of Veterinary Science, Institute for Food Hygiene, H–1078 Budapest, István út 2. Hungary
[b] National Institute of Food Hygiene and Nutrition Science, H–1097 Budapest, Gyáli út 3/a. Hungary

Summarising the number of cases and tendency of food-borne infections and intoxications over 40 years, indicates a steady level of 4–5 thousands per year. In the middle of sixties and by the end of eighties a higher number of cases 6–7 thousands could be observed. Distribution of pathogens has shown a homogenous pattern for the last ten years with growing tendency of the infections by *Salmonella.*

The ratio of *Salmonella* among the pathogens identified in laboratories was consequently 50% and that were followed by *Staphylococcus aureus* on the second place and *Bacillus cereus*, *Streptococcus faecium* and *Campylobacter jejuni*, respectively. The number of epidemics and related events and cases, percentage of diseases due to *Salmonella* has been gradually grown in the last ten years. It is known that the occurrence of *Salmonella enteritidis* has become determinant since 1986. Most frequent transmitters are the meat products but the role of egg products has apparently increased since 1992. The Salmonella transmitter role of poultry meat manifested mainly in the households.

The infections caused by *Campylobacter jejuni* and *-coli* were significant in the past ten years. Examinations proved that *Salmonella* and *Campylobacter* occur together in the intestinal tract of poultry.

The botulism occurs almost every year in our country. Typically, the food is the transmitter of epidemics caused by *Shigella sonnei.* Transmitters of infections are the milk, the cottage cheese, mainly from sheep, noodles with cottage cheese and occasionally, other milk products.

Hungarian examinations pointed to the infections of meat products by *Listeria.*

We have to call the attention to the importance of training and information of people working in food processing, trading and culinary fields. They have important role in prevention of food-borne diseases.

USE OF Z-AGAR FOR THE ISOLATION OF *PSEUDOMONAS AERUGINOSA* AND OTHER *PSEUDOMONAS* STRAINS FROM FOOD

R. KISS[a] and G. SZITA[b]

[a] National Institute of Food-Hygiene and Nutrition, H-1097 Budapest, Gyáli út 3/a. Hungary
[b] University of Veterinary Sciences, Budapest, H-1078 István u. 2. Hungary

Besides the well known *Salmonella*, *Shigella*, *Yersinia*, *Escherichia coli*, *Campylobacter*, and other enteropathogenic bacteria also *Pseudomonas* strains may cause enteric diseases.

In food processing environments, *Pseudomonas* can contaminate the surfaces – being ubiquitous microbe proliferate and biofilms may form.

In 1991 we had 2 familial *Pseudomonas aeruginosa* outbreaks caused by eating foods prepared with meat and mushroom components.

In 1993 there was an other outbreak caused by *Pseudomonas aeruginosa* contaminated food, too. In this case 174 children had enterocolitis.

Using the HACCP system – in the course of food examinations and the control of environment, isolation of facultative pathogenic *Pseudomonas* strains is improved by the use of Z-agar.

Z-agar is a minimum medium – contains acetamide as sole carbon source. As *Enterobacteriaceae* can not use acetamide as carbon source, we introduced the medium for the selective isolation of *Pseudomonas* from contaminated food.

For controlling selectivity of this medium, the following oxidase positive and oxidase negative bacteria were cultured on Z-agar *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloaceae*, *Citrobacter freundii*, *Proteus vulgaris*, *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Aeromonas caviae*, *Aeromonas veronii*, *Aeromonas schubertii*.

Incubation was performed at 37 °C for 48 h. The above listed bacteria did not form colonies on Z-agar.

In the course of our examinations, parallel to Z-agar the following media were used: GSP agar – *Pseudomonas*, *Aeromonas* medium – (FLUKA), Eozin-methylenene blue agar, Brillant-green agar, and blood agar.

After 24 h incubation *Pseudomonas* sp. form small, circular, round colonies on Z-agar.

The *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Pseudomonas syringae*, *Pseudomonas fluorescens* show fluorescence in UV light – after 48 h, also the medium itself becomes green.

In the course of the examination from samples of sea and fresh water fish, crustaceans, polyp, meat, poultry, sheep's cottage cheese, deep-frozen vegetables, lettuce prepared with dried fruits, spices, dried fruit the following strains were isolated:

Pseudomonas aeruginosa (41),
Pseudomonas diminuta (1),
Pseudomonas fluorescens (3),
Pseudomonas mendocina (4)
Pseudomonas putida (3),
Pseudomonas stutzeri (1)

According to our results, using Z-agar together with the GSP-agar – in comparison with other media – positivity ratio increased by 20%.

On other media, *Pseudomonas* colonies were often covered by colonies of other microbes or hemolysis disturbed the differentiation of the colonies, thus false negativity was observed.

Z-agar also helped the differentiation of *Pseudomonas* and *Aeromonas* strains, because of the parallel use of GSP-agar. Thus, from 5 samples, isolation of both species was successful.

The use of the Z-agar provides considerable benefits.

We wish to complete our examinations with searching for the new food-borne pathogen *Shewanella putrefeciens* and *Shewanella alga* strains, because they earlier belonged to *Pseudomonadaceae* and, may be, they can also grow on this medium.

EFFECT OF COMBINED TREATMENTS ON THE *BACILLUS CEREUS* SPORES

I. VIDÁCS, J. BECZNER, E. N. SZERDAHELYI and GY. HAJÓS

Central Food Research Institute, H–1022 Budapest, Herman Ottó út 15. Hungary

The aim of combined treatments is the adequate reduction of the number of spore-forming bacteria with simultaneous (or subsequent) application of otherwise not effective doses to ensure safety. The industry requires fast feed-back, therefore rapid microbiological methods get in the foreground. However, cells injured as a consequence of treatments might arise unexpected problems using rapid methods for determination of cell counts.

The number of *Bacillus cereus* T spores surviving the combined treatments (heat, irradiation) was determined by impedance measurement (MALTHUS instrument) and traditional plate count technique, and the differences between the results are discussed.

To study the effect of treatments on the proteins of spores, SDS-PAGE was used. The heat-treatment decreased the amount of the largest protein band of spores as a function of heat-treatment time in absolute value, however not relative to the other protein fractions.

PROTEIN COMPOSITION OF PEA VARIETIES CULTIVATED IN HUNGARY

I. SCHUSTER-GAJZÁGÓ

Central Food Research Institute, H–1022 Budapest, Herman O. út 15. Hungary

Dry peas are important source of protein for animal feed as well as possible raw material for processing into human food. This protein source has been studied from many aspects in European countries, but about pea varieties cultivated in Hungary there are no data in the Hungarian literature.

Pea proteins are composed of several thousand specific protein. About 70–80% of the crude protein in legume seeds is storage protein. The nonstorage proteins are enzymes, enzyme inhibitors, hormones, transporting, structural and recognition proteins.

The main protein fractions of legume seeds are albumins and globulins which can be separated into two major fractions, vicilin and legumin. The pea albumin has a well balanced amino acid profile and is relativelly rich in S-amino acids. The two globulin fractions differ in their amino acid composition and their physico-chemical properties.

High variability of pea protein composition has often been reported and related to genetic characteristics and environmental factors.

The nutritional value of pea proteins is determined by its albumin and globulin content, and proportion of vicilin because of its low sulphur amino acid content.

The functional properties of pea proteins for food industry using purified globulins or isolates have also been related to the vicilin-legumin ratio.

In the course of our research work 5 pea varieties were examined, determining the protein content, albumin and globulin as well as the legumin and vicilin content and their ratios. The amino acid content of protein fractions was also determined. Protease inhibitors, trypsin- and α-chymotrypsin inhibitor activity of pea varieties were measured.

The pea varieties (Junak, Hunor, Türkis, UM–1073, UM 1095) were supplied by Crops and Vegetable Institute, Újfehértó, Hungary.

The protein content of pea varied between 25–27%. The protein was extracted by 0.1 M, pH 7.0 phosphate buffer according to Gueguen. The buffer solubility of protein

of pea varieties ranged from 80 to 90%. 17–20% of buffer soluble protein was albumin and 55–69% was globulin depending on the variety. The ratio of the main protein fractions varied between 3.1 and 3.9.

The legumin content of varieties showed high variation, 49–67% of globulin was determined as legumin, the vicilin fraction of globulin ranged from 24 to 29%. The globulin contained 1.9–2.8 times more legumin than vicilin.

The albumin fraction differed in its amino acid composition from globulin. The albumin fraction contained significantly more THR, GLY, ALA, TYR, LYS, PRO and less GLU and ARG than globulin.

The pea varieties differed in their protease inhibitor activity (2.0–5.7 TIU mg^{-1}), the trypsin inhibitor activity of varieties was about two times higher than the α-chymotrypsin inhibitor activity. A significant linear relationship was established between trypsin- and α-chymotrypsin inhibitor activity of pea varieties. The protease inhibitors were determined only in the albumin fraction, though from nutritional point of view the amino acid composition of albumin is very advantageous but its protease inhibitor content reduces the nutritional value of it.

IMPORTANCE OF THE MICROBIOLOGICAL RESEARCH IN THE QUALITY CONTROL FOR FOODS

I. FÁBRI

FAO/WHO Hungarian Codex Committee on Food Hygiene, H–1125 Budapest, Diósárok u. 12/b. Hungary

The "Guidelines of the Application of HACCP system" to ensure the safety of food was edited by FAO/WHO Codex Alimentarius Commission on 1993 and its adoption in the food industry is mandatory in the European Union and USA. The essential basic implementation of HACCP is the application of the "General Principles of Food Hygiene" worked out also by the FAO/WHO Codex Alimentarius Commission.

It is obvius that the pathogenic and spoilage microorganisms are the most important hazardous factors threatening the food safety and stability, its suitability for consumption. To prevent the harmful life – functions of the microbes require a thorough research work in the field of microbial- ecology, biotechnology, gene-technique etc. moreover the development of rapid methods etc.

The basis of these microbiological work is given in Hungary for decades, the next task is the continouos development.

ANTIOXIDANT AND FREE RADICAL SCAVENGING PROPERTIES OF POLYPHENOLIC COMPOUNDS OF PLANT ORIGIN

A. LUGASI, E. DWORSCHÁK and J. HÓVÁRI

National Institute of Food Hygiene and Nutrition, H–1097 Budapest, Gyáli út 3/a. Hungary

Natural antioxidants are primarily plant polyphenolic compounds that may occur in all parts of the plant. In particular flavonoids, phenolic acids and their derivatives have shown marked antioxidant activity. The phenolic compounds are the secondary products of plant metabolism, their main role is to prevent the plant cell structure against oxidative degradation caused by UV light and to inhibit the deterioration by plant pathogenes. In the last decade the antioxidant and free radical scavenging properties of several plant polyphenols have been proved in in vitro and in vivo systems, as well. There is no doubt about that to establish the antioxidant and free radical scavenging activity of plant products intended to use either as food supplementation or as medicine is essential for their safe application.

Authors have worked out a complex test system for description of the antioxidant and free radical scavenging character of pure phenolic compounds, plant extracts, new food products with supposed antioxidant activity, and drugs intended to use for medical treatment in several diseases where reactive oxygen species are involved. The following methods were used: reducing power according to the Fe^{3+}/Fe^{2+} transformation, H-donating ability in the presence of 1,1-diphenyl-2-picrylhydrazyl free radical, ferrous and copper ion chelating activity with the use of tetramethylmurexide, inhibition of lipid peroxidation in β-carotene/linoleic acid system according to blenching of β-carotene and with thiocyanate method. All previous measurements were carried out spectrophotometrically. Scavenging activities of the polyphenolic compounds on H_2O_2 and superoxide radical formed in adrenalin/adrenochrome system were determined by chemiluminescence method. With the use of this system it is possible to compare the in vitro effect of molecules with different structures, well-known and new antioxidant components, natural and synthetical antioxidants, and useful information are given about how to manage experiments to know the in vivo effects of these compounds.

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A COMPARATIVE STUDY OF THE QUALITY PARAMETERS OF TOMATOES GROWN IN SOILLESS CULTURE AND IN GLASSHOUSE SOIL

M. GILINGER-PANKOTAI,[a] J. FELFÖLDI[b] and A. FORRAY[c]

[a] University of Horticulture and Food Industry, Department of Vegetable Growing,
H–1118 Budapest, Villányi út 35–43. Hungary
[b] University of Horticulture and Food Industry, Department of Physics and Control,
H–1118 Budapest, Villányi út 35–43. Hungary
[c] Flóratom Ltd., Szeged. Hungary

The tomato is one of the favourite vegetables in our country. It is demanded as an out-of-season article in winter and spring, too. The consumption mainly depends on the considerably fluctuating price. It is generally believed that eating tomatoes in winter is very healthy. The vegetable supply is rather poor in the winter season and tomatoes rank first among the crops regarding its food value.

However, because of inadequate information, there is a widely held view that tomatoes from forcing are poisoned with different chemicals, they have a watery taste and contain useful ingredients next to nothing. Particularly, Dutch tomatoes are branded like this. Recently, soilless hydroculture has spread on farm scale in Hungary, too. The aversion to this technology is especially strong and there is much less correct information available.

For protecting Hungarian tomato forcing and for supporting the new technologies, the authors have carried out several series of examinations. They were aimed at the objective demonstration of the seasonal quality trends in market tomatoes grown by different technologies. A comparison with foreign (Dutch, Spanish, Italian) tomatoes has been made, too. The differences in the quality parameters (nutritive value, sugar, vitamin C, mineral elements, nitrate, chemical residues) of tomatoes grown in soil as compared to tomatoes from soilless culture will be demonstrated. Emphasis will be laid on the differences in keeping quality.

THE EFFECT OF PREPARATION, PROCESSING AND PRESERVATION ON SOME NUTRIENTS OF CARROT

Á. PARRAG-SALLAI, I. VARSÁNYI, É. SZÁNTÓ-NÉMETH and A. KÖRMENDY-DOMJÁN

University of Horticulture and Food Industry, Faculty of Food Industry, Department of Canning Technology, H–1118 Budapest, Ménesi út 45. Hungary

Food habits have changed a lot during the past decades. The demands of consumers are even greater of food quality, they look for more "healthy foods". For these requirements the food producers wish to develop special technologies to improve the nutritive value of foods avoiding the nutrient losses.

The objectives of our experiments performed were to investigate the effect of preparation, processing and preservation on nutritive components of fruits and vegetables in laboratory scale. As model foods peach, sour cherry, carrot and pepper (*Capsicum annuum* var. *pomiferum*) were chosen and we wish to introduce our results obtained by carrots.

Two carrot varieties, NANTI and BOLERO bred at Research Station of Tordas were studied. First carrot pulp was prepared in our laboratory and then juice. We have measured β-carotene, dietary fibre and mineral element content as a function of the main steps of technology: washing, peeling, cutting, blanching (3 min, 100 °C), chopping, finely, heat-treating (20 min, 100 °C).

The β-carotene content of carrot was 15.5±0.66 mg/100 g (NANTI, N) and 20.5 ±0.64 mg/100 g (BOLERO, B). After vapour blanching it changed to 14.7±0.75 mg/100 g (N), 18.2±0.66 mg/100 g (B), that means 4.5–12.0% loss. Heat-treated carrot pulp had 13.17±0.78 mg/100 g (N) and 18.0±1.44 mg/100 g (B) β-carotene, and that of carrot juice was 3.9±0.34 mg/100 g (N) and 5.9±0.23 mg/100 g (B). Carrot juice from blanched carrots looks a good source of β-carotene.

The dietary fibre content of carrot was 3.9±0.34 mg/100 g (N) and 3.7±0.05 mg/100 g (B). The heat-treated pulp contained 3.8±0.04 mg/100 g (N) and 3.6±0.13 mg/100 g (B). It maybe stated that heat-treatment did not cause significant losses neither in dietary fibre nor in mineral content.

SELENIUM-ENRICHED BREAD

I. SARUDI[a], ZS. LASSU-MERÉNYI[a], GY. RUMI[b] and J. KELEMEN[a]

[a] Faculty of Animal Science, PANNON Agricultural University, H–7400 Kaposvár, P.O. Box 16. Hungary
[b] Kaposi Mór County Hospital, H–7400 Kaposvár, Tallián Gy. u. 29–34. Hungary

In the present study *Saccharomyces cerevisiae* grown on a specific culture medium, containing ca. 1 μg per g selenium, was used by itself or in bread. Selenium in various samples was determined by the fluorometric method recommended by HOFFMAN and co-workers (1968).

In the first case, the yeast mentioned above and sodium selenite as selenium sources were compared in pigs each weighing ca. 85 kg at the start of the trial. In both cases the trials were performed at a selenium intake level of 1 mg/day/animal. After feeding selenium supplement for 40 days, the animals were killed and different organs were analysed for selenium content. It was found that skeletal muscle, cardiac muscle and liver accumulated significantly more "bioselenium" than inorganic selenium ($p<0.05$).

Afterwards bread produced with selenium-enriched yeast was given to ten volunteers (40 μg Se per 100 g bread). The daily bread ration contained approximately 100 μg of selenium. The subjects' mean whole blood selenium concentration (52 ± 16.7 μg/l) was indicative of a suboptimal selenium status. After two weeks of supplementation, the subjects' mean whole blood selenium level increased significantly (to 66.1 ± 11.9 μg/l). The relative increase of selenium concentration negatively correlated with selenium concentration before supplementation ($r=-0.711$).

Consumption of selenium-enriched bread seems to be an easily implementable and promptly acting method of selenium status improvement, but for long-term or wide supplementation we recommend bread with a lower selenium content.

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DEVELOPMENT OF DIETETIC AND "LIGHT" MEAT PRODUCTS IN VIEW OF HEALTH POLICY

É. RÉKASI and A. NAGY

Hungarian Meat Research Institute, H–1097 Budapest, Gubacsi út 6/b. Hungary

The study is dealing with the development of dietetic and "light" products for the purpose of preventing serious diseases which a large group of Hungarian population is suffering from.

Four product groups were choosen: cooked sausage type product, liver sausage, cooked and cured ham and semi dry sausage.

The aim of the technology-development was either to reduce fat and cholesterol content and to add some dietary fibre and essential amino acids to cooked and liver sausages, or to reduce sodium-chloride content in cooked, cured ham products and to reduce fat and sodium-chloride content or to add dietary fibre into semi dry sausage.

Preliminary study was made with the help of model experiments, and the results got there were used to develop the meat products.

The products and their characteristics are listed below:

Dietetic cooked sausage: reduced in calorie, fat and cholesterol content, contains some added dietary fibre. Fat was replaced by carbohydrate based material, which is inexpensive and has good water binding ability and nutritional advantages. The taste, colour and texture of the product are favourable.

"Light" cooked sausage: comparing to the traditional product it is reduced in calorie, sodium-content and contains much less fat. The sensory attributes of the product are fairly good.

Dietetic liver sausage: reduced in calorie, fat and cholesterol content, contains some added dietary fiber and essential fatty acids. The taste, colour and texture of the product are favourable.

"Light" liver sausage: reduced in calorie and salt content, contains much less fat and cholesterol than the traditional products.

"Light" ham products: reduced in salt content by 30 and 50%, are produced with 40% brine.

"Light" dry sausage: comparing to the traditional products it contains much less fat and salt. The fat was replaced by textured soy protein and rye flake.

INVESTIGATION OF UTILIZATION OF MÜSLI-MIXTURE MINERALS

P. TOLNAY, E. SIMON and M. HARKAY-VINKLER

University of Horticulture and Food Industry Department of Food Chemistry and Nutrition, H-1118 Budapest, Somlói út 14-16. Hungary

The results of the investigations regarding cereal flakes and müsli-mixtures have been shown. Investigations aimed at the most important essential mineral components (Ca, Mg, Cu, Zn, Fe) and their inhibitors (phytic acid, phytate, fibers). The mineral composition has been measured by ICP, phytic acid and phytate by an indirect method elaborated by the authors.

The ratio of the utilization of mineral components of müzli-mixtures and cereal flakes calculated by estimation has been shown and the role of these foods supplying the human body with minerals and fibers has been discussed. It can be established, that the 300 g consumption of these meals covers the 1–18% of the daily requirement of the minerals and 50–65% of the fibers.

The authors emphasised the importance of the proper nutrition and the disadvantages of the one-sided consumption.

INVESTIGATION OF GLIADIN CONTENT OF CEREALS AND DIFFERENT PLANT SEEDS*

E. AUBRECHT[a], M. HORACSEK[b], É. GELENCSÉR[a] and E. DWORSCHÁK[b]

[a] Central Food Research Institute, H-1022 Budapest, Herman O. út 15. Hungary
[b] National Institute of Food Hygiene and Nutrition, H-1097 Budapest, Gyáli út 3/a. Hungary

Some percent of population can not tolerate certain cereals like wheat, rye, triticale, barley, oat. The name of the disease is coeliac disease or non-tropical sprue, or gluten sensitive enteropathie.

The only therapy of the disease is the strict gluten-free diet, therefore we sought for new sources of gluten-free foodstuffs.

We measured the gliadin content of wheat varieties, wheat products, and different plant seeds by sandwich ELISA method. Their ethanol soluble protein fraction (prolamins) were studied with SDS PAGE as well.

* The detailed paper of this abstract will be presented in the next issue of Acta Alimentaria

The gliadin concentration of amaranth seed (red and white) buckwheat and millet was under the limit, permitted in gluten-free foodstuffs. In our examinations the prolamin content of shorgum approximated the limit, permitted in gluten-free foodstuffs. The toxic prolamin bands of different plant seeds like amaranth (red and white), millet were diffuse on the gel slab and could be found on the low molecular mass area. The seeds containing oil (sesame, flax) did not contain any prolamin band and its concentration was not detected by ELISA method.

Our examinations revealed that seeds of amarant (red and white) buckwheat, millet can be source of gluten-free foodstuffs. Their prolamin content was low enough for feeding patients with an injured gut membrane, provided that antinutritive effect is negligible.

DETERMINATION OF CHOLESTEROL IN FOODS

S. TÖMÖSKÖZI[a], I. BERECZ[a,c], R. MENDI[b], T. KOVÁCS[a] and F. ÖRSI[a]

[a] Technical University, Budapest, Department of Biochemistry and Food Technology, H-1111 Budapest, Műegyetem rkp. 3. Hungary
[b] Technical University, Budapest, Department of Agricultural Chemistry, H-1111 Budapest, Műegyetem rkp. 3. Hungary
[c] National Institute of Food Quality, H-1095 Budapest, Mester u. 81. Hungary

The relationship between the dietary intake of cholesterol and the artheriosclerotic diseases are unambiguous. Because of these effects the determination of cholesterol in foodstuffs has become a major concern in the food analysis. On the other hand, the indication of cholesterol level is obliged by the law of food labelling.

The first methods of cholesterol determination were applied in clinical diagnostics, and the usually used procedure in food analysis were adopted from this area. The very important difference is the sample handling methods: in cases of food materials different extraction procedures are used for elimination of matrix effects.

In our research work enzymatic Flow Injection Analysis (FIA) as a new automatic analytical method was applied for cholesterol determination. The cholesterol content of different type of foods (like egg meal, egg yolk, liver, liver paté and pasta) was determined by polarographic (Fig. 1A) and colorimetric (Fig. 1B) FIA procedures (FERNANDEZ-ROMERO et al., 1987, YAO & WASA, 1988). The sample pretreatment procedures i.e. conventional Folch-extraction (FOLCH et al., 1957) and the direct saponification (AL-HASSAN et al., 1993) were also studied. The FIA results were compared with results obtained by conventional Lieberman-Burchard colorimetric

(GÖRÖG, 1983), manual enzimatic (FERNANDEZ-ROMERO et al., 1987) and official GC (A.O.A.C., 1990) methods (Table 1).

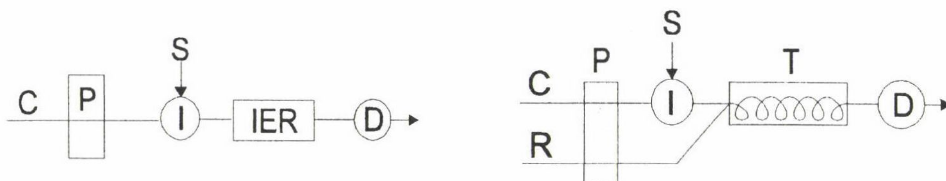

Fig. 1. Determination of cholesterol with A: polarographic-, B: photometric-FIA method.

A:
- C: flow rate: 2 ml min^{-1}
- IER: Immobilized cholesterin oxidase reactor (20/8mm)
- D: polarographic O_2 electrode (YSI 5331)
- Cycle time: 6 min

B:
- C: flow rate 1.5 ml min^{-1}
- R: Enzyme complex in 10% Triton-water solution; flow rate: 0.8 ml min^{-1}
- T: Thermostate; T=35 °C; reaction coil: 100 cm with 0.7 mm i.d.
- D: photometer, wavelength: 500 nm
- Cycle time: 2 min

Our results show, that

A. The direct saponification coupled with liquid-liquid extraction is a suitable procedure for pretreatment of different food matrices. It can eliminate the cholesterol esters and triacyl glycerols from matrix. At the same time there are two factors which have to be taken into account in this pretreatment:

1. The water: solvent ratio is a very important factor in solvent extraction, and it depends on the type of the given food matrix.
2. The type of effective saponification is also matrix dependent.

Table 1

Results of cholesterol determination obtained by different methods (mg/100 g)

	Liebermann-Burchard	Manual enzymatic	GC	Polarographic FIA	Colorimetric FIA
Egg meal	1251	1085	1186	1427	1395
Egg yolk	1442	1533	1411	1686	1567
Pig liver	310	357	363	308	328
Liver pate	164	193	185	181	195
Pasta	112	153	106	121	101
Recovery % (+2mg chol. in egg)	105	95	102	97	103
r.s.d. (%)	<5	<4	<2	<4	<3
Corr coeff. with GC	0.9986	0.9912	–	0.9978	0.9976

average of 3 parallel measurements

B. Both FIA methods are suitable for determination of cholesterol. For example the correlations with the results of the official GC method (Table 1) are very good (R^2 is 0.9978 for polarographic FIA and 0.9976 for photometric FIA). Comparing these methods it can be stated, that the colorimetric procedure is less time and work consuming. However the advantage of polarographic method is less enzyme consuming, because of the application of immobilized enzyme reactor.

It can be concluded that the proposed enzymatic FIA methods are applicable for food analysis, but the further improvement of sample pretreatment efficiency, reproducibility and sensitivity is necessary.

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CHANCES FOR HEALTH-PROTECTIVE FOODS IN HUNGARY

E. DWORSCHÁK, A. LUGASI, J. HÓVÁRI, A. GERGELY, É. BARNA and P. CZUCZY

National Institute of Food Hygiene and Nutrition, H–1097 Budapest, Gyáli út 3/a. Hungary

Health-protective foods can be an alternative term of Function Foods (Designer Foods, Pharmafoods, Nutraceuticals) which were defined by I. Goldberg in 1994 as follows:

"Functional foods, in addition to their basic nutritive value and natural being, will contain the proper balance of ingredients which will help us to function better and more

effectively in many aspects of our lives, including helping us directly in the prevention and treatment of illness and disease."

In Japan the following legislation guidelines are laid for Food for Specified Health use:

1. The food should improve the diet and health.
2. The health and nutritional benefit of the food on the specific ingredient must have a solid scientific basis.
3. The appropriate daily intake quantity of the food or the ingredient must be established by medical and nutrition experts for approval.
4. The food or the ingredient should be safe with regard to a balanced diet.
5. The ingredient should be characterized by its physical and chemical properties; detailed analytical methods for its characterization should be given.
6. The ingredient should not reduce the nutritive value of the food.
7. The food must be consumed in a normal way.
8. The food should not be in the form of tablets, capsules or powder.
9. The ingredient should be a natural compound.

The health-protective foods could be categorized on the base of ingredients in the following way: 1. Dietary fibres; 2. Oligosaccharides and sugar alcohols; 3. Amino acids, peptides and proteins; 4. Vitamins; 5. Bacteria producing lactic acid; 6. Macro- and microelements; 7. Polyunsaturated fatty acids; 8. Plant materials and antioxidants.

Two possibilities are introduced for the home application of health-protective foods.

The meats of Broiler and Transylvanien naked-neck chicken kept under natural circumstances showed the following advantages as compared to those kept in intensive way:

– higher water-holding capacity,
– higher Fe, Zn, B_1- and B_2-vitamin concentration,
– decreased lipid peroxidation.

In ground porks containing spice mixtures the retardation of lipid peroxidation was observed after storage at 4 °C. This procedure gives also a potential chance for developing health-protecting foods in Hungary.

INVESTIGATION OF COLOUR PIGMENTS OF TOMATO FROM THE ASPECTS OF STABILITY

P. K. BIACS[a] and U. WISSGOTT[b]

[a] Department of General and Analytical Chemistry, Technical University, Budapest, H–1111 Budapest, Szent Gellért tér 4. Hungary
[b] Nestlé Research Centre, CH–1000, Lausanne 26. Switzerland

During food industrial preservation process and long time storage tomato products mainly undergo aroma and colour losses in comparison of the original character of the fresh tomato. This is caused by oxydation and thermal decomposition in consequence of light exposure and reactions with oxygen and prooxydative compounds catalysed by enzymes, which results transformation and decomposition of aroma ingredients and carotenoid pigments.

Reduction of viable microbial counts and decrease of enzyme activity to a minimal level were elaborated by using high pressure technology, a new method of preservation among optimal experimental conditions. Carotenoid pigments (licopene, beta-carotene) were stabilized by addition of spices, containing antioxydants (ascorbic acid, citric acid, tocopherols, flavonoids) obtained from natural sources and by mixing with ground tomato seeds, a waste product of tomato processing.

During this experiment samples were submitted to different storage conditions as a model of a normal grocery shop: in frozen stage of –20 °C degree, in closed plastic bag under vacuum or in open plastic cup, in artificial light or in dark for 12 h a day.

Carotenoid compounds were identified by HPLC, quantitative changes were determined by UV/VIS absorption. Colour changes of tomato pulp were measured by instrumental colorimetric system of CIELAB.

It was found, that exposure to higher hydrostatic pressure and long treatment intensified the colour of tomato products whereas it changed and reduced their aroma. Frozen storage didn't blocked the degradation of carotenoid pigments, only decreased slowly the enzymatic and oxydative processes. This diminishing of carotenoid pigment content of tomato was in close linear correlation with the storage time and became apparent in the oxydative environment. The ground tomato seeds with antioxydative ingredients and the addition of spices could stabilize carotenoid pigments after longer time frozen storage. The commercial spices rosemary and marjoram significantly reduced the pigment degradation, their quantity (0.02%) did not change the original color and taste of tomato pulp.

THE INNOVATION ACTIVITY OF THE FOOD INDUSTRY IN ORDER TO MANUFACTURE NEW PRODUCTS

K. PÓDER-ŐSY

National Committee for Technological Development, H–1052 Budapest, Szervita tér 8. Hungary

In the past decades innovation ability and activity have become one of the most important factors of international competitiveness. In Hungary, following the first stages of the economic and political change of regime, the issue of innovations, specifically the maintainable renewal of the Hungarian food industry was put on the agenda once again. The synergic effect of economic, scientific and human factors determining technical development has a significant role in this renewal.

In 1994–95 an OMFB (National Committee for Technological Development = NCTD) study was made in order to survey the innovation performance of economic organizations (companies). The survey, in which the questionnaire-method was used, was made by SZONDA-Ipsos. The processing and analyses of the data were managed by workteam formed in the MTA (Hungarian Academy of Sciences) Research Centre of Social Conflicts. Among others, the study covered the R&D activities of companies and the introduction of new products and technologies. Within the studied food industrial companies (500) the ratio of Hungarian ownership was 80%. Thirty eight % of the food industrial companies surveyed gave an account of some kind of R&D activity. In 1993–94 new products and new technologies were introduced in 73% and 54% of the companies, respectively. It was found that from the innovation point of view this branch of industry belongs to the most progressive sectors. In the period of the survey 37% of the newly developed products and 26% of the newly introduced technologies were novelties in the whole country. Regarding the financing of developments, 4% of the companies surveyed indicated the application of NCTD sources.

The innovation activity of the food industry aimed at introducing new products was encouraged in 1993 by the national project which was started at the initiation of NCTD and then announced jointly with the Ministry of Agriculture. The subject of the project was the "supporting of the development of new, internationally marketable and highly processed food industrial products containing real national originalities and meeting special demands". The interest of Hungarian companies in the project was well indicated by the high number of prequalification competitions (94). In the end, NCTD had concluded 17 contracts with a total Central Technical Development Fund (CTDF) support of HUF 236.84 million. Because of the high degree of market-orientedness the financial investment of the contracting partners is also significant (HUF 231.85 million). It is a positive feature of the project that the amount of money spent and to be

spent on marketing activities is HUF 46.7 million, which represents 9.96% of all the costs. The introduction of new products and technologies is facilitated, among others, by the support of the applied R&D activity and export development from the financial source of CTDF.

Regarding the future, we have to be prepared for a situation where competition will become more intense on both the domestic and foreign markets. The goals set in the national project will remain very important tasks for the future. Important aspects are the guarantee for food safety and for the reliability of quality assurance, and, in order to improve the image of Hungarian foodstuffs, the considerable enhancement of marketing activities. Thus, in order to lay down the foundations for the future, it should further be emphasized that foodstuffs can be the source of both health and disease. In the interest of health protection the full elimination of foreign substances requires the introduction and application of an integrated food safety programme in the field of foodstuff base material production, processing and distribution. In the field of product innovation it is increasingly necessary to use the widening nutritional, physical, chemical, microbiological, etc. knowledge. In order to facilitate this activity the task of the state is to support in the long run such activities which

- either fall outside of the circle of company objectives spurred by profit making,
- or require such a long-term or high R&D investment which can not be financed by the individual companies.

COMPARISON OF EFFECTS OF IRRADIATION AND TRISODIUM-PHOSPHATE DIP ON MICROFLORA OF THE CHICKEN MEAT

I. F. Kiss, J. Farkas, M. Kiss-Brückner, M. M. El-Emam, A. Simon and É. Andrássy

University of Horticulture and Food Industry. Dept. Refrig. and Livest. Prod. Techn., H–1118 Budapest, Ménesi út 45. Hungary

The shelf-life and the microbiological safety of chicken meat depend on its microbiological contamination, slaughterhouse processing and hygienic conditions. In order to reduce the viable cell counts different physical, chemical and combined treatments are used. Some of these techniques are very effective however they have been introduced in practice only in few places. The other part of these methods needs further work for improving the efficiency or clarifying some unresolved problems.

Our subject was to study the effects of a 10 per cent trisodium-phosphate (TSP) dip and gamma irradiation with 2 kGy, and storage at 2, 4 and 10 °C, resp. The effect of those treatments on the natural microflora of chicken skin was investigated,

furthermore, survival and growth of an artificial contamination by *Listeria monocytogenes* (*L.m.*) as well as pH and spoilage–odour development were studied as a function of the treatments.

The mesophilic aerobic viable cell count was reduced by two orders of magnitude (from 10^4 CFU cm^{-2} to 10^2 CFU cm^{-2}) by TSP treatment and the residual level didn't change at 2 °C and 4 °C temperature for minimum 15 days, whereas at 10 °C temperature up to 7 days. At the beginning the *L.m.* cell count was 5.10^4 CFU cm^{-2}. This number after TSP treatment was similarly reduced as the mesophilic aerobic viable cell count while it reached the level of 10^6 CFU cm^{-2} on the untreated samples. The pH of the TSP treated samples increased to pH=9.0. On the base of spoilage–odour development in 50 per cent of samples the shelf-life of untreated batch samples was 4 days at 10 °C, 5 days at 4 °C and 7–8 days at 2 °C. The same level of spoilage–odour development of TSP-dipped samples occurred on the 17th day at 10 °C and after more than 3 weeks when the samples were stored at 2 °C and 4 °C temperature, respectively.

The mesophilic aerobic viable cell count of irradiated samples showed the same decrease as that of TSP treated samples. After 16 days of storage, the cell count of irradiated samples was 10^6 CFU cm^{-2} at 2 °C and 10^7 CFU cm^{-2} at 4 °C. The latter level was found at 10 °C after 8 days of storage time. The irradiation (2 kGy) reduced the CFU of *L.m.* by 2.5 log cycles. The number of *L.m.* was increased from 10^2 CFU cm^{-2} to 7.10^5 CFU cm^{-2} at 10 °C during 7 days, or to 3.10^6 CFU cm^{-2} and to 10^3 CFU cm^{-2} during 15 days at 4 and 2 °C temperatures, respectively.

*

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MONITORING OF PLANT PHYSIOLOGICAL PROCESSES BY NIR SPECTROSCOPY

A. SALGÓ[a], J. NAGY[a], GY. DELY-SZABÓ[a], D. NYERGES[a] and A. TARLÓS[b]

[a] Department of Biochemistry and Food Technology, Technical University,
H–1111 Budapest, Műegyetem rkp. 3. Hungary
[b] Concordia Co. Ltd.

The quality and technological characteristics of food raw materials can be influenced by their physiological status which changes during processing and storage.

The physiological changes are the result of simultaneous biochemical, enzymatic, functional, colloid chemical and microstructural modifications in plant material.

The NIR spectroscopy is a sensitive tool for investigation of complex qualitative and quantitative changes based on its fast and non-destructive character.

The aim of the present work was to study the germination and ageing process in wheat and barley.

Inhibition and germ reactivation phases of germination were followed by two parameters (moisture content, germination time). Calibration equations were developed for detection of both parameters and the differences in extent (dinamics) of germination were shown.

The changes in the status of water species, lipid composition and metabolically active proteins were sensitively indicated in NIR spectra.

Viabilities of wheat and barley seed were also detected by NIR technique using biological and TTC (tetrazoliumchloride) dehydrogenase tests as reference methods.

Viability was detected in 65–100% region with low accuracy (SECV=2.8%) based on NIR spectra. The developed calibrations have only limited applicability in routine analysis.

STRUCTURAL AND MORPHOLOGICAL CHANGES IN APPLE STARCH

E. KOVÁCS[a], T. M. EADS[b] and I. DÉKÁNY[c]

[a] Central Food Research Institute, H–1022 Budapest, Herman O. út 15. Hungary
[b] Purdue University, West Lafayette, IN. USA
[c] JATE, Institute for Colloid Chemistry, Szeged. Hungary

Ripeness stage of apple (*Malus domestica* cv. Mutsu) could be characterized by starch iodine pattern. Morphological changes of starch granules (area, perimeter, circularity) were determined by image analyzer (Semper6, Synoptics) interfaced with a standard light microscope (Olympus, BH_2) (CCD: 1.25×1.6×40) (315 mm=786 pixel) using bright field illumination.

The distribution of area, perimeter and circularity was calculated by the Distribution Fitting Statistical program; Normality of frequency distribution of data (X^2-test), average and standard deviation, t-values were presented. All data were evaluated by PC Analysis.

X-ray and C-13 NMR spectra were determined. Gelatinization of starch from 25 °C to 100 °C was measured. The size degradation of starch granules were observed by SEM. The content of amylose was determined, too.

Summarizing the results it was established that the starch granules were very inhomogen. Beside round shape granules a number of irregular shaped granules could

be observed, too. With increasing ripeness of apple the number of round shape granules increased, holes or cavities were not detected regularly.

According to the DSC, NMR, X-ray results apples did not differ each other as a function of harvesting dates. The content of amylose was about 19–20%, and the amorphous fraction of starch (NMR results) varied between 10–14%.

MICROELEMENT ENRICHMENT IN NON-GROWING YEAST CELLS

J. HEGÓCZKI[a], B. JANZSÓ[b], Á. SUHAJDA[b] and G. VERECZKEY[a]

[a] Department of Bioengineering. Central Food Research Institute, H–1111 Budapest, Budafoki út 59. Hungary
[b] Department of Agricultural Chemical Technology, Technical University of Budapest, H–1521 Budapest, Szt. Gellért tér 4. Hungary

Yeasts are capable of enriching and forming organic bonds with microelements. These microelement enriched yeasts can be utilised as new natural microelement resources in human nutrition and in various paramedicinal preparations.

In our experiments we used two accumulation methods: enrichment in growing cells and enrichment in non-growing cells.

In the first method the addition of microelements was in the exponential phase of yeast growth. Cultivation was carried out in 10 dm^3 laboratory scale fermenters with 5 dm^3 actual broth volume at 30 °C, 700 r.p.m. agitation, batch technique, 1 $vv^{-1}min^{-1}$ aeration. The amount of inoculum was 10% of the broth volume.

In the second method ("resting cell method") we used separated baker's yeast milk (*Saccharomyces cerevisiae*). The uptake period was an essentially non-growth period where the yeast cells in the fermenter were in an aqueous suspension in the presence of the dissolved microelement salt. The circumstances (aeration, agitation, initial pH, volume) were same as that in the growth phase accumulation. This method has numerous advantages in comparison to "classic" growing cell accumulation:

- the required addition of trace element can be calculated exactly;
- inhibitory effect of trace element can be eliminated almost completely;
- the fermentation technique becomes significantly simple:
  - reduce fermentation time (4–8 h);
  - it can be carried out semi-sterile;
  - the fermentation can be monitored by the measurement of solved oxygen level;
- the economy of production is improved.

Microelement enrichment in non-growing yeast cells ("resting cell method")

Microelement	Uptake in yeast (μg/g)	Microelement	Uptake in yeast (μg/g)
Cu	1694	Ni	352
Co	723	Zn	4493
Fe	7343	Ti	840
Mn	529	Si	420
Mo	11127	Cr	512

We calculated the theoretical microelement addition basing the dry material content of the used separated yeast milk (*Saccharomyces cerevisiae*) which was about 20 percent. In the experiments we added twice or three times the amount of the theoretical microelement addition.

We show our best results with resting cell method in the next table. The concentration of microelements in yeast was determined by inductively coupled plasma atomic emission system (ICP-AES).

DECOMPOSITION OF THE BEETROOT BETACYANINS DURING JUICE AND NECTAR PRODUCTION

GY. PÁTKAI, J. BARTA and I. VARSÁNYI

University of Horticulture and Food Industry, H–1118 Budapest, Ménesi út 45. Hungary

The beetroot is an important source of different nutritional and natural colouring factors. The retention of natural colourings (betanin and vulgaxanthin I) during juice and nectar production, first of all after blanching, peeling, pasteurization and storage was investigated. Three different beetroot sorts (Bordó pt, Bordó tk and Rubin) were used for investigations. These sorts were improved by breeding in the Research Institute for Vegetable Growing in Kecskemét. Juice and nectar production was carried out on pilot-plant scale.

Sequence of the mentioned sorts regarding the betacyanin concentration was: Rubin>Bordó pt>Bordó tk.

During processing of the filtered juice a small but significant loss of colour was registered already after clearing and filtration. Considerable decrease of the betacyanin concentration was observable after adding citric acid to the juice and after pasteurisation.

Following betanine retention was registered during nectar production:
1) raw material: 100%, 2) blanching: 99.8%, 3) peeling: 99.4%, 4) crushing, homogenisation, filling: 91.6%, 5) pasteurisation: 501%, 6) storage (20 °C, 60 days): 31.3%, 7) storage (5 °C, 60 days): 46.9%.

Vulgaxanthine retention after the same processing steps was as follows: 1) 100%, 2) 84.1%, 3) 82.9%, 4) 67.52%, 5) 42.66%, 6) 36.4%, 7) 45.24%.

Authors investigated the decomposition of the main beetroot colouring – betanine and vulgaxanthine – as a function of temperature and pH value in acetate and citrate buffer solutions in a temperature range between 60 °C–80 °C and pH range of 3.3–6.2. First order reaction rate constants between –0.0047 min^{-1} and 0.0621 min^{-1} and activation energy of 77.5 kJ mol^{-1} were calculated for the heat degradation of betanine.

Retention values calculated on the basis of reaction kinetic constants and measured retention values during different steps of the juice and nectar processing were compared. The betacyanine retention in the natural beetroot juices and nectars during pasteurization was much better (45–50% after 125 min, 80 °C) than in buffer solutions (63% after 20 min, 80 °C).

By measuring the heat penetration during pasteurisation F_0 values were calculated. The F_0 value for filtered juice pasteurisation was 2.3×10^{-2} min, F_0 value for nectar pasteurisation was 8.11×10^{-2} min, both heat equivalents are significantly higher than recommended for pasteurisation of vegetable juice with similar pH values.

Summarized: most important chance of the optimal betanine retention is to avoid too high heat equivalents during heat treatment and too low pH values during processing of beetroot products.

PRESENTATION OF AN ALTERNATIVE, WASTE-FREE PROCESSING TECHNOLOGY ILLUSTRATED BY THE EXAMPLE OF INULIN CONTAINING CROPS

J. Barta, Gy. Pátkai, B. Gion and A. Körmendy-Domján

University of Horticulture and Food Technology, H–1118 Budapest, Ménesi út 45. Hungary

Objective of the research work was to develop different product groups and processing technologies, suitable for complete utilization of inulin containing plants, successfully cultivated under Hungarian climatic and soil conditions, like Jerusalem artichoke and chicory.

The inulin content of Jerusalem artichoke is 16–18%, the inulin yield is 1.4 t/ha.

Products suggested for production from Jerusalem artichoke:

- Sweetening products, rich in fructose (natural Jerusalem artichoke concentrate, purified concentrate, hydrolysed Jerusalem artichoke pulp).
- The natural concentrate, rich in fructose and minerals has excellent nutritional properties. It can be produced by enzymatical hydrolysis of Jerusalem artichoke pulp, considerate juice purification and concentration. Purified syrup is a most valuable carbohydrate source, liquid sugar, suitable for sucrose substitution. It can be produced by acid hydrolysis of Jerusalem artichoke juice, juice purification by ion exchange and concentration.

Inulin degradation inhibiting technology was developed to produce inulin concentrate.

Inulin rich products (natural Jerusalem artichoke pulp, dried Jerusalem artichoke cubes, fiber rich inulin flour) can be produced partly by trituration or cutting in cubes and drying of the fresh Jerusalem artichoke tubers.

The natural and the hydrolysed sweet pulp is suitable to increase the dietary fiber content and to substitute partially the fruit component of fruit juices.

The fiber-rich inulin flour can be used in bakery products as quality improving additive. Syrups and pulps rich in fructose can be added to dairy products as sweeteners and fiber-content increasing additives.

Inulin-content of the chicory-root is 11–12%, the inulin yield is 0.9 t/ha. Products for industrial processing:

- Granulated or instant chicory produced by cleaning, crushing, drying and grinding.
- Syrup, rich in fructose, produced by hydrolysing the chicory juice or natural chicory concentrate, produced by juice extraction and concentration. The press-cake, by-product of juice extraction can be also utilized after drying or trituration. This technology belongs also to the research field of the authors.

QUALIFICATION OF FLEXIBLE PACKAGING MATERIALS FOR SPICES

J. MONSPART-SÉNYI, I. VARSÁNYI and A. KÖRMENDY-DOMJÁN

University of Horticulture and Food Industry, H–1118 Budapest, Ménesi út 45. Hungary

One of the main tasks of packaging is to preserve the quality and quantity of packed foods until consumption. In case of dried powdered spices the aroma barrier

behaviour and the oil and fat resistance of packaging materials were determined, respectively.

In the frame of activities experiments were performed with flexible packaging materials, made in Hungary. The aim of investigations was to test the mechanical properties of the packaging materials (tensile strength, elongation, bursting test and sealing test), and the barrier properties (water vapour, oxygen and carbon dioxid gas permeability). Storage test was launched for shelf life determination of spices (paprika and marjoram) according to the practice of marketing (time, temperature, relative humidity and light).

On the base of packaging material tests and the commercial aspects two packaging foils were chosen:

- Paper-polyethylene combined packaging material:
  (70 g m^{-2} paper and 20 g m^{-2} HDPE/Lopalux/)
- Multiple-layer Alu-polymer foil:
  (60 g m^{-2} natron paper and 15 g m^{-2} LDPE 9 μm Al and 35 g m^{-2} PE)

The reference packaging of storage tests was glass vessel with glass stopper. The storage temperature was ambient (25±2 °C) and cooled (5±2 °C), the relative humidity was 60+5% and 35+5%, storing in light and in dark, respectively.

The storage tests were started at the end of December of 1995, it means that the shelf life will be determined at the end of this year.

COMMUNICATION WITH MARKINGS PLACED ON FOOD PACKAGES

E. SZABÓ[a] and Z. LAKNER[b]

[a] Central Food Research Institute, H-1022 Budapest, Herman O. út 15. Hungary
[b] Department of Food Economics, University of Horticulture and Food Industry, H-1118 Budapest, Villányi út 35-43. Hungary

Marking placed on food packages is an important mean of influencing the market. In line with the growing differentiation and complexity of products, producers are providing more divergent informations to the consumers in order to give them better orientation to make their choice.

Two aspects from the same rank should be met by the practice being under change due to the development: the fairness of the competition and the protection of consumers' interests, the correct information of consumers.

According to our food act realizing the EU harmonization, the information carriers of marking are words, numbers, brand-marks, pictures, illustrations and marks.

The kinds of communication with graphic markings, their possibilities and problems are discussed in this lecture.

Graphic marking is the visual form of information placed on food package, being well separated from its environment, including in some cases some legends, or accompanied by them, the primary function of which is the rapid interpretation of some essential and complex information.

Graphic markings are ranked into six groups: markings indicating quality, markings controlling action, markings helping commercial selling, accrediting markings, markings motivating the consumer and markings supporting environmental protection.

Each type of the markings is discussed in the lecture. The content of the message included into markings, the enforcement of aspects of consumer protection have been studied and the potential future role of the types of markings and in this connection the aspects of regulation of the theme have been analysed.

CHANGES IN FOOD CONSUMPTION IN HUNGARY AND THEIR RELATION TO THE ECONOMY AND NUTRITION–HEALTH

J. LAJOS and M. SZŐKE

Central Food Research Institute, H–1022 Budapest, Herman O. út 15. Hungary

The overall level and main features of consumption and nutrition are considered as significant indicators of social and economic development. As a general rule, significant trends in consumption patterns may only be perceived within a few years, or even decades. Evidently, these processes may accelerate, as we have been witnessing during the midnineties. The main purpose of this paper is to study the effects of these changes.

Statistical analysis, based upon representative surveys by the Central Statistical Office and other institutions, show how far these changes in nutrition have affected the economy and the overall state of health of the population.

The analysis covers the average quantity and value of food consumed by each household. As a result of the overall developments taking place in the lifestyle of Hungarians, their financial position and continuous retail price increases, food consumption has been reduced.

Beside the tendencies having been formed in the total food consumption, in the details positive processes can be observed, as well. The general effect of the picture is

unfavourable, but within main processes, a strong differentiation can be observed in the part processes.

The defensive strategy of the households is determinated today essentially by the degree of inflation pressure and the social state of the households i.e. the families, where numerous factors thus the size of the family, the property status and the income, etc. are of decisive character.

Nutrition is in close connection with the health level of society. The nutrition-related diseases can be prevented with the elimination of risk factors. On this domain, the lack of knowledge, the nutritional habits and the growing unequalities in food consumption can all be made responsible. It is important to stress, that the indirect effects are the result of some very complicated complex phenomena, where nutritional level is only one influencing factor. In this aspect, we speak of long-distance social interests, and today we hardly are at the work of forming the boundaries, of revealing the phenomena. For this reason, it would be highly important e.g. to strengthen the connections between nutritional science and food consumption research and finding the relations with the results of the demographic researches.

Studies regarding the negative and positive impacts on food consumption cannot be considered as complete and finished, as further investigations are still needed in this area.

ROLE OF FOOD PHYSICS IN ASSURANCE OF FOOD QUALITY

A. S. SZABÓ

Department of Food Chemistry and Nutrition, University of Horticulture and Food Industry,
H–1118 Budapest, Somlói út 14–16. Hungary

The lecture deals with the question of factors, determining the quality of foodstuffs, e.g. sensory and chemical parameters, packaging, labelling. It analyses the possibilities of application of measurement techniques – e.g. near infrared reflectance (NIR), nuclear magnetic resonance (NMR), instrumental neutron activation analysis (INAA) – based on physical principles in the qualification of foodstuffs.

Information is given about the necessity of education and research of food physics. Food physics is a special field of science, belonging both to food science and applied physics. The lecture deals with development and topics (e.g. physical parameters of foodstuffs, investigation of foodstuffs with physical methods) of food physics. Special attention is paid to the up-to-date food technologies (e.g. irradiation, microwave treatment) application of which is the base of modern food processing and production.

Finally information is given about the present radioactive contamination of the Hungarian foodstuffs, emphasizing the low level radioactivity, which has the same order of magnitude in 1996 as before the Chernobyl disaster.

APPLICATION OF THE PATTERN RECOGNITION METHODS FOR THE EVALUATION OF SENSORICAL INVESTIGATIONS

F. ÖRSI

Department of Biochemistry and Food Technology, Technical University, Budapest, H–1111 Budapest, Műegyetem rkp. 3. Hungary

The mathematical-statistical methods of pattern recognition were applied for evaluation of the point of view of consumer in determining sensorical attribute of the food quality by scoring.

The investigations were made with the aim of

- checking the weighting factors of the sensorical qualification system for apricot preserves
- separation and determination of various kinds of errors
- using the PRIMA computer program for controlled classifying.

The work was made by getting assistance from OTKA (contract No. 2639).

The investigated data include the individual results of apricot preserves by sensorical method (13 samples were investigated by a panel of 5 members for 4 characteristics namely for outside: form, surface and wholeness together; for flavour; colour and consistency). Every single characteristic was measured by a 5 points scale.

In the first step the data were investigated by a self-produced MANOVA (Multivariate analysis of variation) computer program. This program can investigate the deviation of groups (in this case the samples), taking into consideration all four variables (the sensorical points), at the same time by mathematical-statistical method. The Hoteling T2=4.24 and from that the calculated F=4.35 show significant differences at a security level of 95%.

The deviations were further investigated by principal component analysis. There were few significant correlation connections among the variables, for this reason 3 principal components are needed to describe the system. A loose but significant correlation exists between the outside and consistency, it seems that the samples with a too soft consistency were susceptible to damages and got worse points on the outside. The relationship can be characterised by the next equation:

$$\text{outside} = 2.3 + 0.26^{*}\ \text{consistency}$$
$$r = 0.264$$

A loose but significant correlation exists between colour and flavour, too. It indicates the speculation that a product with a better colour is evaluated as a product with a better flavour, too. The relationship can be characterised by the next equation:

$$\text{flavour} = 2.25 + 0.38^{*}\ \text{colour}$$
$$r = 0.363$$

The comparison of mean values of samples and the single values show three outliers during the first three trials. It is possible the panel member who gave an outlier was warned since later there was no outlier. The three outliers were eliminated from the further investigations.

The 13 apricot preserves were classified in 5 groups on the basis of:

– cluster analysis for the principal components calculated from the sensorical scores and

– the MANOVA results for the by pairs made comparison of the samples as well as

– the three dimensional pictures of samples mean in the space of the first three principal components:

1st group consist of samples numbered as 2, 3, 4, 5, 7, 8, 9 and 10.
2nd group consists of samples numbered as 1 and 12.
3rd group consists of sample numbered as 13.
4th group consists of sample numbered as 6.
5th group consists of sample numbered as 11.

On the basis of the statistical characteristics (mean and standard deviation) of sensorical scores group No. 4 proved to be the best one, groups No. 1 and 2 represented medium qualities and groups No. 3 and 5 gave the worst statistical characteristics. The difference between groups No. 1 and 2 seems to be in the colour and outside sensorical characteristics. In the group No. 1 all two characteristics showed medium score but in the group No. 2 the colour got a very good (5) score, the outside is worse than 3. The groups No. 3 and 5 showed significant difference in the outside characteristics (the means are 2.6 and 4.0, respectively).

The discriminant analysis was applied to investigate whether the groups are distinguishable with mathematical-statistical method. The five groups may distinguish from another by two discriminant equations applying the original variables.

Applying the discriminant equation for classifying the samples in the 5 groups, the samples which originally stayed in the group 1 were ranked again with a 61% probability into the first group, the degree of efficiency in the second group is 89% and

it is 100% in the other groups. The cause of the worse degree of efficiency may be connected with the higher number of data and their distribution in the first and second groups.

The quality of samples shows a correlation with the 1st discriminant variable which was calculated from the original variables. Applying in the first discriminant equation given standardised multiplicators the weighting factor of outside ought to change from 0.5 to 0.7, the weighting factor of colour from 1.0 to 1.7, the weighting factor of flavour from 2.0 to 1.4 and the weighting factor of consistency from 0.5 to 0.2. The summarised weighted value (total score) calculated by the previously given weighting factors shows a strong correlation with the first discriminant variable:

$$\text{totalscore} = 14 + 1.28 * \text{DSCR1}$$
$$r = 0.957$$

The panel proved to be very homogeneous on the basis of standard deviations calculated by the MANOVA. The standard deviations were for the outside in the range of 0.4–0.5, for the colour in the range of 0.5–0.6, for the flavour in the range of 0.46–0.61, for the consistency in the range of 0.44–0.69. When the range of standard deviation was calculated for all samples the ranges were higher for the outside 0.0–0.7, for the colour 0.4–0.7, for the flavour 0.0–0.8 and for consistency 0.0–1.1 but this standard deviation does not differ significantly from the standard deviations characterising the accuracy of the panel members and gives the error of the sensorical investigation.

The samples were classified in 5 quality groups on the basis of the weighted total score calculated by the previously given weighting factors and the suitability of the PRIMA controlled classifying program for the qualification of samples. When the training sample contained all samples the original and estimated quality classes were identical. The quality definition made on the basis of the weighted total score proved to be excellent for the controlled classifying.

SUPERCRITICAL FLUID EXTRACTION OF OIL SEEDS

B. SIMÁNDI[a], E. RÓNYAI[a], A. DEÁK[a], S. TÖMÖSKÖZI[b], ZS. WEINBRENNER[c], I. ZÁMBÓ[d], ZS. FATÉR[e], M. THEN[f] and G. YANXIANG[a,g]

[a] Department of Chemical Engineering, Technical University, Budapest, H–1111 Budapest, Műegyetem rkp. 3. Hungary
[b] Department of Biochemistry and Food Technology, Technical University, Budapest, H–1111 Budapest, Műegyetem rkp. 3. Hungary
[c] Unilever Rt., Budapest. Hungary
[d] Herbária Herb Processing and Trading Co. Ltd., Budapest. Hungary
[e] Research Institute for Medicinal Plants, Budakalász. Hungary
[f] Institute of Pharmacognosy, Semmelweis University of Medicine, Budapest. Hungary
[g] Central Food Research Institute, Budapest. Hungary

The removal of the vegetable oil from different oil seeds can be accomplished by mechanical pressing and subsequent solvent extraction or by direct extraction only. Commercially available hexane extract is used for producing the oils. Hexane is inflammable however, and the 1990 Amendments to the Clean Air Act (in the U.S.) listed hexane as a hazardous substance. The governmental regulations of solvent emissions and solvent residue vary from country to country. Supercritical fluid extraction (SFE) may be viable alternative to current extraction methods.

Different seeds were extracted with carbon dioxide, in a high pressure apparatus with 5 dm^3 extractor vessel volume. Effects of extraction pressure and temperature on the yields and quality of the products were investigated. The carbon dioxide extracted oils were compared with oils derived through hexane extraction. Fatty acid composition and other properties (acid value, saponification number, ester number, refractive index) were only slightly different for oils obtained by SFE and hexane extraction. Significant differences were found between the phosphorus contents of the oils since phospholipids are insoluble in supercritical carbon dioxide. The removal of phospholipids was possible with the addition of a polar entrainer (2.5–10% ethanol) to supercritical CO_2. The extraction time decreased significantly when ethanol was added, because the solubility of the oil in the supercritical fluid under the same temperature and pressure conditions was greatly enhanced.

Due to the high capital costs of SFE plant and the high carbon dioxide consumption removal of oil from commodity oil seeds (sunflower, corn germ) does not appear economical. SFE may be economical in the extraction of high-value oils (pumpkin seeds, wheat germ), produced in relatively small quantities and often used in various pharmaceutical preparations.

Using CO_2 the thermolabile active components (tocopherols, carotenoids) can be extracted without any degradation (e.g. poppy seeds, buckthorn seeds). The seed oils of

spices (e.g. fennel, parsley) can be separated into essential oil rich and fatty oil rich products by stagewise precipitation of the extracts.

*

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DETECTION OF RADIATION TREATMENT OF PAPRIKA POWDER USING THE NIR PQS TECHNIQUE

S. Barabássy

Department of Refrigeration and Livestock Products Technology, University of Horticulture and Food Industry, H–1024 Budapest, Ménesi út 45. Hungary

Gamma irradiation is increasingly used for decontamination of spices in many countries because this treatment causes minimal chemical alteration and minimal, if any, detectable changes in the flavour of spices.

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.

First the IAEA co-ordinated ADMIT research collaboration started investigations, then the EU co-ordinated COPERNICUS program continued to elaborate methods for the identification of irradiated foodstuffs.

The aim of our research was to extend – based on our earlier results – the NIR polar qualification system (PQS) for the irradiated paprika powder.

For the investigations we used paprika grown in the southern part of Hungary, cultivated and harvested in 1995 in the vicinity of Szeged.

The homogenised paprika powder samples were packed into plastic bags (30 g). Then the samples were irradiated in three different institutions: Central Food Research Institute, Institute of Isotopes of the Hungarian Academy of Sciences and Agroster (an industrial company). Each institution used Cobalt-60 as radiation source. 33 samples were prepared in each institution. The samples were irradiated with 0 and 5 kGy doses.

The diffuse reflectance spectra of the samples were measured immediately after irradiation and then from time to time during storage at ambient temperature and humidity. The storage time of the samples were 6 month (guaranteed storage-time of the product).

The changes in the main components of samples caused by the irradiation were investigated on a SPECTRALYZER 1025 type spectrophotometer in the near infrared wavelength region between 1000 and 2500 nm. The data processing was performed using the polar qualification system PQS – a new data reduction method.

The Fig. 1 shows the log(1/R) NIR spectrum of an untreated paprika powder sample. From our earlier results (presented in Aberdeen 1992) we know that the most characteristic changes caused by irradiation effect appear in the 1900–2400 nm wavelength region, characteristic for the water, carbohydrate and oil components.

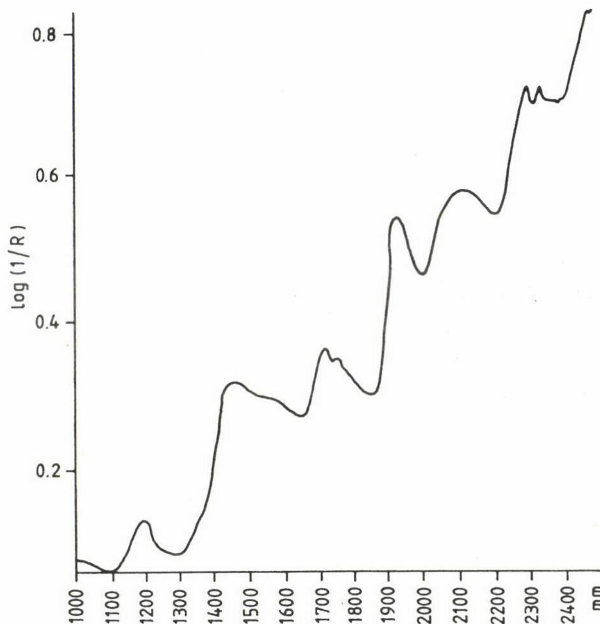

Fig. 1. The log(1/R) NIR spectrum of an unirradiated paprika powder sample

In the present investigations we used the polar qualification system PQS, as a possible rapid test to distinguish the irradiated paprika from the unirradiated one.

The "quality points" of the samples obtained as the centre of gravity of the NIR spectra represented in polar diagram concentrated all information characteristic to the investigated samples (Fig. 2).

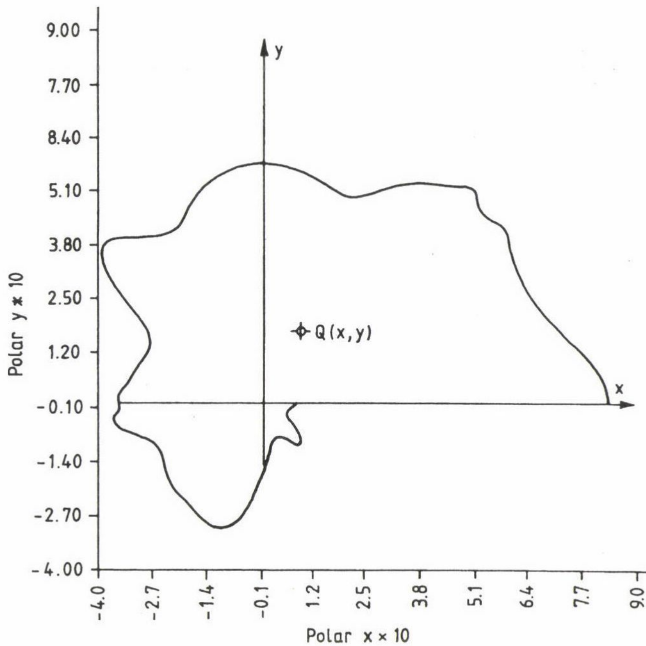

Fig. 2. The log(1/R) polar spectrum of paprika powder in the 1000–2500 nm wavelength range. Q(x,y): gravity point (quality point)

The centre of gravity of the polar spectrum can be expressed in a rectangular (Descartes) coordinate system.

The results for the most characteristic wavelength region two weeks after the irradiation treatment are given in the Fig. 3. The Fig. 3 shows the quality points of the paprika samples determined by the wavelength range optimisation. The centre of gravity points derived from the second derivative NIR spectra for samples unirradiated and irradiated with 5 kGy doses can be distinguished, namely the points in the quality plane are far from each other.

The distance between the quality points expresses the changes in the "quality" after the irradiation treatment. So from the position of the quality point it can be defined whether an unknown sample has been irradiated or not.

For the three different irradiators the samples showed approximately the same result.

This paper presents the results achieved two weeks after the irradiation treatment. Our earlier studies showed that during the storage period the best characteristic wavelengths changed, so we need further investigations for the different storage time.

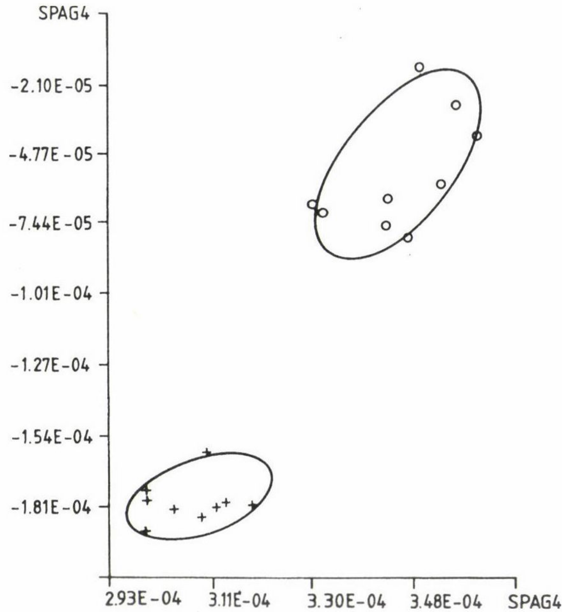

Fig. 3. The quality points of the paprika samples irradiated at C.F.R.I. determined by the wavelength range optimisation method. SPAGH (2.99E–0.4, –1.90E–04). o: unirradiated samples; +: irradiated samples

*

Supported by grant from the Hungarian Scientific Research Fund (OTKA) No. T14371.

IDENTITY CONTROL OF PEPPERS BY GC-MS MEASUREMENTS

ZS. MEDNYÁNSZKY, K. KORÁNY and A. S. SZABÓ

Department of Food Chemistry and Nutrition, University of Horticulture and Food Industry, H–1118 Budapest, Somlói út 14–16. Hungary

Pepper (*Piper nigrum*) is one of the most widely spread and sold spice by the World Trade. In the Eastern-European countries spice import started to grow from the end of the seventies and recently Hungary is also considered a significant pepper consuming country. This growing consumption leads to adulteration and often causes the deterioration of quality.

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The present Hungarian qualification methods aiming at to control the visual purity and microbiological state of the pepper important for the foreign trade are not sufficient. They contain information neither on the quality nor on the quantity of the aroma and flavour compounds giving the real value of the spices. The lack of measuring methods capable of the investigation of the aroma-profiles of spicy and medicinal plants makes necessary the thorough research of the relating analytical fields. Elaborating the procedure proper for the determination of the characteristic aroma-structures the next tasks had to be solved:

- forming a steam distillation method that produces a distillate representing the real pepper aroma-character without distortion,
- determining the optimal separation conditions of flavour and fragrance compounds,
- the identification of the compounds of different black and white pepper varieties by HP 5971/A GC-MS,
- creating a new evaluation method for the chromatograms of the individual samples.

In order to know and describe the peppers' aroma-structures more precisely we tried to identify as many compounds as possible. In the samples 61 components could be recognized with a match quality of 70% or better. It has been suggested that by this mode of data handling a whole lot of components will be found individually characteristic of the peppers' cultivated variety. In reality only a few of them, not enough for the recognition existed, e.g. isoterpinolene, camphor, junipene.

The lack of marker compounds initiated a relative spectra creation method to be worked out. The retention times have been converted into RRT-s dividing them by the RT of an every sample compound (in our case trans-caryophyllene, ~26.6 min). The peak areas have been transformed into relative intensity data dividing the individual areas by that of the largest peak. This conversion led to a diagram equal to a relative mass-spectrum from physical point of view. By this solution aroma-chromatograms gained under standardized conditions of sample preparation turned into individual "aroma-spectra" highly characteristic of the cultivated variety (c.v.) and the provenance of the pepper samples. Seven black and white peppers of different origin and c.v. (Indonesian, Malaysian, Brasilian, Vietnamese, Chinese, Muntok, Lampong, Sarawak, Sungai Budi etc.) could definitely be distinguished and identified. Our new method is capable of the control of peppers' identity based on the aroma-profile measurements of the trade samples.

QUALITY ASSURANCE AND QUALITY IMPROVEMENT IN MILK AND DAIRY FOOD PRODUCTION USING UP-TO-DATE MICROBIOLOGICAL TECHNIQUE

J. ERDÉLYI and E. TARDY

Hungarian Dairy Research Institute, Raw Milk Quality Control Laboratory,
H–1113 Budapest, Bartók B. út 120/22. Hungary

Requirements of food are getting more exact throughout the world. Bacteriological contamination decisively determines the qualitative parameters both for dairy and whole food industry.

Determination with traditional methods is time consuming and needs serious laboratory background and special professional knowledge.

Petrifilm (3M, USA) and Hygicult (Orion Diagnostica, Finland) are ready to use testkits, suitable for very important analyses as Total Flora, Coliform, E. Coli, Enterobacteriaceae and Yeast & Mould.

Our Raw Milk Quality Control Laboratory in Budapest – where the number of annual examinations are approximately 14 000 – compared the results obtained by these products with those of the traditional reference standard method. The fecal bacterial contamination in raw milk samples was examined using Petrifilm Aerob Count Plate, Hygicult TPC, E/Gluc (*Enterobacteriaceae* and *E. coli*) vials.

Evaluating the results we found that the MSZ ISO 6610 standard "Determination of total germs at 30 °C by colony counting" and the Petrifilm give well comparable figures.

Hygicult also proved reliable for estimating scale number of several microbes.

These methods are suitable for making a transition between the traditional, extremely labour-consuming methods and those new technologies which require very expensive equipments.

Authors would like to give help to specialists, who work for safe food producing and quality improving.

Acta Alimentaria, Vol. 26 (1), pp. 103–105 (1997)

BOOK REVIEWS

Foods and packaging materials – Chemical interactions

P. ACKERMANN, M. JÄGERSTAD & T. OHLSSON (Eds)

The Royal Society of Chemistry, 1995. ISBN 0-85404-720-4.
XVII + 231 pages

The publication collects the proceedings of an International Symposium on Interaction: Foods – Food Packaging Materials and it was held on 8–10 June, 1994, in Lund (Sweden). The organizers were The Lund Institute of Technology, Lund University and The Swedish Institute of Food Research (SIK) in Göteborg. The editors are well known scientists on the field of food packaging science and technology and the contributors are eminent representatives of institutions from European countries, but authors are from Israel, Japan and USA, too. The publication covers various aspects of interactions between foods and their packaging materials and it helps to survey the consequences of the phenomena on food quality changing during storage.

The papers were ordered in five topics as chapters of the present book. Chemical interaction – General approach is the first part and it has three presentations as overviews of the basic phenomena. The second topic is Sensory problems: Off-taste, Aroma sorption, Flavour scalping. Eight papers are dealing with the aroma sorption of various polymer-based packaging materials and with the methods of aroma compound determinations. The largest part has the title of Mechanical interaction/Barrier properties. It contains twelve papers about the different aspects of food quality and safety if various packaging materials and packages are used. Two papers focused on the edible films as New packaging materials. The last part is the Active packaging as one of the newest trend of packaging science and technology to keep the quality of packed products. Six papers are in the last part of three of them are dealing with the advantages of modified atmosphere packaging in connection with meat products. A subject index helps the effective and rapid work of readers.

This new book of food packaging focusing on the problems of chemical interactions between packaging materials and packed foods contains many practical and theoretical considerations for development with regard to the problems of food quality assurance. It may be well used by the researchers of food and package industries and by the students of universities and high schools to solve an adequate problem.

I. VARSÁNYI

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Food irradiation
A guidebook

M SATIN

Technomic Publishing Company, Lancaster and Basel. 1996, second edition,
ISBN No.1–56676–344–4. XXIII + 211 pages

Probably non of the technologies which are able to improve keeping quality and microbiological safety of food have been so thoroughly investigated and scrutinized by scientists before implementation than food irradiation. Nevertheless, in spite of more than fifty years of systematic research and the abundance of favourable scientific and technological information available, the practical uses of this physical process are still sporadic and creates a lot of controversy. This is mainly due to the psychological deterrent connoted with its name, the activity of vocal groups of some self-appointed consumerists, and the consequent reluctance of marketing experts of the industry to venture into the introduction of irradiated food.

The aim of this book was to provide factual and the same time easy-to-understand information on all aspects of food irradiation. The author, who has a solid scientific background and not only a broad expertise in product development and marketing but also extensive international experience, presently as a leading officer in a subdivision of the Food and Agriculture Organization of the United Nations, is eminently suited to make this very useful contribution. It is a sign for the success of the first edition and a changing attitude towards commercial utilization of food irradiation, mainly due to a increasing concern connected with growing problems of food safety, that this second, extended edition has been published within a relatively short interval between editions.

The first chapter briefly explains the terms of irradiation, radioactivity, the properties and units of ionizing radiations, their basic effects, and the technological fundamentals comparing them with other food processes. A short history of food irradiation research and development, including international projects completes this chapter.

The second chapter deals with the need for and early work on pasteurization of food, well illustrating the difficulties of technology transfer by the history of heat pasteurization of milk, and the relationship between pasteurization and irradiation of specific solid foods.

Chapter 3 illustrates the necessity of alternative food safety measures by the increasing incidences of food-borne diseases and their economic consequences.

Chapter 4 summarizes the reasoning, why use of irradiation should be considered to prevent some food-related infections, with particular emphasis given to radiation processing of poultry and spices.

The fifth chapter deals with the extent of food losses due to various factors, and illustrates the feasibility of irradiation as a viable alternative process for disinfestation of plants and plant products, control of sprouting and germination of tubers, bulbs and malting barley, respectively, or, shelf-life extension of perishable food.

The most important parts of the book are Chapter 6 ("Advocacy Objections to Food Irradiation"), Chapter 7 ("Irradiated Foods and the Consumer") and Chapter 8 ("Irradiation and the Food Industry").

Chapter 6 lists of and appropriately respond to objections of "consumer" advocacy groups and demonstrates that many of the complaints concerning food irradiation are similar to those voiced about heat pasteurization 100 years ago. Additional complaints of anti-irradiation lobbyists, e.g. problems of formation of free radicals and radiolytic products, mutagenic effects, lack of tests for detection of irradiated food,

occupational safety and environmental damage are also discussed and clarified in other sub-chapters in a realistic context.

Chapter 7 summarizes consumer perception of food issues and the encouraging results of various marketing tests on irradiated foods. This chapter deals also with the Government's role in establishing proper food policies, the necessity of legislation and proper consumer information, and the task of media to educate and not mis-inform the public.

Chapter 8, which is a welcome addition in the second edition emphasizes the need of a changing attitude of the food industry towards radiation processing, suggesting careful analysis of risk versus benefits, examining liabilities versus assets using this technology.

Chapter 9 ("Some Final Thoughts") emphasizes that people must be allowed free choice, particularly, when the weight of evidence indicates a benefit for a particular product or technology, as specific applications of food irradiation do.

The concise and well-written book is completed with a list 296 literature references and a detailed subject index. Together with the former "landmark" publications, such as Prof. J. F. Diehl's book on "Safety of Irradiated Foods" published first in 1990, and the WHO's document on "Safety and Nutritional Adequacy of Irradiated Food" published in 1994, this book is a "most" for individuals and organizations who are seeking proper advice and relevant information on food irradiation.

J. FARKAS

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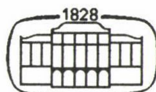

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Acta Alimentaria, Vol. 26 (2), pp. 107–115 (1997)

STUDIES ON DEVELOPMENT OF REDUCED SUGAR APPLE JELLY

VISHAL SINGH BARWAL[a] AND MANORANJAN KALIA

Department of Food Science and Nutrition, HPKV – Palampur, 176 062. India

(Received: 20 March 1996; accepted: 31 October 1996)

Low sugar apple jellies of same sweetness were prepared using non-nutritive sweeteners viz., aspartame, cyclamate and saccharin with sweetness proportion (sucrose equivalent) of 25, 50 and 75 per cent along with sugar. Physico-chemical and sensory observations were recorded at different intervals during storage period of 150 days at ambient conditions. Jelly with 25 per cent aspartame sweetness did not differ significantly from standard jelly. Calorie reduction could be achieved upto 23 per cent per serving without compromising quality. There were no significant differences between jellies sweetened with 25 and 50 per cent cyclamate, 25 per cent saccharin and 50 and 75 per cent aspartame.

Keywords: apple, jelly, non-nutritive sweetener, additive

Apple (*Malus domestica* Borkh) has been grown since time immemorial in many parts of the world and the old adage 'An apple a day keeps the doctor away' rightly illustrates its beneficial therapeutic effects, besides being a source of nutrition. In India, apple is being cultivated in Himachal Pradesh, Jammu & Kashmir and hills of Uttar Pradesh with an annual production of 1.238 million tonnes (FAO, 1994).

Apple is processed into a variety of products (HANG, 1987), amongst which juice concentrate, jam, jelly, candy and dehydrated & canned rings are important. Most of these products are found to be concentrated source of sugars which provide a quick burst of energy and large amount of calories. Unfortunately, more energy present in product become a liability in the present day context because, major part of our society (real consumers) has sedentary habits and excess calorie consumption is considered partially responsible for obesity (ANDERSON, 1989), increased incidence of diabetes mellitus (RIMM et al., 1975) and hypertension (ABRAHAM et al., 1971). In India, every third man and woman above 30 years in the middle class urban population tends to be obese (BERRY et al., 1973). There were 14 million people suffering from diabetes in

[a] Corresponding author: Department of Postharvest Technology, University of Horticulture & Forestry, Solan, 173 230 (HP) India

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1990 in India and this figure is likely to go upto 35 million by year 2000 (DASH, 1995). The major cause of this increase is attributed to the traditional dietary habits.

However, sweet taste is one of the fundamental sensations for which people continue to demonstrate their desire. Deprivation of it leads to a feeling of guilt, discontent and sometimes depression. Keeping in view the above facts, there is an urgent need to develop dietetic products for the benefit of masses in general and sufferers in particular. The present investigation has therefore been undertaken to develop a low calorie dietetic apple jelly for health conscious and obese subjects.

1. Materials and methods

'Golden Delicious' apples were purchased from the local retail market. Non-nutritive sweeteners viz., aspartame (aspartyl phenylalanine methyl ester) and cyclamate (cyclohexane sulfamic acid) were imported from the USA through SIGMA Chemicals Inc.

1.1. Preparation of fruit juice ingredient

Fruits were washed, cut into slices and kept in air for 20 min for enzymatic browning. Fruit juice ingredient was prepared from these slices by boiling with water (1:1) for 25 min and filtered through muslin cloth. The filtrate (fruit juice ingredient) was used for the preparation of jelly.

Standard jelly was prepared using fruit juice ingredient and sugar (3:2) and cooking to the required soluble solids concentration (SSC). Additives viz., pectin, agar-agar and guar gum at 1 per cent level in combination with half the quantity of sugar were used for the preparation of low solids jelly up to calculated SSC (Table 1) to study the setting/gel formation process. After screening additives, dietetic jellies were prepared with different sweeteners as per detail in Table 2. Benzoic acid (0.01%) was dissolved in small quantities in the hot syrup and added to the boiling mix. On attaining required SSC, the jelly was filled hot in pre-sterilized and dried glass jars, sealed with silver foil, capped and kept at ambient conditions (12.95 °C minimum, 20.51 °C maximum and 55.5% RH). Fifteen samples of each treatment were prepared and analysed on 0, 30, 60, 90 and 150 days for storage stability studies.

Table 1

Ingredients used for setting/gel formation process of jelly

Treatment	Fruit juice ingredients[a] (ml)	Sugar (g)	Additive (1%)	Cooking terminated at SSC (°Brix)
T_1	300	200	Pectin	65±1
T_2	300	200	Agar-agar	65±1
T_3	300	200	Guar gum	65±1
T_4	300	100	Pectin	50±1
T_5	300	100	Agar-agar	50±1
T_6	300	100	Guar gum	50±1

[a] SSC (°Brix) = 7.70±0.35; Acidity (%MA) = 0.22±0.03; pH = 3.59±0.37

Table 2

Sweeteners used for preparation of dietetic apple jelly

Treatment	Per cent sweetness equivalent used			Cooking terminated at SSC (°Brix)
	Sucrose sweetness		Non-nutritive sweetness	
JT_1	100 (Standard)		–	65±1
JT_2	75	+	25 (Aspartame)	59±1
JT_3	75	+	25 (Cyclamate)	59±1
JT_4	75	+	25 (Saccharin)	59±1
JT_5	50	+	50 (Aspartame)	50±1
JT_6	50	+	50 (Cyclamate)	50±1
JT_7	50	+	50 (Saccharin)	50±1
JT_8	25	+	75 (Aspartame)	37±1
JT_9	25	+	75 (Cyclamate)	37±1
JT_{10}	25	+	75 (Saccharin)	37±1

1.2. Physico-chemical determinations

SSC (°Brix), pH and titrable acidity [per cent malic acid (%MA)] were determined according to methods outlined by A.O.A.C. (1984). Total solids (TS) were determined by drying in an oven (RANGANNA, 1986) and expressed in percentage. Caloric value was measured in a bomb calorimeter.

1.3. Sensory evaluation

Low solids jellies obtained in setting experiment were evaluated for sensory descriptive attributes by a panel of 10 judges for appearance, texture, cut surface and setting on popular terms (SZCZESNIAK, 1983). The judges were selected at random and were same for all the evaluations. The dietetic jelly samples were presented for evaluation to overall acceptability on a 9-points Hedonic Scale (AMERINE et al., 1965) to a panel of 10-judges.

1.4. Economics

The economics of dietetic jelly prepared with different non-nutritive sweeteners contributing 25 per cent sweetness was worked out based on the cost of all the ingredients plus flat cost of processing. To reduce error, the per unit cost was determined on a bulk (10 kg) basis.

1.5. Statistical analysis

The data were analysed according to the design and procedure of PANSE and SUKHATME (1967).

2. Results and discussion

The fruit juice ingredient fulfils requirements for the preparation of standard jelly (WOODROOF & LUH, 1975) as given in Table 1.

2.1. Effect of additives on descriptive sensory attributes of low solids jelly

Each additive had different sensory qualities than that of T_1 (standard). But T_5 (agar-agar) had more or less similar characteristics as that of T_1 (Table 3). However, T_6 (guar gum) had soft and gummy texture which was disliked by the panelists. Moreover, pectin and guar gum with half sugar level produced mobile and loose, and mobile and syneresis jellies, respectively.

Table 3
Effect of ingredients (additive) on sensory attributes of jelly

Treatment	Descriptive attributes of physical quality			
	Appearance	Texture	Cut surface	Setting
T_1	Transparent, tender	Soft, gel	Immobile, clean	Firm
T_2	Glossy, crumby	Hard, brittle	Immobile, clean	Firm
T_3	Glossy, pasty	Soft, gummy	Immobile, hard	Firm
T_4	Cloudy	Soft, sticky	Flowy, mobile	Loose
T_5	Transparent, crumby	Soft, crumby	Immobile, clean	Firm
T_6	Dull, pasty	Soft, gummy	Mobile	Syneresis

2.2. Effect on SSC

The different treatments (Table 2) produced overall highly significant results for SSC (Table 4). The SSC was highest on the day when jellies were packed in all the treatments, which subsequently decreased during storage. The decrease in SSC could be attributed to Maillard reaction and other chemical reactions of sugars in presence of acid during storage (SHRESHTHA & BHATIA, 1982; YOUSIF et al., 1990). JT_1 (standard) had highest SSC and differed significantly from other treatments (Table 4). JT_2 and JT_4 were non-significant during storage intervals but differed significantly from rest of the treatments. The SSC decreased in finished jelly with increasing sweetness share of non-nutritive sweetener (Table 4), it is because of non-addition of SSC by alternative sweeteners (PANDEY & NIGAM, 1987).

Table 4
Mean SSC (°Brix) of jellies at different storage intervals

Treatment	Days after preparation				
	0	30	60	90	150
JT_1	64.17	63.50	63.00	62.50	62.13
JT_2	59.33	58.67	58.33	58.17	57.77
JT_3	60.17	60.00	59.33	59.17	59.07
JT_4	58.50	58.17	57.67	57.33	57.23
JT_5	50.50	50.00	49.67	49.17	48.67
JT_6	51.33	50.67	50.33	50.33	50.10
JT_7	49.67	49.17	49.00	48.67	48.57
JT_8	37.33	36.83	36.67	36.33	35.77
JT_9	37.83	37.33	37.17	36.50	36.47
JT_{10}	37.00	36.17	35.67	35.67	35.47
CD at 1%	0.96	0.88	1.19	0.69	0.43

2.3. Effect on TS and caloric value

TS and calorie value were highest in JT_1 (standard) and lowest in JT_8, JT_9 and JT_{10} (Fig. 1). Non-nutritive sweeteners have high sweetness intensity and contribute comparatively less to TS and calorie values (GIESE, 1993). Among these, saccharin sweetened treatments had even lower TS and caloric value (Fig. 1). Very little work seems to have been conducted so far to prepare low solid dietetic jellies by use of non-nutritive sweeteners. KOSMARK (1992) had prepared low calorie candy with alternate sweeteners. In the present investigation 25 and 50 per cent level of non-nutritive sweetener's sweetness have reduced caloric value upto 10 and 23 per cent, respectively per serving without compromising quality.

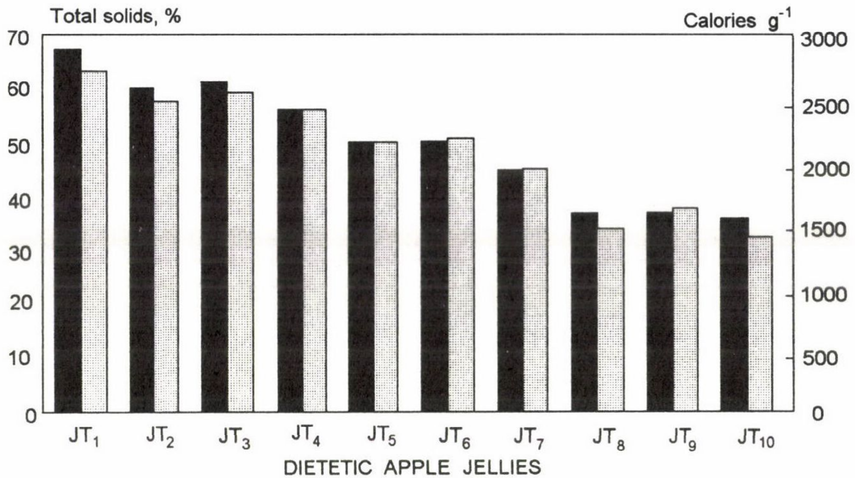

Fig. 1. TS and caloric value of jellies. ■: Total solids; ▒: calories

2.4. Effect on overall acceptability

The highest overall acceptability was obtained by JT_1 and the lowest by JT_{10} (Table 5). JT_1 was statistically non-significantly with JT_2, JT_3, JT_4 and JT_5 during storage period except on or after 90 days of storage for JT_4 and on/or after 150 days of storage for JT_5 where it differed significantly. Even 50 per cent sweetness of non-nutritive sweeteners has produced jellies of above like slightly overall acceptability (Table 5). Among non-nutritive sweeteners, aspartame sweetened jelly was significantly more acceptable than cyclamate and saccharin sweetened jelly.

2.5. Economics of the dietetic jelly

Aspartame sweetened jelly was the costliest followed by cyclamate jelly and saccharin jelly (Table 6). The production cost of aspartame sweetened jelly was higher because of the import of sweetener from USA which included custom duty, postal duty, extra packaging charges and the extremely high value of US Dollar in relation to Indian Rupee (Rs). The per unit cost worked out and reported here for different jellies are for the purpose of comparison, only.

Table 5

Sensory mean score of overall acceptability of jellies at different storage intervals

Treatment	Days after preparation				
	0	30	60	90	150
JT_1	8.8	8.7	8.5	8.5	8.4
JT_2	8.3	8.0	7.7	7.4	7.3
JT_3	7.7	7.3	7.0	6.7	6.8
JT_4	7.3	6.8	6.4	5.9	6.1
JT_5	7.3	6.9	6.6	6.4	6.6
JT_6	6.8	6.2	5.8	5.3	5.1
JT_7	6.2	5.8	5.4	5.2	4.9
JT_8	6.5	5.9	5.6	5.3	5.1
JT_9	5.5	5.2	4.9	4.6	4.6
JT_{10}	5.3	4.8	4.3	4.0	4.3
CD at 1%	2.16	2.27	2.09	2.31	2.16

On a 9-points Hedonic scale: 1 = dislike extremely;
9 = like extremely

Table 6

Economics of dietetic jellies (10 kg) prepared with different sweeteners

Ingredient	Rate (Rs)	Aspartame		Cyclamate		Saccharin	
		Quantity required	Amount (Rs)	Quantity required	Amount (Rs)	Quantity required	Amount (Rs)
Apple fruit	12/kg	10.200 kg	122.40	10.200 kg	122.40	10.200 kg	122.40
Sugar (trust)	16/kg	4.400 kg	70.40	4.400 kg	70.40	4.400 kg	70.40
Aspartame	367/g	9.145 g	2989.21	–	–	–	–
Cyclamate	7.52/g	–	–	48.875 g	367.54	–	–
Saccharin	0.35/g	–	–	–	–	3.258 g	1.14
Agar-agar	1.50/g	100 g	150.49	100 g	150.00	100 g	150.00
Tartaric acid	0.37/g	53.40 g	19.75	53.40 g	19.75	53.40 g	19.75
Sodium benzoate	0.28/g	2 g	0.56	2 g	0.56	2 g	0.56
Glass jar	4.60/jar	20 number	92.00	20 number	92.00	20 number	92.00
Total cost of ingredients			3444.32		822.65		456.25
Processing cost (flat)			100.00		100.00		100.00
Total preparation cost			3544.32		922.65		556.25
Total yield (10 kg) produced			20 jars		20 jars		20 jars
Cost/jar or 500 g: pack			177.22		46.13		27.81

3. Conclusions

– Agar-agar was proved to be a suitable additive for the development of dietetic jelly of suitable descriptive sensory attributes.

– The development efforts had successfully reduced caloric value upto 23 per cent per serving without compromising quality parameters.

– Aspartame was found to be superior in comparison to cyclamate and saccharin for the production of low calorie jelly.

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PREDICTION OF DURUM WHEAT QUALITY FROM ELECTROPHORETIC AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY PATTERN OF GLIADINS

S. A. TAHA

Department of Biochemistry, Faculty of Agriculture, Zagazig University, Zagazig. Egypt

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Gliadin proteins of twenty five durum wheat varieties of diverse origins have been extracted with 70% ethanol and analysed by both acetic acid polyacrylamide gel electrophoresis (AA-PAGE) and reversed-phase high-performance liquid chromatography (RP-HPLC) techniques. Based upon the established unbroken relationship between electrophoretic γ-gliadin bands 45 or 42 with gluten strength or weakness, respectively, the studied varieties have been classified into high (type-45) and low (type-42) quality varieties. Four chromatographic peaks designated P1, P2, P3 and P4, eluted at retention times of 38, 39, 43 and 49 min, respectively, could be used to classify durum varieties into two groups in a way analogous to that in which electrophoresis may be used. This classification depends on the presence or absence of RP-HPLC peaks, namely P3 that corresponds to PAGE 42-γ gliadin band, and peaks P1, P2 and P4 that correspond to 45-γ gliadin band. However, only the relative areas of peaks P1 and P3 were highly correlated with durum quality ($r = 0.68^{***}$ and -0.89^{***}, respectively), suggesting that they correspond to PAGE γ-gliadin bands 45 and 42, respectively. Therefore, and because of its convenience and rapidity, RP-HPLC can be recommended as an analytical procedure alternative to electrophoresis for predicting quality and screening for durum varieties with unacceptably weak gluten characteristics when large numbers of samples must be analysed, as in early generations of wheat breeding programs.

Keywords: durum wheat, quality prediction, gliadin PAGE, RP-HPLC

Assessing the pasta color, pasta-making and cooking quality of durum wheat varieties is very important to the breeders as well as the manufacturers. The ability of a cultivar to be processed into a yellow-amber-color pasta is readily evaluated, but predicting the decisive criteria for cooking quality is still a critical problem, specially during the early generations of breeding programs (FEILLET, 1980; AUTRAN, 1981).

Because protein quantity and quality of durum wheat grains were decisive factors of pasta cooking quality, more attention has been paid, in the last decade, to specific components of storage proteins and their relationships with the elastic recovery and pasta cooking quality.

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Biochemical methods, i.e. poly-acrylamide gel electrophoresis (PAGE) and reversed-phase high-performance liquid chromatography (RP-HPLC) have been recommended for quality assessment and prediction at the breeding stage (PAYNE et al., 1982; HUEBNER & BIETZ, 1987; GALTERIO et al., 1993; FEDERMANN et al., 1994). Such methods also allow better understanding of quality at the molecular level. Several studies have described an unbroken association between durum wheat quality and the presence of specific γ-gliadins: the presence of the electrophoretic component γ-45 is associated with strong gluten, high elasticity, dough strength and superior cooking quality, while the presence of another allele (γ-42) is associated with poor quality properties (DAMIDAUX et al., 1980; KOSMOLAK et al., 1980; DU CROS et al., 1982; PAYNE et al., 1984; TAHA & SÁGI, 1987; AUTRAN & GALTERIO, 1989a; 1989b; WILLIAMS et al., 1990; FEDERMANN et al., 1994; KAAN et al., 1993). Many investigations have been also carried out on the relationships between durum wheat pasta quality parameters and the electrophoretic patterns of low molecular weight (LMW) glutenin subunits (AUTRAN & GALTERIO, 1989a; 1989b; GALTERIO et al., 1991; 1993; FEDERMANN et al., 1994; KOVACS et al., 1995) and/or high molecular weight (HMW) glutenine subunits (KOVACS et al., 1993; GALTERIO et al., 1993; KAAN et al., 1993). These electrophoretic procedures are useful since they require small amounts of sample; however, they are time-consuming and are restrictive when rapid analyses are desired or when large numbers of samples must be studied.

Recently, an alternative method, RP-HPLC, have successfully been applied in separating complex mixtures of cereal proteins (BIETZ, 1983; HUEBNER & BIETZ, 1987; MARCHYLO et al., 1989). The relative amounts of ω-gliadins, α-, β-, γ-gliadins, HMW and LMW glutenin subunits were quantified as a new tool for wheat variety identification (BIETZ et al., 1984b; BIETZ & COBB, 1985) and/or for potential quality prediction (BURNOUF & BIETZ, 1984; HUEBNER & BIETZ, 1987; MARCHYLO et al., 1989). Thus it was suggested that RP-HPLC could be used for quality prediction of wheat during breeding stages.

The purpose of this work was to develop a new tool that facilitates the accurate prediction of pasta cooking quality in durum wheat cultivars grown in Egypt using both PAGE and RP-HPLC patterns of gliadin proteins. The biochemical and technological quality attributes as well as their interrelationships of these cultivars have been evaluated and reported elsewhere (TAHA, 1996b).

1. Materials and methods

1.1. Grain samples

Twenty five varieties of durum wheat (*Triticum durum Desf.*) were used in this study. Four Egyptian varieties were obtained from the Seed Division, Ministry of Agriculture, Cairo, Egypt. The remaining 21 varieties have been supplied by ICARDA, and adapted under the Egyptian conditions during 1993 and 1994 in the nursery of the faculty of Agric. Zagazig Univ., Egypt. Of these 21 varieties (harvested in 1994), 12 were from Syria, 6 from Mexico and 3 from Libya. Information on the chemical composition, gluten strength, rheological and cooking qualities of the most of varieties have been described elsewhere (TAHA, 1996b).

1.2. Gliadin extraction

Kernels were milled using a Cienceware, Bel-Art Products micro mill before extraction. Samples (300 mg) of flour were extracted with 1 ml of 70% ethanol. After brief agitation in a vortex mixer, the suspensions were left to stand at room temperature for 1 h, then centrifuged at 4000 r.p.m. for 20 min. The clear supernatant was transferred into a 2 ml sealed vial and stored at –20 °C.

1.3. PAGE

Two drops of glycerine containing the tracking dye pyronine-G were added to a 400 µl of gliadin extracts used for continuous acetic acid PAGE according to the procedure of CLEMENTS (1988) as modified by TAHA (1992). The electrophoresis apparatus, power supply, gel preparation, electrophoretic conditions, staining and destaining procedures have been described in detail elsewhere (TAHA, 1996a). Briefly, the gel solution (100 ml) of 12% total acrylamide with 3% cross linkages (12% T, 3% C) was polymerized in the precooled gel former (25 cm wide × 15 cm long × 1.5 mm thick) and the comb was inserted immediately. A DESAPHOR VA vertical slab gel electrophoresis cell consisting of two buffer reservoirs of 1.5 l (upper) and 5 l (lower), with a circulating bath and power supply (500/500) was used. Buffers containing only acetic acid 1.6 ml l^{-1} and 2.6 ml l^{-1} were poured into the upper and lower reservoirs, respectively. A layer of 10 µl of gliadin extracts was loaded onto wells. Gels were electrophoresed for 30 min at 10 mA per gel followed by 7 h at 20 mA per gel with temperature maintained at 20 °C. Gels were then fixed and stained in 250 ml of 12% trichloroacetic acid containing 10 ml of aqueous 0.5% Coomassie Brilliant Blue R 250 for 2–3 days. Gels were destained with two short (2–3 min) rinses in 50% aqueous methanol/10% acetic acid followed by agitation in 5% aqueous methanol/7% acetic

acid until the gel background became clear for photography (2–4 h). Gliadin components were numbered using the nomenclature of BUSHUK & ZILLMAN (1978), choosing band 51 as a reference.

1.4. RP-HPLC

The remaining 200 μl of gliadin extracts were membrane-filtered into HPLC autosampler vials and the gliadin proteins were analyzed according to the RP-HPLC procedures of BIETZ (1983), BURNOUF & BIETZ (1984) and BIETZ et al., (1984a, 1984b). An HP 1090 M HPLC apparatus equipped with an automatic sample injector, an autosampler, a PV 5 solvent-delivary systems controlled by a solvent programmer, a temperature controlled column compartment and a variable-wavelength detector was used. A 250×4.1 mm Synchropak RP-P column (C 18) was used for analytical runs. Chromatography was carried out using mixtures of two solvents, designated A and B, which contained acetonitrile (ACN), trifluoroacetic acid (TFA) and water. Solvent A was 15% ACN + 0.1% TFA, whereas solvent B was 80% ACN and 0.1% TFA. Solvent pH was approximately 2.2.

The column was equilibrated with a solvent mixture containing 80% solvent A and 20% solvent B. The proteins were eluted using a linear 55 min gradient from 80% solvent A + 20% solvent B to 45% solvent A + 55% solvent B, followed by a 10 min hold at final gradient conditions (total run time = 65 min). Column temperature was 31 °C and flow rate 1 ml min^{-1}. Components eluted from the column were detected continuously by measuring the absorbance at 210 nm. Data were recorded and simultaneously stored in a computer system for subsequent integration and replotting.

1.5. Statistical analyses

Linear regression analyses were performed by a computer program according to SVÁB (1981).

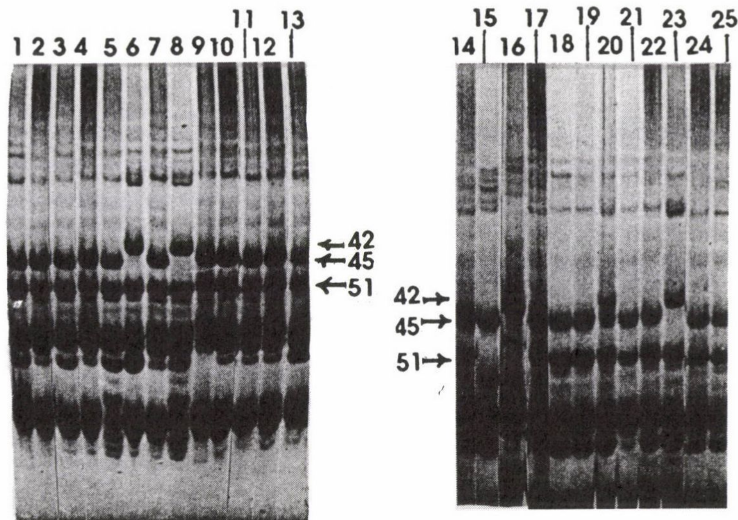

Fig. 1. Continuous acetic acid PAGE patterns of gliadins extracted from 25 durum wheat varieties grown in Egypt. The positions of bands 42 and 45 as well as of band 51, which is used as a reference, are indicated.

1 – Brachoua 2 – Om Rabi 5 3 – Tensift 1 4 – Om Rabi 9 5 – Cedifla
6 – Chahda 88 7 – Ruff 8 – Cham 1 9 – Snip/Fg/Ato 10 – Om Rabi 3
11 – Awl 1/Mrb 20 12 – Awl 2/Bit 13 – Ru/Mrb 12 14 – Stork-s 15 – Belik H2
16 – Gueraru 1 17 – Lahn 18 – Haurani 19 – Bani Sweaf 1 20 – Suhag 2
21 – Suhag 1 22 – Ofm/Somo 23 – Daki 24 – Ru/Mrb 15 25 – Cham 3

2. Results and discussion

2.1 PAGE patterns

Gliadin PAGE patterns (electrophoregrams) of 25 durum wheat varieties grown in Egypt are shown in Fig. 1. The gliadin components 42-γ and 45-γ were identified on the figure choosing band 51 as a reference (BUSHUK & ZILLMAN, 1978). It is now demonstrated that presence of the 45-γ gliadin component is linked to strong gluten and consequently to a high cooking quality wheat variety, whereas the presence of the 42-γ gliadin component is linked to a weak gluten and poor quality variety (DAMIDAUX et al., 1980). This suggests that durum wheat cultivars can be classified into either strong-gluten or weak-gluten types if their gliadin PAGE electrophoregrams contain γ-gliadin bands 45 or 42, respectively (KOSMOLAK et al., 1980). Strong-gluten varieties generally produce pasta with superior cooking quality. Results showed that the majority of the examined varieties contain 45-γ gliadin band. Only five varieties (lanes 6, 8, 16, 20 and 23) contain 42-γ gliadin band. Both 42-γ and 45-γ bands are present in two cultivars of

them (lanes 16 and 20). The studied varieties have been classified according to their PAGE patterns into 45-γ type and 42-γ type as listed in Table 3.

FEILLET (1980) suggested that this relationship could be due to : 1) a linkage of genes coding for 45-γ (or 42-γ) and for components responsible for quality; or 2) a functional type of relation in which the presence or absence of these gliadin bands affects the pasta behavior during cooking. In an inheritance study, DU CROS & HARE (1985) found that dosage effect of both alleles controlling gliadin bands 45-γ and 42-γ were present, however, band 42 allele displayed a greater degree of dominance than band 45 allele. This could explain why the varieties containing both bands have poor gluten quality inspite of the presence of band 45 even at a relatively higher concentration compared to band 42.

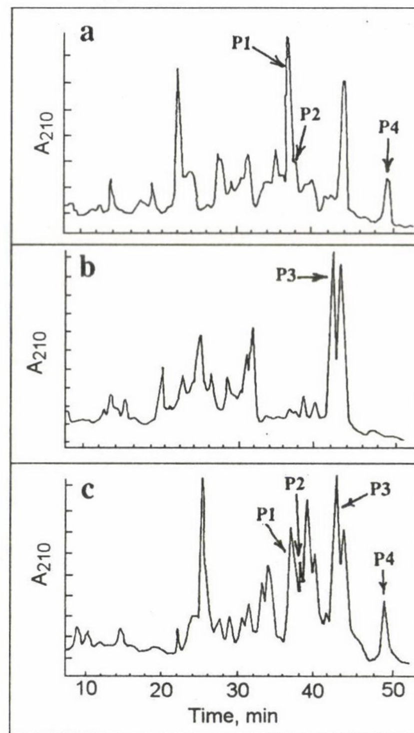

Fig. 2. RP-HPLC separation of gliadins extracted by 70% ethanol from durum wheat varieties (a) Cedifla (b) Cham 1 and (c) Suhag 2. The chromatographic conditions were as described in experimental section. Peaks P1, P2, P3 and P4, which distinguish durum varieties on the basis of gluten strength and cooking quality, are indicated by arrows.

2.2. RP-HPLC patterns

Figure 2 shows, for example, three RP-HPLC patterns of gliadins extracted from the durum varieties Cedifla, Cham-1 and Souhag-2. On average, 20–23 major peaks were observed in each chromatogram, out of a total of about 70 major and minor peaks detected by the computer system. Three distinct types of pattern could be distinguished among all durum varieties. The first type was characterized by peaks P1 (eluted at a retention time 38 min), P2 (39 min) and P4 (49 min) (Fig. 2a). The second type contained only peak P3 (eluted at a retention time of 43 min) (Fig. 2b), and the third contained all peaks of both first and second types, i.e. P1, P2, P4 and P3 (Fig. 2c). The first and second types of patterns often mutually occurred. None of the varieties examined was found to have any other combinations of peaks P1–P4; suggesting that proteins from P1, P2 and P4 may be coded for by linked genes on the same chromosome, whereas that of P3 may be coded for by an allelic form of one of these genes. The presence of peaks P1, P2 and P4 together with P3 (type 3) occurs as a result of hybridization between type 1 and type 2. These results are in a good agreement with those obtained by BURNOUF & BIETZ (1984) and HUEBNER & BIETZ (1987), except that they have classified the RP-HPLC patterns into only two types corresponding to the PAGE 45-γ and 42-γ types; so they have reported that the two types of patterns were mutually exclusive. This could be explained on the basis that the varieties examined by them probably were free from any mixed genotypes.

Table 1

Correlation coefficients between parameters and major RP-HPLC peak areas of durum wheat varieties

Quality parameters	RP-HPLC peak area % of total area				
	P1	P2	P3	P4	P0
Total protein content	0.24	0.10	0.16	–0.04	–0.32
Water insoluble protein	0.15	0.30*	–0.02	–0.001	–0.32
0.5 mol NaCl insoluble protein	0.22	0.36**	0.04	0.01	–0.30
70% ethanol insoluble protein	0.25	0.38**	–0.13	–0.03	–0.16
0.1 mol acetic acid insoluble protein	0.23	0.34**	–0.19	–0.06	–0.13
SDS-sedimentation volume	0.49***	0.46***	–0.19	0.22	–0.23
Mixograph developing time	0.28*	0.35**	–0.24	0.21	–0.12
Mixograph maximum consistency	0.49***	0.28*	–0.05	0.14	–0.20
Mixograph curve area	0.48***	0.34**	–0.15	0.25	–0.28
Alveograph value (normal cooking)	0.24	0.34**	–0.22	0.01	–0.001
Alveograph value (overcooking)	0.23	0.41***	–0.21	0.13	–0.10
Alveograph total value	0.24	0.39**	–0.23	0.07	–0.05

Correlation coefficients above 0.393, 0.304 and 0.257 are significant at ***$P<0.01$, **$P<0.05$ and *$P<0.1$ respectively

The correlation coefficient values between quality parameters and RP-HPLC areas of the investigated varieties (Table 1) show that P1 and P2 positively correlated with quality parameters while P3 negatively correlated. However, P2 shows highly significant correlations with most of quality parameters, while P1 shows only highly significant correlations with gluten strength (SDS-sedimentation test) and mixing properties (developing time, maximum consistency, curve area). All negative correlation values of P3 with quality parameters were not significant.

Grouping durum varieties according to RP-HPLC peaks P1–P4 corresponded exactly to that based on the presence of band 45-γ or 42-γ in PAGE patterns (Fig. 1). Durum varieties having PAGE 45-γ gliadin band exhibited RP-HPLC peaks, P1, P2 and P4, whereas those having PAGE 42-γ gliadin band exhibited RP-HPLC peak P3. Thus, the presence or absence of peaks P1–P4 in RP-HPLC patterns can be used in a way analogous to that involving the presence or absence of γ-gliadin bands 45 or 42 in PAGE patterns to segregate durum varieties according to gluten strength and cooking quality. Finding three peaks (P1, P2 and P4) in the type 45 varieties, suggests that several gliadins, and not just band 45, may be associated with quality as demonstrated by Du Cros and co-workers (1982).

2.3. Prediction of pasta cooking quality

The division of durum wheats on the basis of chromatographic peaks is identical to that based on γ-gliadin electrophoretic bands 45 and 42, which predicts durum gluten strength and weakness, respectively. The studied varieties, listed in Table 3, were divided, accordingly, into high quality varieties (45-γ type) and poor quality varieties (42-γ type). Durum gliadins extracted from 21 varieties were fractionated by RP-HPLC and their chromatograms are shown in Fig. 3. It is clear that chromatograms of poor varieties (PAGE 42-γ type) are characterized by the presence of major peak P3 and absence of peaks P1, P2 and P4, whereas chromatograms of high quality varieties (45-γ type) are characterized by the presence of P1, P2 and P4 and absence of P3.

Table 2

Correlation coefficients between the relative areas of five major RP-HPLC peaks for 21 durum varieties

	P1	P2	P3	P4	P0
P1	1.00	0.266	−0.681***	0.563**	−0.251
P2		1.00	−0.541*	0.165	−0.080
P3			1.00	−0.433*	0.057
P4				1.00	−0.348
P0					1.00

Correlation coefficients above 0.665 are very highly significant (***P<0.001), those over 0.549 are highly significant (**P<0.01), and those over 0.4329 are significant (*P>0.05)

Table 3

Correlations between RP-HPLC peak areas and quality of durum varieties classified according to PAGE and RP-HPLC

Durum variety and source[a]		Quality[b]	RP-HPLC peak area% of total area				
			P1	P2	P3	P4	P0
45-γ Type							
Brachoua	(S)	1	9.2	10.6	1.2	0.1	14.6
Om Rabi-5	(S)	1	12.0	4.8	0.9	2.3	30.9
Tensift-1	(M)	1	16.7	3.7	0.8	6.9	10.6
Om Rabi-9	(L)	1	13.1	4.4	0.3	1.8	13.6
Cedifla	(S)	1	11.4	3.3	0.2	2.5	11.3
Ruff	(S)	1	5.5	4.2	0.3	2.3	11.5
Snip/Fg/Ato	(M)	1	14.8	5.0	0.3	2.5	12.4
Om Rabi-3	(L)	1	11.3	6.0	3.0	2.6	12.3
AWl 1/MRB20	(S)	1	14.6	3.6	2.2	1.9	12.8
AWl 2/BIT	(S)	1	9.2	9.2	2.3	2.4	13.2
Ru/MRB 18	(S)	1	9.4	4.5	2.4	2.0	11.2
Stork-S	(E)	1	–	–	–	–	–
Belik H2	(S)	1	–	–	–	–	–
Lahn	(M)	1	–	–	–	–	–
Haurani	(S)	1	–	–	–	–	–
Beni Sweaf-1	(E)	1	15.2	4.5	1.3	0.9	15.0
Suhag-1	(E)	1	13.4	10.6	0.2	2.4	13.7
Ofm/Somo	(M)	1	10.7	3.7	3.1	2.6	13.3
Ru/MRB 15	(S)	1	20.2	4.0	1.8	1.5	11.2
Cham-3	(M)	1	10.0	10.0	0.01	3.4	12.8
42-γ Type							
Chahda-88	(M)	0	1.5	1.0	13.4	0.01	17.0
Cham-1	(S)	0	1.4	2.9	13.1	0.1	14.3
Guerau-1	(S)	0	12.7	4.0	13.4	3.8	6.5
Suhag-2	(E)	0	2.9	1.8	13.0	0.3	17.8
Daki	(M)	0	1.9	2.3	14.0	0.1	17.0
Correlation coefficient	(r)		0.675***	0.330	–0.891***	0.369	–0.049

[a] S, L, M and E mean that the origin country of variety is Syria, Libya, Mexico and Egypt, respectively

[b] Arbitrarily defined as 1 = good and 0 = poor quality

*** Very highly significant (P>0.001)

RP-HPLC generates a large amount of data for each sample. Both qualitative and quantitative differences existed among chromatograms, and each possessed a unique pattern. Thus, computers are necessary to adequately store data, perform integrations

and calculations, detect nonobvious relationships to quality, and determine correlation coefficients through linear or multiple regression analysis. Correlation coefficient values presented in Table 2 confirm the association of RP-HPLC peaks P1, P2 and P4. Only P1 and P4, however, showed significant association to each other (r=0.563**). P3 was negatively correlated with each of P1, P2 and P4. These correlations were highly significant with P1 (r=0.681***), but less significant with P2 and P4 (r=–0.541* and –0.433*, respectively), supporting the hypothesis that the occurrence of the two types is mutually exclusive (BURNOUF & BIETZ, 1984; HUEBNER & BIETZ, 1987).

The data in Fig. 3 and Table 3 reveal that the RP-HPLC computer data can relate to durum wheat quality. Relative areas of five major peaks (P1, P2, P3, P4 and P0 in Fig. 3, eluted at retention times of 38, 39, 43, 49 and 45 min, respectively) in 21 varieties were determined and illustrated in Table 3. The relative areas were correlated with quality by linear regression analysis. The major peak P0 (not related to quality) was very poorly correlated with quality (r=–0.05). Peaks P2 and P4 correlated to quality but not significantly (r=0.33 and 0.37 respectively), whereas peaks P1 and P3 correlate highly with quality (r=–0.68***, and –0.89*** respectively). Thus, RP-HPLC peaks P1 and P3 correspond to PAGE γ-gliadin bands 45 and 42 respectively. Therefore, the segregation of durum cultivars can depend on the presence or absence of the two chromatographic major peaks, P1 and P3 (Fig. 3) for superior and inferior quality, respectively. So, RP-HPLC of gliadin proteins can be recommended as an analytical procedure for predicting durum quality during early generations of wheat breeding, and could be used as a routine test for pasta processing quality.

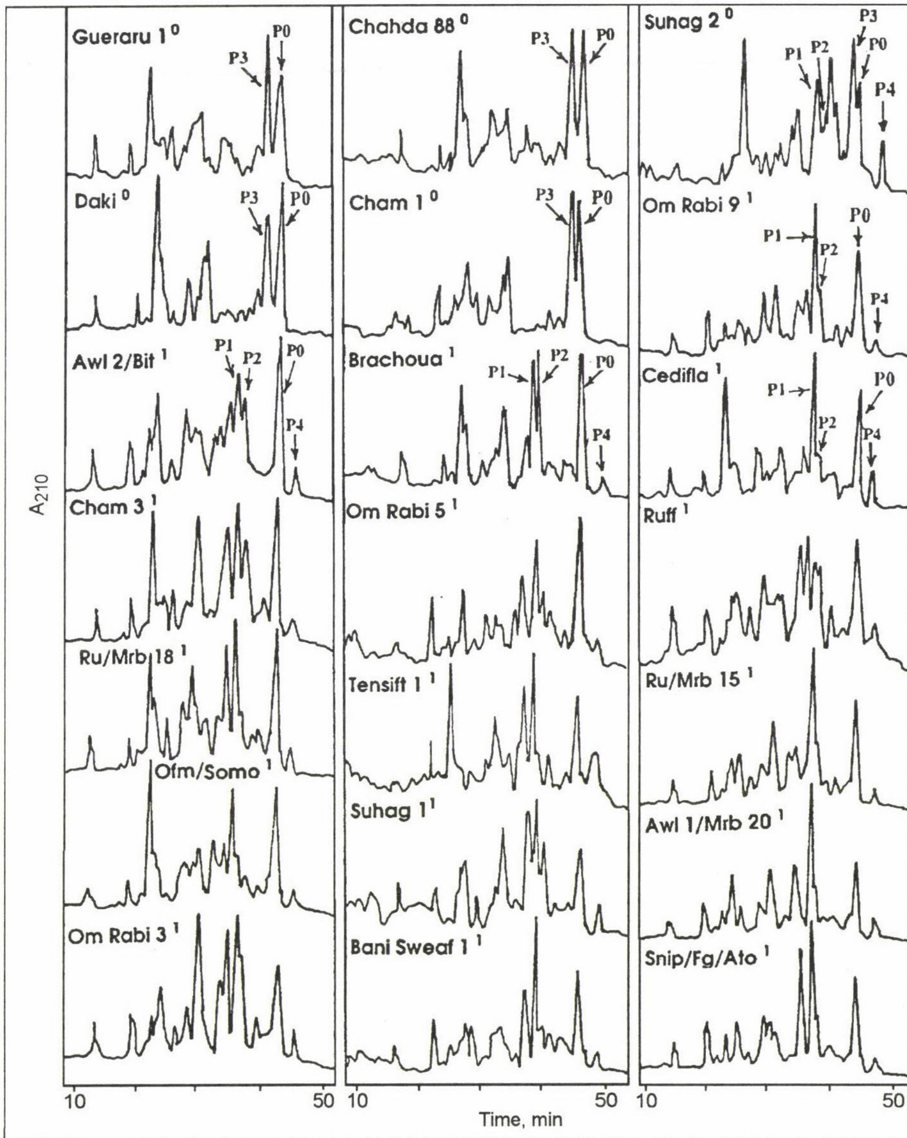

Fig. 3. RP-HPLC patterns of gliadins extracted with 70% ethanol from low (0) and high (1) durum wheat varieties. Chromatographic conditions were as described in experimental section. Major peaks P1, P2, P3, P4 and P0 are indicated by arrows. Low quality varieties (42-γ gliadin type) are characterized by the absence of P1 and presence of P3, whereas high quality varieties (45-γ gliadin type) are characterized by the presence of P1 and absence of P3

3. Conclusions

The results obtained demonstrate that durum wheats can be divided into two groups of varieties according to RP-HPLC as well as PAGE of gliadins. This classification depends on the presence or absence of chromatographic peaks, namely P3 that corresponds to electrophoretic gliadin band 42-γ, and peaks P1, P2, P4 that correspond to electrophoretic gliadin band 45-γ, suggesting that several gliadins, and not just band 45, may be associated with quality. Furthermore, it has been established that RP-HPLC peaks P1 and P3 correspond to PAGE γ-gliadin bands 45 and 42, respectively.

The main objective of this study was to distinguish between the varieties having poor quality (band 42 present, 45 absent) and those having superior pasta quality (band 42 absent, 45 present). Thus, classification of durum varieties depending on RP-HPLC is exactly analogous to that depending on PAGE patterns. Because the PAGE band patterns offer a way for eliminating the weak varieties, it is apparent that the RP-HPLC data can also be used to segregate durum varieties based on the presence and absence of peaks P1 and P3 for high and low quality, respectively. Computer analysis is necessary to fully use the large amount of data generated by RP-HPLC. The segregation of durum wheat varieties according to RP-HPLC data agreed with the published results of DAMIDAUX and co-workers (1980) and with quality evaluation of the studied varieties by SDS-sedimentation test, mixograph analysis and aleurograph test (TAHA, 1996b). Relative areas of P1 and P3 correlate highly with quality (0.68 and –0.89, respectively).

Although PAGE is considered as a useful tool for prediction of durum quality at breeding stage, RP-HPLC technique can be recommended as a valuable tool for half kernel analysis in durum wheat breeding. It is easily performed, rapid and allows numerous determinations per day. Furthermore, HPLC can be automated and requires less technical attention than does electrophoresis, enabling analysis to be performed unattended overnight or during weekends. So, it may be more convenient than electrophoresis for breeding purposes. As previously proposed for PAGE, RP-HPLC can also be used to predict pasta cooking quality in order to quickly eliminate the undesirable progeny and select lines having superior cooking quality at the early generations of breeding programs. Nevertheless, RP-HPLC technique needs a very expensive computerized apparatus and with the models of relatively lower costs, there is no possibility to run parallelly a large number of samples as can be made by PAGE.

*

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HYGROMETRIC EVALUATION OF PERMEABILITY OF PLUM CUTICLE AFTER PRETREATMENTS TO DRYING

D. MASTROCOLA[a], M. DALLA ROSA and C. R. LERICI

Dipartimento di Scienze degli Alimenti, Università degli Studi, Via Marangoni, 97, 33100 Udine, Italy

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A non destructive hygrometric measuring procedure capable of quantifying in advance the effectiveness of different pretreatments to whole fruit drying was developed. The results obtained with hygrometric measurements were verified by monitoring the drying time of plum samples submitted to the same pretreatments.

The permeability evaluation required only a short time for the analysis (20 min) and, under our experimental conditions, had a good repeatability and discriminated between samples with slight differences in permeability.

From hygrometric data a "permeability index" (b') was calculated. The sample submitted to dipping in NaOH solution showed b' values 5 times higher than raw sample.

The products which had a higher skin permeability also showed the shortest drying times. In particular, raw plums ranged between 940 and 960 min plums dipped in ethyl oleate emulsion between 760 and 820 min and plums dipped in hot NaOH solution between 630 and 670 min. In this way, the hygrometric tests could be used to predict the drying behaviour of whole fruit.

Keywords: plums, dehydration, hygrometry, permeability, cuticle

All fruits of higher plants are covered by cuticle. This extracellular structure is tightly bound to the outer cell wall of the epidermis. Its thickness varies between several hundred nm and 10 μm or more (BAKER, 1982; BECKER et al., 1986; LENDZIAN & KERSTIENS, 1991). The framework of the cuticle is constituted of cutin, a three-dimensional polymer of various long chain substituted aliphatic acids. The soluble cuticular lipids are a secondary characteristic feature of all cuticles. These wax-like substances are embedded within the cutin matrix and to a varying degree on the outer surface of the cuticle (LENDZIAN & KERSTIENS, 1991).

Cuticle represents a barrier to water and gas permeation; however, it is not impermeable. Depending on the plant species, cuticles show characteristic permeability to water and gases, and they also reduce the leaching of ions and metabolites from the interior to the surface (LENDZIAN, 1984).

[a] To whom correspondence should be sent. Fax number: 0039 432 501637

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The transport of gases and vapour through cuticles occurs, in the absence of stomatal pores (e.g. many fruit surfaces) and cracks, as an activated diffusion, a combination of absorption, diffusion and desorption (SCHONHERR, 1982; LENDZIAN & KERSTIENS, 1991). Obviously, the direction and rate of transport through the cuticle depends on the concentration gradient of gases on both sides.

During the post-harvest storage, the barrier function of the cuticle is very important in prolonging the shelf-life of fresh fruit. In the case of whole fruit subjected to drying processes, the presence of a waxy cuticle appears to be a relevant resistance to the water loss, leading to negative effects on drying time and, consequently, on process economy. In this case, it should be advantageous to submit the whole fruit to pretreatments able to increase the mass transfer during drying (PONTING & MC BEAN, 1970; BARBANTI et al., 1995).

Generally, the effectiveness of drying pretreatments is indirectly and "a posteriori" evaluated by considering their effects on drying time, but, as is well known, drying time is influenced by many other processing conditions such as: air moisture, temperature and velocity; product load, shape and specific surface area (DAUDIN & BIMBENET, 1985; BARBANTI et al., 1994, 1995).

On the other hand, the determination of water permeance of isolated fruit cuticles involves very long procedures and isolated cuticle do not reflect the barrier properties of non isolated cuticles in vivo (SCHONHERR, 1976, 1982).

The present research work was undertaken to develop an accurate and non destructive hygrometric measurement technique to quantify in advance the effectiveness of different pretreatments to drying. The results obtained by hygrometric measurements were verified by recording the drying time of plum samples submitted to the same pretreatments.

1. Materials and methods

1.1. Materials

Trials were performed on medium sized plums (*Prunus cerasi*, cv. Stanley) at the same stage of ripeness. The fruits were obtained during the 1994 summer season from the experimental centre "Mario Neri", located in Imola, Emilia Romagna, a central-north region of Italy.

For each fruit, the major and minor axis was measured in order to calculate the surface area and volume by using the equations of a prolate spheroid (solid formed by the rotation of an ellipse about its major axis) (PERRY et al., 1963; BARBANTI et al., 1994).

The trials were carried out on fruit with the same average surface area (59.1± 1.9 cm^2) and volume (23.6±0.7 cm^3). Fruit with cracks on the cuticle were not used in the experiments.

1.2. Sample preparation

Fruit was stored at 4 °C until use. They were then removed from the refrigerator and equilibrated at room temperature for at least two hours.

Two different pretreatments to drying were carried out (PONTING & MC BEAN 1970):

– dipping in an aqueous solution of sodium hydroxide (Carlo Erba RPE) (1:50 w/v) for 10 s at 90 °C, followed by equalisation in a solution of citric acid (Carlo Erba RPE) (1:5:50 w/v) at 20 °C for 2 min (called: "NaOH dip");

– dipping in an emulsion of ethyl oleate (Carlo Erba FU-BP) (30 g/l), potassium carbonate (Carlo Erba RPE-ACS) (25 g/l) and distilled water for 10 min (called: "oil dip") and then drying in air flow (25 °C) for 10 min.

Some fruits were hand peeled by knife.

1.3. Evaluation of fruit cuticle permeability by hygrometric measurements

When a whole fruit is placed in the measuring cell of an electric hygrometer, the time required by the instrument to give a constant value of relative humidity (RH) depends on the rate of migration of water vapour from the fruit pulp to its head space and, consequently, on the permeability of the barrier (cuticle) placed in the fruit pulp/ambient atmosphere interface.

The assumption of our research was that, in the case of a wet coated product, the rate of RH(%) is increasing and consequently the attainment of hygrometric equilibrium in a small closed environment is directly correlated with the permeability of the coating to the water vapour.

The other factors which influence the values of the relative humidity and the rate of attainment of the hygrometric equilibrium are:

– temperature;
– surface area of the sample;
– integrity of the fruit cuticle;
– cell volume fruit volume ratio.

In our trials, water vapour permeance of plum cuticle was determined by calculating the rate of RH(%) increase in the hygrometer cell containing the sample during the first 20 min of measurement. In fact, in this period was observed the highest rate of RH(%) increase whereas, continuing the measurement up to the equilibrium, very low increase of relative humidity was noticed. Under our experimental conditions,

in order to reach the equilibrium (no change in hygrometric value at least for 20 min) about 3–4 h was needed.

In this way the permeability evaluation required only a few minutes and did not involve the destruction of the sample, that could be used afterwards for other measurements, too.

1.3.1. Apparatus. A hygrometer ROTRONIC – HYGROSKOP DT equipped with a ROTRONIC DMS-100 measuring cell (Pool Bioanalysis Italy, PBI – Milan – Italy) thermostated at 25 °C and connected to a recorder (LKB – BROMMA 2210 – Milan-Italy) was used for measurements. The hygrometer was placed in a chamber (50 ×50×40 cm) maintained at constant relative humidity (22%) with saturated solution of potassium acetate (Carlo Erba RPE).

The instrument was calibrated with saturated solutions of different salts of known water activity.

Before each measurement, the empty cell was equilibrated to the relative humidity of the chamber. After placing the sample, the cell was hermetically closed and the recording of relative humidity (RH in %) started.

The variability of the hygrometric measurements was estimated by measuring each sample ten times and the average value of coefficient of variation was 2.4%.

1.3.2. The "permeability index". The permeability (b) can be expressed by the following equation (KAMPER & FENNEMA, 1984; KESTER & FENNEMA, 1986; PASCAT, 1986; HAGENMAIER & SHAW, 1991):

$$b = \frac{QLS}{A\,\Delta pt} \tag{1}$$

where:

S: solubility coefficient;

Q: amount of penetrating element;

A: surface of the membrane;

L: thickness of the membrane;

t: time;

Δp: vapour pressure gradient between the two surfaces of the membrane.

In case of water vapour, using dW/dt to indicate the rate of diffusion across the film, the equation can be written as follows:

$$b = \frac{L\,S\,dW}{A\,\Delta p\,dt} \tag{2}$$

If the driving force (Δp) is expressed as the difference between the relative humidity at the equilibrium (RHeq: RH values when no hygrometer display change was observed at least for 20 min) and the actual relative humidity (RH), the rate of diffusion of the water could be expressed by the rate of change of the relative humidity (dRH/dt).

Combining all the constants, the equation becomes:

$$b' = \frac{(dRH / dT)}{(RHeq - RH)} \quad (3)$$

where b' the "permeability index" of a film with surface A and thickness L and solubility coefficient S, is a function of temperature and total pressure.

1.4. Drying trials

A laboratory cabinet drier (Memmert mod. ULE 400 Schwabach-Germany) was used to air-dry the plums. Air temperature was maintained at 70 °C during the entire process and the plums were arranged in a single layer. Specific drying kinetics were evaluated by weighing samples at different time intervals throughout the drying cycle. This drying cycle was repeated three times for each sample and the average value of coefficient of variation was of 3.1%.

Water content determination was carried out in a vacuum oven for 8 h at 70 °C (A.O.A.C., 1980).

2. Results and discussion

In Fig. 1 the curves indicating the increase of relative humidity (RH%) during recording time in the head space of the measuring cell containing raw plums, those submitted to different pretreatments and peeled ones are reported. It is evident that the RH(%) values of the "peeled" sample, for the entire length of the reported measurement period, was significantly higher than those of the raw sample. This behaviour highlights the barrier role of the cuticle and the ability of the hygrometer to show this phenomenon.

In case of samples with the same specific surface, the rate of variation of RH(%) in the head space of a whole fruit also allows the evaluation of the effectiveness of some drying pretreatments performed to increase skin permeability. In fact the curves plotted in Fig. 1 clearly show how the effect of dipping in hot NaOH solution led to a remarkable increase in RH(%) values. The fruit submitted to dipping in ethyl oleate had a slight increase of relative humidity in the measuring cell. This behaviour could be linked to the presence of pruin – a non-continuous layer of wax-like crystals – on the plum cuticle which would decrease its permeability. The "dipping oil" fills the spaces between the wax crystals and so makes pruin more hydrophylic (PONTING & MC BEAN, 1970).

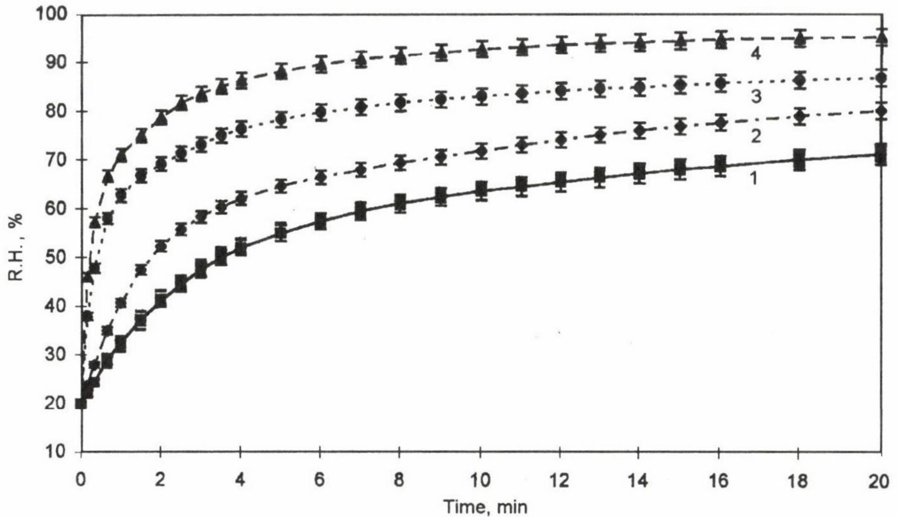

Fig. 1. Increase of relative humidity (RH%) vs recording time in the measuring cell containing raw and differently pretreated plums. 1: Raw; 2: oil dip; 3: NaOH dip; 4: peeled. Bars represent the standard deviation of the mean of ten replications

The narrowness of the standard deviation intervals, reported as bars in Fig. 1, clearly demonstrates the good repeatability of the hygrometric measurements.

The rate of change of the relative humidity in the measuring cell (dRH/dt) as a function of driving force – the difference between the relative humidity at equilibrium (RHeq) and actual relative humidity (RH) – are plotted in Fig. 2. The curves have a linear portion in accordance with equation 3 (Fig. 2). For values of RHeq-RH lower than 30–40 the dRH/dt ratios in all samples tended to zero because the changes of RH near the equilibrium are extremely slow and not entirely dependent on the cuticle permeability.

The slopes (b') of the linear portions of the curves plotted in Fig. 2, calculated by linear regression, together with the correlation coefficients, are reported in Table 1. The regression slopes, referring to homogeneous material and arising from measurements carried out under the same conditions, could be considered "permeability indicators" of the plum cuticle.

Table 1

Permeability index (b') of plum skin and correlation coefficient (r^2) of linear portion of curves reported in Fig. 2

Treatment	b'	r^2
Raw	0.326±0.012	0.982
NaOH dip	1.886±0.22	0.992
Oil dip	0.560±0.017	0.960

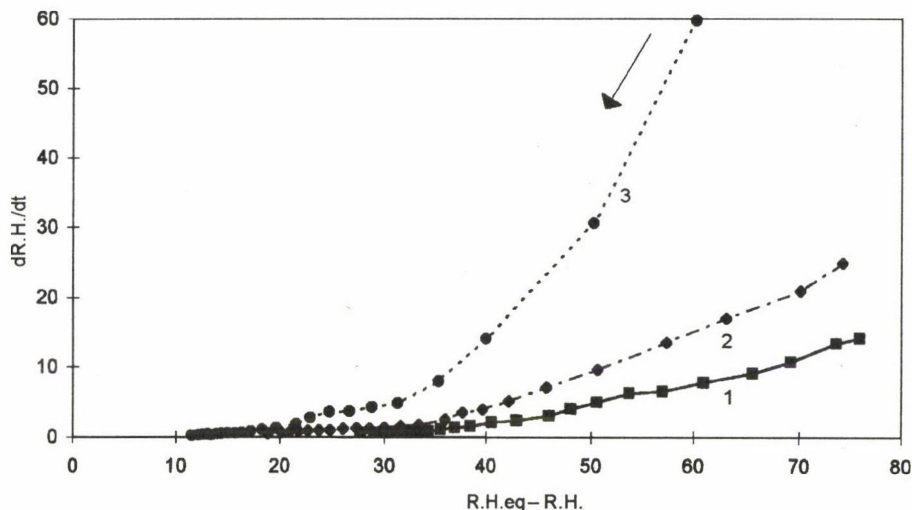

Fig. 2. Rate of change of the relative humidity (dRH/dt) vs the difference between the relative humidity at the equilibrium (RHeq) and actual relative humidity (RH) in the measuring cell containing raw or pretreated plums. 1: Raw; 2: oil dip; 3: NaOH dip

Raw and pretreated plums were air-dried under standard conditions and Fig. 3 reports their specific drying kinetics. It is evident that each sample required different drying times to reach a water content (M) of about 0.25 g H_2O/g D.M. In particular, drying times for raw plums ranged between 940 and 960 min, for plums dipped in ethyl oleate emulsion between 760 and 820 min and for plums dipped in hot NaOH solution between 630 and 670 min. The drying results confirmed the indications obtained through the hygrometric measurements. Also in this case the narrowness of the standard deviation intervals, reported as bars in Fig. 3, clearly demonstrates the good repeatability of the drying trials.

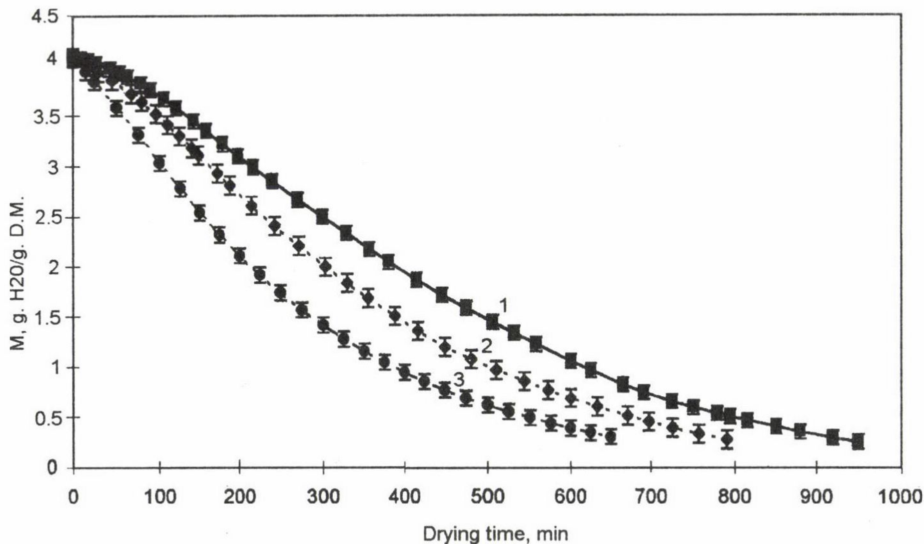

Fig. 3. Drying curves (water content vs drying time) of plums submitted to different drying pretreatments. 1: Raw; 2: oil dip; 3: NaOH dip. Bars represent the standard deviation of the mean of three replications

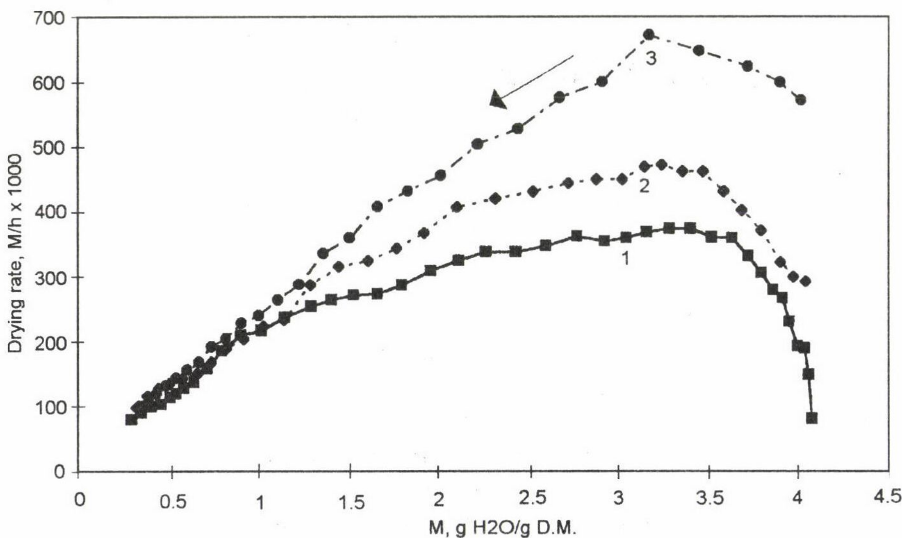

Fig. 4. Drying rate vs water content of plums submitted to different drying pretreatments. 1: Raw; 2: oil dip; 3: NaOH dip

When the specific drying rates were plotted as a function of water content (Fig. 4), the curves were superposed for moisture values (M) lower than 1.2 g H_2O/g D.M. In the first phase of drying, when the water vapour pressure in the inner of the fruit is very high, the limiting factor for the evaporation is probably the cuticle permeability. When the water content of the fruit has been reduced (g H_2O/g D.M.<1.2), the water binding capacity of the pulp becomes predominant and the barrier effect of the cuticle ceases to influence the evaporation rate.

3. Conclusions

The hygrometric measurements of whole fruit give a "permeability index" for its cuticle. The present method of permeability evaluation requires only short analysis times (20 min) and does not lead to the destruction of the sample. Under our experimental conditions, the method had good repeatability and allowed the discrimination between samples with slight differences in cuticle permeability. Products with higher skin permeability also had the shortest drying times. In this way, the hygrometric tests could be used to predict the drying behaviour of whole fruit.

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THE ASSESSMENT OF CURRENT PROTOCOLS FOR PREPARING EDIBLE CARTHAMIN DYE FROM DYER'S SAFFRON FLOWERS

K. SAITO[a] and Y. FUKAYA

Department of Bioscience and Technology, School of Engineering, Hokkaido Tokai University, Sapporo 005. Japan

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Experimental works have been carried out to assess reported protocols for preparing carthamin dye which is to be used as a red colorant for processed foods. $KMnO_4$-method is most promising – (1) carthamin productivity is high, (2) the dye can be induced speedily and smoothly, (3) the operation process is simple and easy, (4) $KMnO_4$ reacts effectively not only with fresh florets, but also with dry ones to produce carthamin, (5) it is inexpensive. H_2O_2-method is an efficient procedure for yielding a high amount of the red colorant – (1) H_2O_2 acts on both fresh and dry florets to produce carthamin dye effectively, (2) the method is easy, simple and costless, (3) no harmful by-product is generated during the reaction course. Glucose oxidase-method is an appreciable technique, however, it is somewhat disadvantageous – (1) the enzymatic process is sensitive to external factors, in particular, to temperature, pH, dissolved O_2 and certain chemicals, (2) overall process is complicated and hazardous, (3) no positive induction of carthamin synthesis is detectable in dried materials by mixing glucose oxidase sample, (4) the process is too expensive to apply it to the practical field. Above three improved methods are superior to the traditional protocols especially in carthamin productivities and/or in simplified preparatory techniques.

Keywords: carthamin productivity assessment, flower processing technique, edible carthamin dye, food colorant, dyer's saffron (*Carthamus tinctorius* L.)

Carthamin, a red colorant for dyeing processed foods (FRANCIS, 1992), is obtained from matured and reddened flowers of dyer's saffron (*Carthamus tinctorius* L.). The overall process for processing flowers, however, is very complicated and laborious, which has, as a matter of course, reflected on high prices of the colorant (SAITO, 1990). To improve the troublesome and inefficient techniques, three ameliorated procedures have been reported in recent years, two of which are conducted with chemicals (SAITO, 1991, 1992) and one is done by a biological process (WADA & OTA, 1986). In these published techniques, original protocols have been presented in detail, with accompanying advantages. Whereas, no direct comparison to realize the

[a] To whom all correspondence should be sent

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emphasized merits has been shown yet in the literature. It would be helpful for colorant engineers to keep the best protocol ready to their hands, because selection of a useful technique has often became one of the most requisite factors to obtain carthamin dye efficaciously.

The aim of the experiments reported in this paper was to assess that procedures are promising to produce copious amounts of carthamin for dyeing processed foods. Comparative data from the quantitative assays will be presented in the following pages. On evaluating the positive efficacities of the ameliorated techniques, carthamin productivities in commercial materials which were processed by the traditional methods will also be investigated in separate experiments.

1. Materials and methods

1.1. Materials

Carthamin used as an authentic marker was from our private collection. Analytical grade of $KMnO_4$, H_2O_2, *o*-phosphate, acetone and glucose oxidase (EC 1.1.3.4) from *Aspergillus niger* (specific activity 201 units mg^{-1}) were purchased from Wako Pure Chemical (Osaka, Japan). Manganese (III) acetate was obtained from Aldrich Chemical (Milwaukee, WIS, USA). Avicel cellulose was furnished by Funakoshi Yakuhin (Tokyo, Japan). Other chemicals and reagents were supplied by different commercial sources. Beni-Mochi (traditional kneaded/packed flowers) and Koka (traditional reddened/dried flowers) were purchased from a local market (Yamagata, Japan). Fresh flowers were collected from freshly opened flowering heads of dyer's saffron early August in 1994 and stocked immediately at –40 °C in a deep freezer until used. Macerated/reddened flowers were prepared by triturating fresh materials (100 g), which were dried in air before conducting the following experiments. Yellow dried flowers were obtained by boiling fresh flowers (80 g) in distilled methanol for 5 min and dried in an air circulation oven at 30 °C for about 18 h.

1.2. Methods

1.2.1. Reddening of flowers with aqueous solution of $KMnO_4$. Freshly prepared $KMnO_4$ solutions (20–2000 μmol l^{-1}) were used. Fresh flowers (0.5 g each) were put in a mortar to fixed concentrations of $KMnO_4$ in 50 mmol l^{-1} acetate buffer, pH 3.4 (2 ml) and were crushed with a pestle. The flower pastes were left in the air for 30 min at room temperature (23±1 °C). Yellow dried flowers (0.3 g each) were powdered in a mortar with a pestle. To the flower powders, 8 ml citrate buffer (50 mmol l^{-1} pH 3.4), containing 400 μmol l^{-1} $KMnO_4$ was mixed and left for 30 min at 23±1 °C. Each test was repeated from five to seven times.

1.2.2. Reddening of flowers with aqueous solution of H_2O_2. Fresh flowers (0.5 g each) in a mortar were triturated with a pestle in given concentrations of H_2O_2 (260 μmol l^{-1} –100 mmol l^{-1}, 2 ml each) and kept for 30 min in open air at 23±1 °C to develop red coloration in the flower pastes. Yellow dried flowers (0.3 g each) were powdered in a mortar with a pestle. The fine powders were suspended in distilled water (8 ml) containing 515 μmol l^{-1} H_2O_2 and left for successive 30 min at 23±1 °C. Each test was repeated from five to seven times.

1.2.3. Reddening of flowers with glucose oxidase sample. Glucose oxidase (0.05–50000 munits ml^{-1}) solution in 50 mmol l^{-1} acetate buffer, pH 4.8 (2 ml each) was pipetted over fresh flowers (0.5 g each) and triturated in a mortar with a pestle, then the pastes were kept to stand in the air for a successive 30 min at 23±1 °C. For carrying out the incubation with glucose oxidase under O_2 limiting conditions, triturated fresh flowers (0.5 g each) in 50 mmol l^{-1} acetate buffer, pH 4.8 (1 ml) were put in a Thunberg tube. The headspace air was evacuated and replaced by N_2-gas. Enzyme reaction was started just after mixing with 5 munits glucose oxidase solution (1 ml) from the side-wall and incubated at 23±1 °C for 30 min by gentle agitation at 110 strokes min^{-1}. Effect of chemicals on flower reddening was observed, using *o*-phosphate and manganese (III) acetate (100 μmol l^{-1} each). Glucose oxidase assays were carried out using 5 munits enzyme and 0.5 g flowers in 50 mmol l^{-1} acetate buffer, pH 4.8 (2 ml) for 30 min at 23±1 °C. Induction of carthamin synthesis by glucose oxidase was studied at three different temperatures (10, 30 and 70 °C), pH tests were performed at varied pH ranges from 3.0 to 7.0 by the use of 50 mmol l^{-1} acetate or citrate/phosphate buffer. Incubation times, enzyme units and flower weights were the same as described above. Yellow dry flower powders (0.3 g each) were suspended in 8 ml acetate buffer, (50 mmol l^{-1} pH 4.8) containing 5 munits glucose oxidase and incubated for 30 min at 23±1 °C without agitation. All tests were repeated from five to seven times.

1.2.4. Extraction of technically induced carthamin. Reddened pastes from above experiments were suspended separately in 7–8 ml of 0.5% (v/v⁻) K_2CO_3 and stirred for 10 min on a magnetic stirrer. The suspensions were filtered through a paper on a Büchner funnel. The filtrates were retained and the residues resuspended in 7–8 ml fresh K_2CO_3 (0.5%), stirred and filtered. The K_2CO_3 extraction was repeated once again and the combined extracts (20–40 ml in total) were acidified by the addition of solid citric acid (0.2–0.5 g each).

1.2.5. Adsorption and recovery of carthamin. Avicel cellulose (0.1–0.2 g each) was stirred in the acid filtrates on a magnetic stirrer for a few minutes and the

suspension was transferred to teflon tubes. Centrifugation was carried out at $4000\times g_n$ for 5 min. The pellet was resuspended in 25–35 ml distilled water and centrifuged again. The washing was repeated two more times. The reddish Avicel was treated with 60% (v/v) acetone, then centrifuged ($4000\times g_n$, 5 min). The carthamin extracts (100 ml each) thus prepared were used immediately for spectrophotometric determination.

1.2.6. Determination of carthamin content. In the spectrophotometric assays Hitachi, model U-1100 spectrophotometer was used and 60% (v/v) acetone served at reference. The measurements were carried out at 521 nm, and the carthamin content was calculated on the base of the calibration curve. Specific value of carthamin formation was expressed as ng or µg carthamin ml^{-1} min^{-1}. UV/VIS spectra of recovered carthamin were monitored with a Hitachi, model U-3210 spectrophotometer in 65% (v/v) methanol.

2. Results

2.1. Tentative identification of technically induced products

Red products obtained from the technically processed flowers of dyer's saffron were screened with spectrophotometry. The UV/VIS absorption spectra of the products and that of an authentic sample in 65% (v/v) methanol are presented in Fig. 1. All spectra show a characteristic absorbance pattern at 521 nm (Band I), which coincides well with that of a standard carthamin marker, although some discordances are seen especially in shorter UV absorbance ranges from 187 to 213 nm. Based on the experimental data, we confirmed that the technically induced red products are all carthamin dye.

2.2. Assessment of carthamin productivity

2.2.1. Test with $KMnO_4$. Effect of $KMnO_4$ on the reddening of fresh flowers of dyer's saffron is shown in Fig. 1. Out of the eight different concentrations examined, 400 µmol l^{-1} $KMnO_4$ is the most effective. At this concentration, 26.7 mg carthamin was obtained from 0.5 g fresh flower (yield 5.34%, reaction velocity 1.067 µg carthamin ml^{-1} min^{-1}, increment 100.6%). This value is twice as much as that of the blank run conducted with no addition of $KMnO_4$. The efficacity of 500 mmol l^{-1} $KMnO_4$ ranks next (1.6-fold of the blank, velocity 0.87 µg carthamin ml^{-1} min^{-1}, increment 63.8%). Dosages over 1000 µmol l^{-1} $KMnO_4$ are unfavourable, because dark brown precipitates accumulate gradually during incubation, although apparent productivity of carthamin is high compared to that of the blank run (1.6-fold, velocity 0.85 µg carthamin ml^{-1} min^{-1}, increment 59.9% in average).

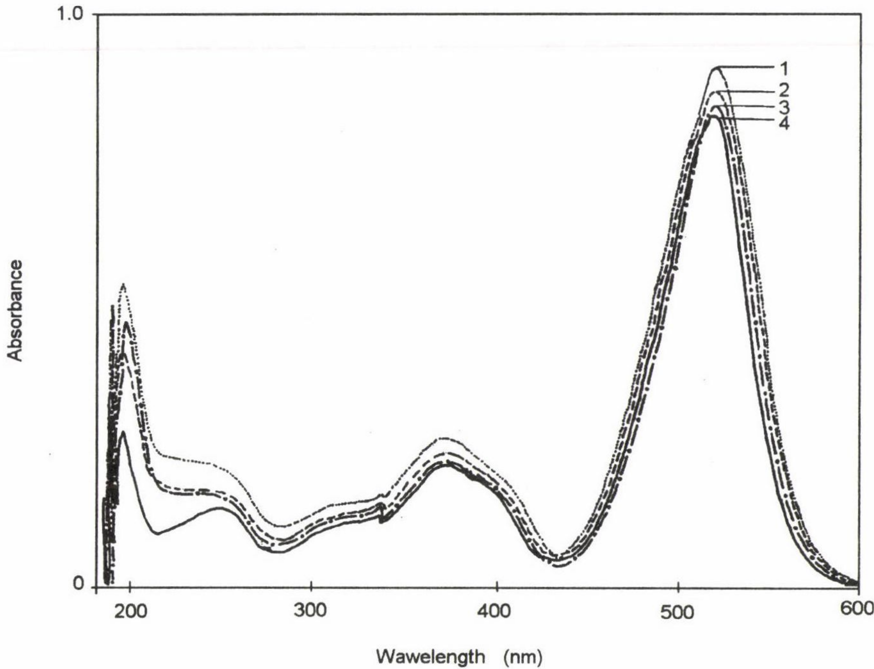

Fig. 1. UV/VIS absorption spectra of technically induced products from flowers processed by various methods. All spectra were recorded in 65% (v/v) methanol. 1: H_2O_2-induced products, 2: $KMnO_4$-induced product, 3: Glucose oxidase-induced product, 4: authentic carthamin

2.2.2. Test with H_2O_2. Carthamin productivities in processed flowers as affected by various concentrations of H_2O_2 are shown in Fig. 3. Initial 260–570 μmol H_2O_2 dosage induces carthamin accumulation most actively (1.2-fold in average, reaction velocity 0.80 μg carthamin ml^{-1} min^{-1}, increment 18.0%). It is followed by the concentrations 620–770 μmol l^{-1}, the efficacities of H_2O_2 axe reduced (1.0-fold on average, velocity 0.68 μg carthamin ml^{-1} min^{-1}, increment 0.36%). At higher levels (3–25 and 50–100 mmol l^{-1}), carthamin yield was inhibited greatly (inhibition 10.9 and 79.0%, reaction velocity 0.61 and 0.14 μg carthamin ml^{-1} min^{-1}, respectively). Time-course of H_2O_2-dependent carthamin production is depicted in Fig. 4. Incubation of 30 min period is the most effective. H_2O_2 hastens the formation of carthamin from its direct precursor, precarthamin by about 1.2-fold of the blank run at the dosage of 515 μmol l^{-1} H_2O_2. Thus, we conducted 30 min incubation throughout the present experiments. The reaction proceeds linearly for 30 min, then the velocity falls gradually.

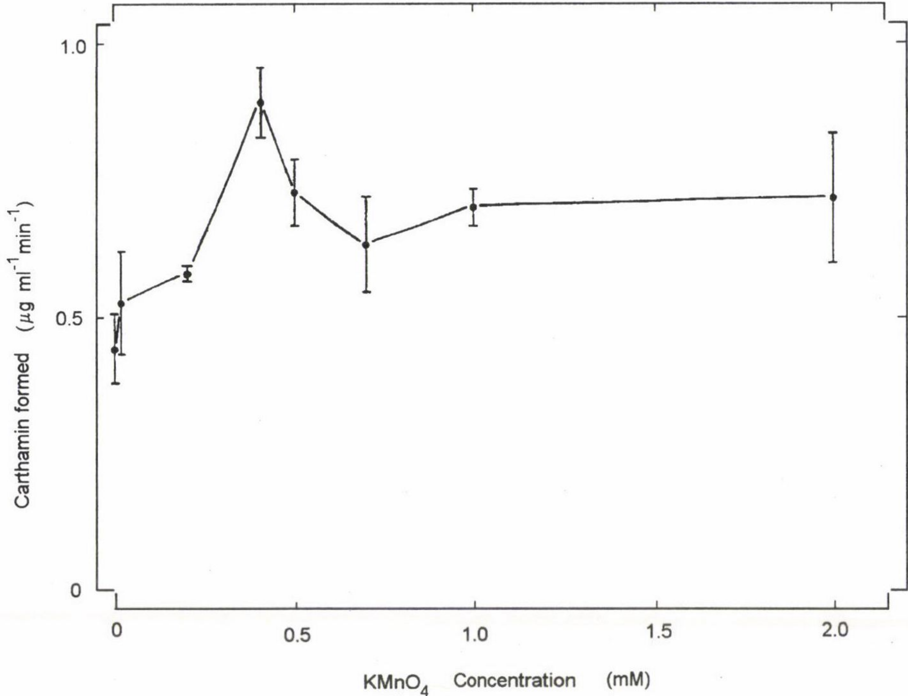

Fig. 2. Relationship between $KMnO_4$ concentration and carthamin productivity

2.2.3. Test with glucose oxidase. Figure 5 shows the relation of glucose oxidase amounts vs productivities of carthamin in reaction systems. At 5 munits dosage of the oxidase, the highest activity is obtainable in carthamin formation (1.2-fold of the blank run, velocity 1.05 μg carthamin ml^{-1} min^{-1}, increment 18.6%). The value decreases with 500 munits (1.1-fold, velocity 0.95 μg carthamin ml^{-1} min^{-1}, increment 7.2%). Higher glucose oxidase concentrations cause reduction in the carthamin productivity (inhibition 21.1% at 50 units dosage). The enzyme process is easily affected by pH, temperature, dissolved O_2 and chemicals. At pH 4.6–5.0, glucose oxidase acts the most actively. Below pH 4.0 and above 5.5, the activity decreases (17.9 and 30.6% of the highest activity at pH 4.6–5.0, respectively). Glucose oxidase-mediated reddening of flowers is temperature dependent, with decrease occurring above and below the temperature at which the enzyme activity is the most prominent (30 °C). The rate of flower reddening directed by glucose oxidase was reduced under O_2 limiting conditions (10–13% of the control test). Both Mn^{3+} and *o*-phosphate act on glucose oxidase-associated carthamin accumulation inhibitorily. At the dosage of each 100 mmol l^{-1}

level, 54.7 and 39.1% inhibition was observed, respectively. Table 1 summarizes the data from above three tests.

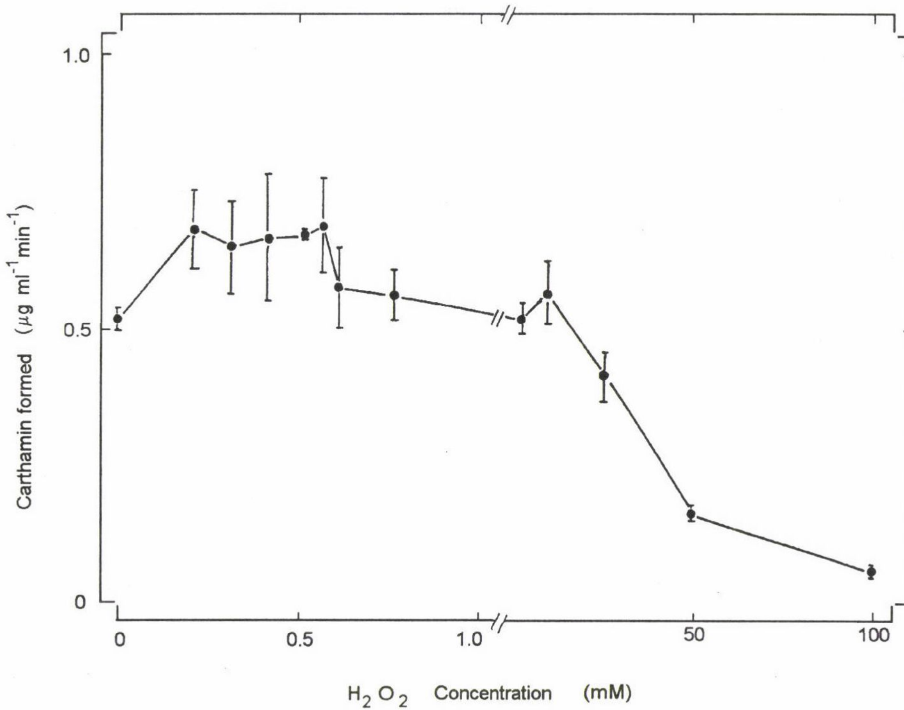

Fig. 3. Relationship between H_2O_2 concentration and carthamin productivity

Table 1

Carthamin production in fresh dyer's saffron flowers processed by different techniques

Material	Carthamin formed ($ng\ ml^{-1}\ min^{-1}$)
$KMnO_4$-processed flowers	1066.6±0.028
H_2O_2-processed flowers	798.0±0.001
Glucose oxidase-processed flowers	1046.2±0.036

The listed experimental data were obtained from five to seven replications. Fresh flowers (0.5 g) were used to each test

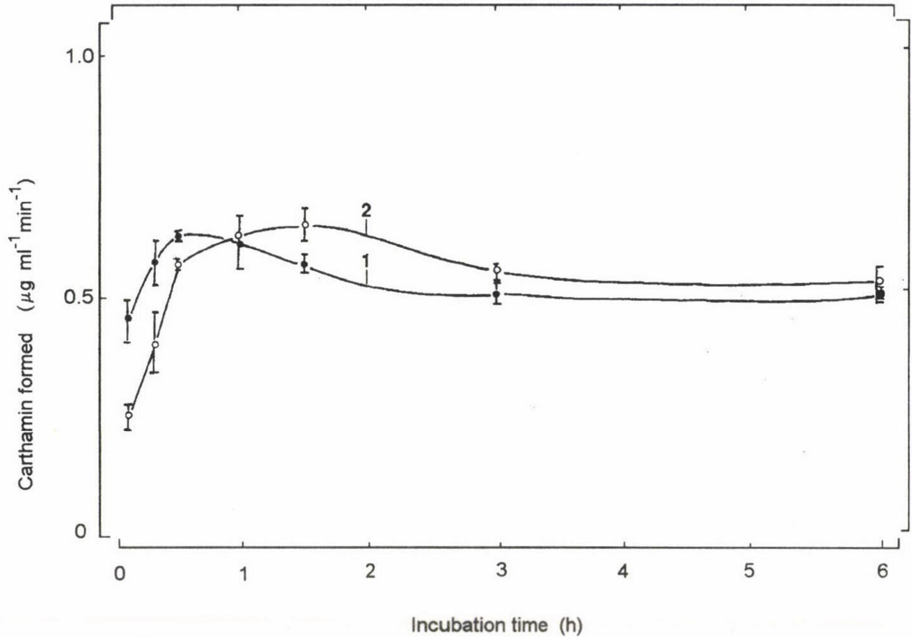

Fig. 4. Time-course of H_2O_2-dependent carthamin formation. 1: H_2O_2-dependent carthamin synthesis, 2: control

2.2.4. Test with flowers processed by the traditional methods. To estimate the productivities of carthamin in processed flowers, kneaded/packed flowers (Beni-Mochi) and reddened/dried flowers (Koka) or macerated/reddened flowers were used, each was produced manually by traditional methods. Table 2 lists the results of the quantitative analyses. Obviously, varying quantities of carthamin were found in the reddened samples processed by different techniques. On the whole, low levels of carthamin yield are brought by treating flowers with the traditional techniques. On assessing carthamin productivities in dried materials, three ameliorated techniques were tested to redden heat denatured yellow flowers (Table 2). $KMnO_4$ promotes carthamin accumulation, yielding a high rate of carthamin dye (increment 278.9%). H_2O_2 is also effective to produce carthamin in dried flowers (increment 40.6%). With glucose oxidase sample, no positive induction of carthamin synthesis can be observed in the yellow dry flowers (diminution 1.3%) (see Table 2).

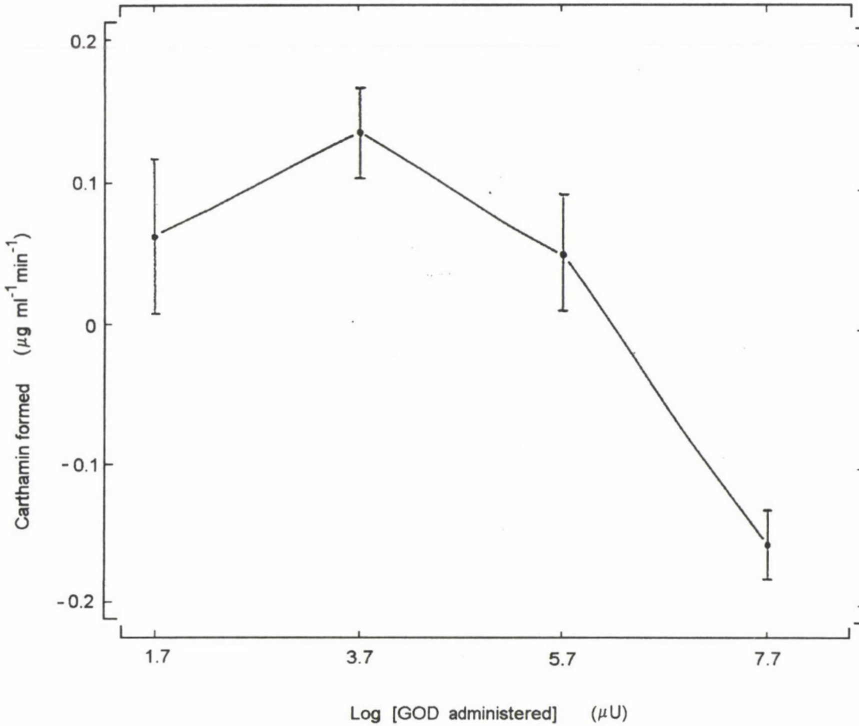

Fig. 5. Relationship between glucose oxidase dosage and carthamin productivity

Table 2

Carthamin production in dried dyer's saffron flowers processed by different techniques

Material	Carthamin formed ($ng\ ml^{-1}\ min^{-1}$)
Beni-Mochi (kneaded/packed flowers)[a]	811.3±0.083
Koka (reddened/dried flowers)[a]	341.5±0.035
Macerated/reddened flowers[a]	644.2±0.026
$KMnO_4$-processed flowers[b]	79.2±0.006
H_2O_2-processed flowers[b]	11.2±0.002
Glucose oxidase-processed flowers[b]	–4.0±0.001[c]

The presented data were obtained from five to seven replications. [a] Dried flowers (0.1 g) were used to each test. [b] Yellow dry flowers (0.3 g) were used to each test. [c] Diminution

3. Discussion

Food colorants comprise a vast variety of structural and tinctorial diversities, in which natural or synthetic dyes are involved. These colorants are all favourable and stimuli towards our sense of vision and/or taste. Among natural red colorants, carthamin is, no doubt, the most attractive and fascinating. The herbal colorant has been used not only for cosmetic goods, but also for colorant of creamy cakes, soft drinks, beverage, fruit jams and so on, which reflect increasingly on requiring ample supply of carthamin dye to the practical fields. Thus, pre-works on specifying efficient techniques are absolutely requisit to quench the quantitative deficiency: a selected protocol has always become one of the most decisive factors for obtaining natural colorants.

The experimental data presented in above paragraphs are comparable with each other, suggesting the varying efficacities in the carthamin productivity of the test samples treated by different procedures.

Externally supplied $KMnO_4$ acts on florets effectively: at 400 μmol l^{-1} dosage, the highest carthamin production is obtained. Below 200 μmol l^{-1}, carthamin yield decreases markedly. At a higher concentration (500 μmol l^{-1} $KMnO_4$), carthamin production reduces greatly. Over 700 μmol l^{-1} $KMnO_4$ feeding, carthamin yield tops out and blackish brown precipitates accumulate in the floral pastes, through which carthamin recovery is hindered, though high carthamin productivities are still kept compared to those of the blank runs. The method is useful: (1) the reaction proceeds speadily, (2) the highest carthamin yield so far can be obtained at a specified concentration of $KMnO_4$, (3) it can be applied to both fresh and dried materials, (4) it is simple and easy, (5) the overall process is performed at a low cost.

H_2O_2 is also a promising carthamin inducer: (1) the highest carthamin yield is obtained at 515 μmol l^{-1} H_2O_2 dosage (2) this method is very simple, easy and not troublesome if the settled concentration of H_2O_2 is used constantly, (3) it is safe because H_2O_2 is easily oxidized to harmless H_2O and O_2 during the operation process, (4) it is applicable in all seasons of the year, to both fresh and dried flowers, (5) it is costless to process large quantities of starting materials.

Glucose oxidase is an appreciable stimulator for carthamin induction: (1) feeding of 4 munits glucose oxidase results in the highest productivity of carthamin, (2) the registered method is a useful technique, however, it comprises several weak points – (a) glucose oxidase reddens fresh flowers to produce carthamin dye, while it shows no positive activity towards dried materials, (b) the activity is sensitive to external factors such as temperature, pH, dissolved O_2, certain chemicals, (c) the method is too expensive to apply in practice, (d) operation processes are somewhat complicated, specified and restricted.

Carthamin productivities in samples produced by the traditional methods are relatively low (see Table 2). The experimental results confirms that the recently improved methods are all superior to those of the usually applied protocols in carthamin productivities and/or in simplified preparatory techniques.

4. Conclusion

– $KMnO_4$-method (SAITO, 1991) is an efficient technique for preparing carthamin dye. The method is easy, simple and not expensive. It can be used to both fresh and dry materials for all seasons in a year. This method is very fit for preparing edible carthamin dye.

– H_2O_2-method (SAITO, 1992) is promising for the preparation of carthamin dye. The method is simple, easy and costless. No harmful by-product is generated during and/or after operation.

– Glucose oxidase-method (WADA & OTA, 1986) is an appreciable procedure for reddening fresh flowers. However, it seems to be somewhat disadvantageous in the following four points mainly: (a) reaction process is easily affected by external factors, in particular, by temperature, pH, dissolved O_2 and/or by certain chemicals, (b) no positive reddening of dried flowers is detectable in the presence of glucose oxidase sample, (c) the overall process is complicated, hazardous and restricted, (c) it is too expensive to apply to the practical fields.

– The traditional methods are troublesome, laborious and complicated to carry out. Carthamin productivities are low.

Abbreviations

UV: ultraviolet light
VIS: visible light

*

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INFLUENCE OF PACKAGING ON THE RHEOLOGICAL CHARACTERISTICS OF KASHKAVAL

V. LAZIĆ and M. CURAKOVIĆ

Faculty of Technology, University of Novi Sad, 21000 Novi Sad, Bulevar Cara Razara 1. Yugoslavia

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The influence of three kinds of packaging materials and three packing conditions on rheologic characteristics of Kashkaval was investigated.

After the production and ripening, Kashkaval was cut into pieces and packed in chosen packaging materials (Cryovac foil, thickness 70 µm, usually used for packing of cut hard cheeses, and two domestic foils: the combined foil polypropylene lacquered with PVDC lacquer/polyethylene, PP (PVDC)/PE, declared thickness 60 µm and polyether/polyethylene foil, PETP/PE, thickness 60 µm). The packing was performed under defined conditions (atmospheric, vacuum and modified atmosphere 90% CO_2 and 10% N_2).

The hardness of packed cheese was determined by INSTRON 4301.

The rheological character of Kashkaval were varying during storage (from initial 120 N to 160 N at the end of the four-month storage). The highest hardness was determined in cheese samples packed in packaging materials with higher water vapour permeability (packaging material 1 – Cryovac foil). The influence of packing conditions is also presented. The hardness of samples packed under vacuum was higher than that of samples packed under atmospheric or modified atmosphere, when packed in the same packaging material.

Keywords: packaging, packing conditions, Kashkaval, rheology

The barrier properties of the packaging materials regulate the transport of moisture and gases between the cheese and the environment. In this way they influence significantly the flow and intensity of complex biochemical processes during ripening and storage of hard cheeses (STEHLE, 1987).

According to a number of authors, the most severe demands are connected with the packaging of hard cheeses (SPARAKOWSKI, 1990; KOSIKOWSKI, 1982; CHOISY et al., 1987). The barrier characteristics of packaging materials influence significantly the quality of hard cheese (SCOTT, 1981; STEHLE, 1987, LAZIĆ, 1994).

Kashkaval is a characteristic hard cheese for the Mediterranian area (CARIĆ, 1993). During ripening and storage the different biochemical processes affect the modification of cheese composition and structure. In this way the values of

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characteristic appearance, consistency, colour, taste and smell for every kind of cheese are formed (SCOTT, 1981; CHOISY et al., 1987).

Some important sensory properties (hardness, elasticity, spreadability etc.), are determined by rheological characteristics (PRENTICE, 1987). The aim of this work was to investigate the influence of packaging materials, different water vapour and gas permeability, as well as different packing conditions on the rheological characters of Kashkaval, expressed by compression force.

1. Materials and methods

Three packaging materials were chosen for the investigations:

I. Cryovac foil, declared thickness 70 µm, "Grace" Italiana

II. PP lacquered with PVDC lacquer/PE foil, nominal thickness 60 µm, "Viscoza" Loznica

III. PETP/PE, thickness 60 µm, "Tipoplastika" Gornji Milanovac

The Kashkaval was produced by the standard technological procedure. After ripening, it was cut to pieces and packed in sacks made of the chosen material, under three different conditions.

Cheese samples were packed under atmospheric conditions (O) and modified atmosphere (S) at the packing line in the milk factory "Mlekoproduct" Zrenjanin with "Hayssen ECON-O-MATIC RT 218" packing machine. The laboratory packing machine AUDION ELEKTRO vacuum type, maintaining the vacuum from 0.7 to 0.8 bar, was used for packing under vacuum (V).

The composition of the modified protective atmosphere was 90% CO_2 and 10% N_2. The samples were kept at 4–7 °C for 4 months.

The samples were investigated after 0, 0.5, 1, 2, 3 and 4 months of storage.

The barrier properties of the packaging materials e.g. permeability of water vapour and gases were investigated.

Water vapour permeability of packaging materials was measured on VAPOR PERMEATION TESTER L-80 (method according to LYSSY, 1975) (DIN 53122).

Permeability of gases (CO_2, O_2, N_2) was determined on Lyssy GPM-200, with the corresponding gas chromatograph Gasukuro Kogyo GC-320 and integrator HP 3396A (method according to LYSSY, 1984) (DIN 53380).

The permeability was investigated at 23 °C. The calculation of gas permeability was performed on the base of sample chromatogram and carbon. The parameters used for the calculation are:

- added mixture amount during calibration
- ratio of CO_2, O_2, N_2 in the mixture
- peak area during calibration

– value of measurement interval
– peak area of the investigated sample

The results of CO_2, N_2 and O_2 permeability are expressed in ml m^{-2} 24 h^{-1} bar^{-1}. The air permeability was calculated using the following equation:

$$P_A = P_{O_2}V_{O_2} + P_{CO_2}V_{CO_2} + P_{N_2}V_{N_2}$$

where
P: permeability of gases determined by the presented method
V: volume ratio of these gases in the air

The rheological characteristics of Kashkaval were followed. Hardness was determined by compression of cylindrical cheese samples. The sample size was: diameter 2.54 cm and height 1.5 cm. The compression was performed till 50% of the initial height at 20 °C, using the Instron Universal Testing Machine, model 4301 (Instron Limited, High Wycomte, Buckinghamshire, England), the speed being 0.1 m min^{-1}. The hardness is defined by the force necessary to compress the cheese samples till a certain height (SPANGLER et al., 1990).

Three sackets made of each packaging material and packing conditions were opened for each investigation. Five samples of cheese were cut for hardness determination using a special tool.

2. Results

The gas permeability chromatograms of the investigated packaging materials are presented in Figs. 1, 2 and 3.

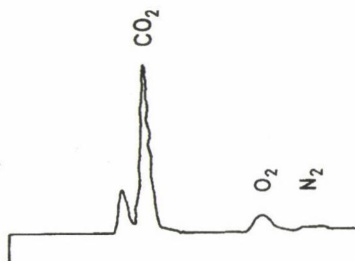

Fig. 1. Gas permeability chromatogram of packaging material I: Cryovac foil. Determination performed at 23 °C, on Lyssy GPM-200

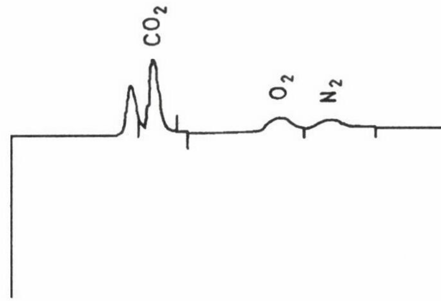

Fig. 2. Gas permeability chromatogram of packaging material II: PP lacquered with PVDC lacquer/PE foil. Determination performed at 23 °C, on Lyssy GPM-200

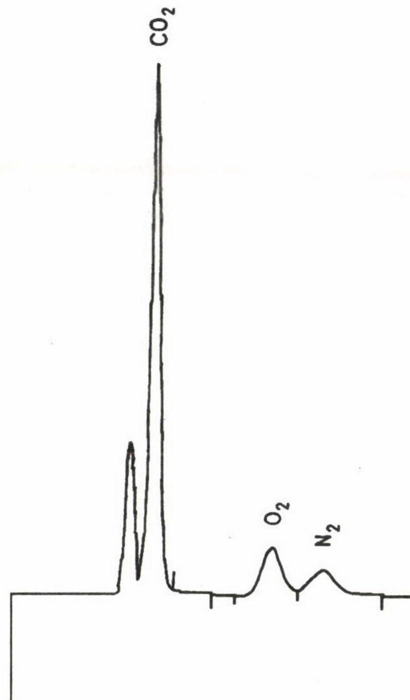

Fig. 3. Gas permeability chromatogram of packaging material III: PETP/PE. Determination performed at 23 °C, on Lyssy GPM-200

As the permeability was determined under the same chromatographic conditions, the obtained results are comparable. The visual observation is quite satisfactory for the estimation of correlation of the permeability of packaging materials. The CO_2, O_2 and N_2 permeability was determined, while the air permeability was calculated on the basis of chromatograms obtained for the samples and during calibration. The results are presented in Table 1.

Table 1

Gas (ml m^{-2} 24 h^{-1} bar^{-1}) and water vapour (g m^{-2} 24 h^{-1}) permeability of the packaging materials

Packaging material	CO_2	O_2	N_2	ir	water vapour
I	163.00	27.88	9.00	13.03	6.15
II	87.07	25.33	11.76	14.06	2.37
III	715.56	98.27	20.73	37.32	5.55

I: Cryovac foil, 70 μm
II: PP lacquered with PVDC lacquer/PE, 60 μm
III: PETP/PE, 60 μm

The barrier characteristics of packaging materials I and II to O_2 and N_2 are similar, while the CO_2 permeability of material I is significantly higher. The permeability of material III for all gases investigated is significantly higher. The obtained results are in accordance with the literature data (TAMINE, 1986).

The water vapour permeability of packaging material II is the smallest due to the PVDC layer. According to literature data (GODDARD, 1990; GRIFFIN et al, 1985), the main property of polyvinylendichlorid (PVDC) is the low gas and water vapour permeability.

The results of investigation of rheological characteristics are presented in Figs. 4, 5, 6.

The alternate decrease and increase of compression force is characteristic for all samples, under all packing conditions applied, with a pronounced increase at the end of storage period.

The initial compression force was 120.5 N and at the end of storage period it reached even 160 N. The biggest difference between certain samples was noticed in samples packed under atmospheric conditions "O". At the end of the investigation period, greater hardness was established in cheese samples packed in packaging materials with higher water vapour permeability (I and III) (Fig. 4). The different water vapour permeability influences the moisture content of the cheese and analogously, the mass loss, resulting in lower cheese hardness in packaging materials with lower water vapour permeability (packaging material II). Values obtained during compression force

determination of samples packed under "V" conditions are somewhat lower, and the difference between certain samples are smaller. A similar trend of changes is also noticed in samples packed in modified atmosphere "S".

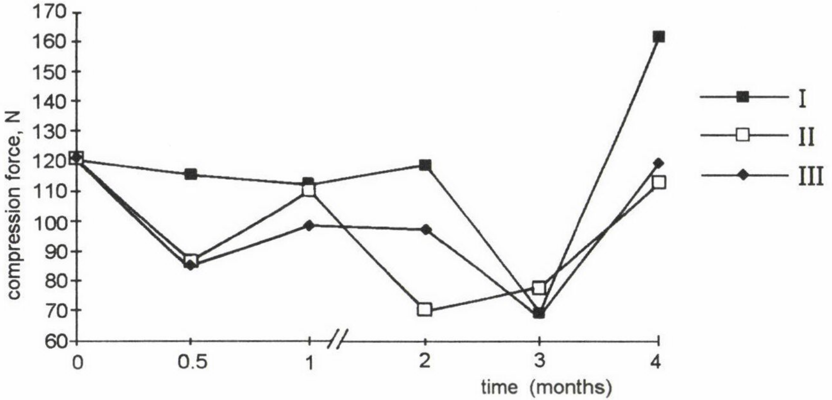

Fig. 4. Hardness change of Kashkaval samples packed under atmosphere "O" conditions. Samples of 1.5 cm high and 2.54 cm in diameter, were compressed till 50% of initial height, at 20 °C, using "INSTRON" 4301 apparatus

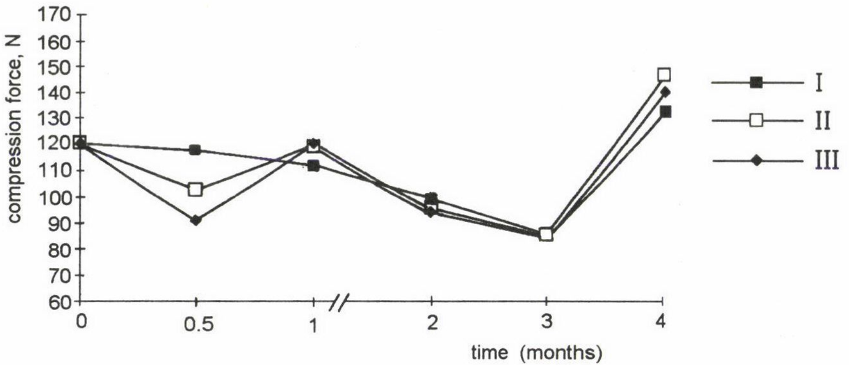

Fig. 5. Hardness change of Kashkaval samples packed under vacuum "V", samples of 1.5 cm high and 2.54 cm in diameter, were compressed till 50% of initial height, at 20 °C, using "INSTRON" 4301 apparatus

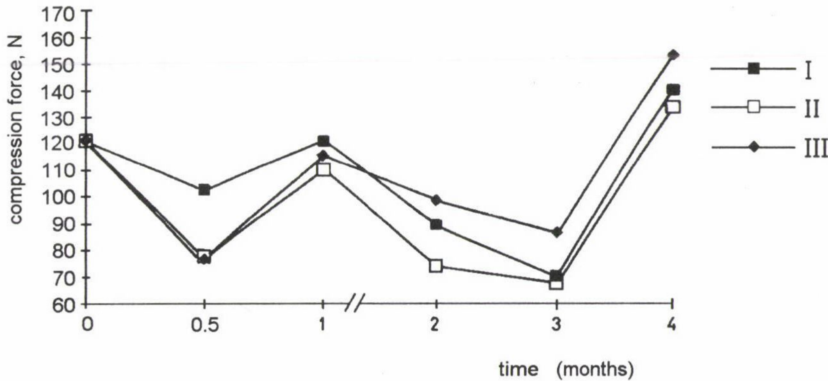

Fig. 6. Hardness change of Kashkaval samples packed under modified atmosphere atmosphere "S". Samples of 1.5 cm high and 2.54 cm in diameter, were compressed till 50% of initial height, at 20 °C, using "INSTRON" 4301 apparatus

At the end of the storage period the hardness ranged from 130 N (packaging material II) to 153 N (packaging material III), as presented in Fig. 6.

Regarding the influence of packing conditions it was found that the compression force for "O" and "S" samples was similar, while the compression force of samples packed under vacuum "V" was higher (Fig. 7).

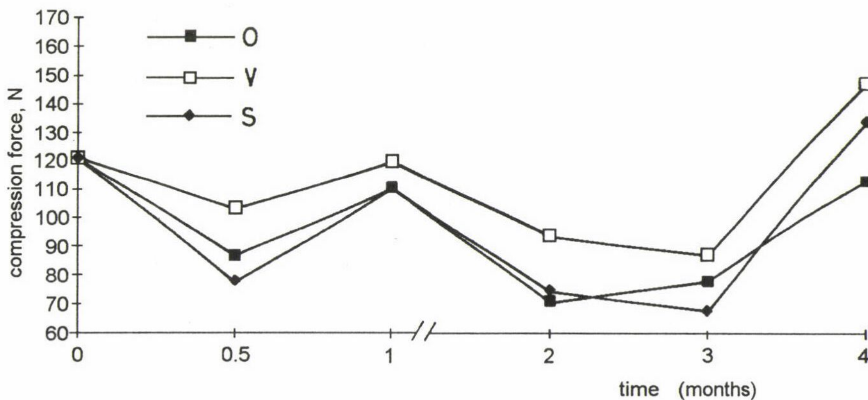

Fig. 7. Change of hardness of Kashkaval samples packed in material II: PP lacquered with PVDC lacquer/PE, under different conditions "O": atmospheric, "V": vacuum, "S": modified atmosphere

The literature data on change of rheological properties of hard cheeses are different. Our results are in accordance with some literature data (CREAMER et al., 1988; WALSTRA, 1987). In case of Cheddar cheese the compression diagram shows an increase till 5 months of storage. Other authors obtained a permanent decrease of compression force during the storage of Gauda cheese (SPANGLER et al., 1990). The decrease of compression force was also observed in case of UF Kashkaval (MILANOVIC, 1993).

3. Conclusions

– The water vapour permeability of all packaging materials is relatively low. The lowest values were found in case of packaging material II (PP lacquered with PVDC lacquer/PP).

– The gas permeability of the applied packaging materials is different. Packaging material II has the best barrier characteristics, while the gas permeability of packaging material III (PETP/PP) is somewhat higher.

– The rheological characteristics of Kashkaval, expressed by the compression force, are changing during the period of investigation. The changes are characterized by alternating drop and increase of compression force values. After the 4-month storage period, an expressed increase of compression force was estimated in all samples. The cheese samples packed in materials characterized by higher water vapour permeability were the hardest, while the lowest hardness was found in cheese samples packed in packaging materials having the best barrier characteristics. The values of compression forces of cheese samples packed under atmospheric condition and modified atmosphere are similar, whereas these for the samples packed under vacuum are higher, e.g. the samples were firmer.

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CHROMIUM AND NICKEL CONTENTS OF SOME COMMON EDIBLE MUSHROOM SPECIES

J. VETTER

Department of Botany, University of Veterinary Sciences, H–1400 Budapest, P.O. Box 2. Hungary

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Ninety three samples of twenty five higher mushroom species were analysed for chromium and nickel contents. The average Cr-content of the samples (gathered in years 1993–1995) was 1.43 mg/kg D.M., the concentrations varied between 0.05 and 4.51 mg/kg D.M. The highest Cr-content was found in *Lactarius deliciosus*, the smallest one in *Tricholoma scalpturatum* and *Xerocomus subtomentosus* (0.05 mg/kg D.M.). The nickel level of the samples was between 0.81 (*Hypholoma capnoides*) and 9.9 mg/kg D.M. (*Tricholoma terreum*) the average concentration of the mushrooms was 2.79 mg/kg D.M. The measured Cr- and Ni-concentrations are under the toxicological limits (for Cr: 5.0, for Ni: 20 mg/kg D.M.).

Keywords: chromium, nickel, edible mushrooms

The mineral composition of the fungi is an interdisciplinary question. The concentration of toxic elements has food chemical and toxicological importance. The following toxic elements were already investigated: arsenic and cadmium (VETTER, 1990; 1994a), copper, manganese and zinc (VETTER, 1994b), boron (VETTER, 1995) and others. Their presence in food plants are connected with contaminations of environment and thus with ecological factors. According to earlier and sporadic data from Italy (SANTOPRETE & INNOCENTI, 1984), Sweden (TYLER, 1982a, 1982b) and from Ukraine (SOLOMKO, et al., 1986) bioaccumulation of chromium and nickel was not found. The determined concentrations were not high. According to international data (KALAC & STASKOVA, 1994; TYLER, 1980), the average chromium concentration is between 0.1 and 1 mg/kg D.M. The nickel concentration of Scandinavian mushroom samples is 1.4 mg/kg D.M. (TYLER, 1982b) that of Bohemian samples from contaminated areas is 9 mg/kg D.M. (LEPSOVA & KRAL, 1988). In our earlier works (VETTER, 1990) an average concentration of 2.0 mg/kg D.M. was published. The aim of the present investigations (1993–1995) was to analyse and compare the chromium- and nickel contents of the most important, edible wild mushroom species.

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1. Materials and methods

The fruiting bodies of fungi were gathered from different sites of Hungary, in the years 1993, 1994 and 1995. The edible character of the fungi was judged according to the worldwide known book of MOSER (1978). At far as possible the same sites were visited and the same species were gathered. In some cases more than one sample were collected, from one site. The fruiting bodies were dried, milled and treated (in: HNO_3:H_2O_2=1:1, in specific, closed teflon bombs), than the Cr- and Ni-contents were determined with ICP methods (VETTER, 1989) in four parallels per samples. The metal contents are characterised with the arithmetical mean (mg/kg D.M.) and with standard deviation (±s).

2. Results and discussion

The chromium and nickel contents of the analysed mushrooms are given in Table 1. The different mushroom species have different metal contents. For easier comparison and orientation the average data of the species (under the data of samples) are also presented. The number of samples varied between 2 and 14.

Table 1

Cr and Ni content of some edible, common mushroom species of Hungary

Species and year	Site of gathering	Cr content (mg/kg D.M.)		Ni content	
		$\bar{x}$	±s	$\bar{x}$	±s
Armillaria mellea					
(Vahl. in Fl. Dan.: Fr.)					
1993	Mt. Budai	0.05	(0.01)	0.92	(0.02)
1993	Miskolc/3	0.05	(0.01)	0.02	(0.00)
1993	Tatabánya/3	0.05	(0.01)	0.39	(0.04)
1993	Tatabánya/4	0		0	
1994	Tatabánya/2	2.29	(0.10)	4.00	(0.25)
1994	Miskolc/1	0.27	(0.03)	1.29	(0.14)
1994	Miskolc/1	0.96	(0.07)	3.85	(0.23)
1994	Miskolc/2	0.62	(0.04)	2.08	(0.26)
1995	Mt. Budai	0.83	(0.04)	2.26	(0.14)
1995	Miskolc/3	1.99	(0.09)	4.69	(0.35)
1995	Tatabánya/1	1.87	(0.14)	3.20	(0.15)
1995	Tatabánya/2	1.17	(0.11)	2.66	(0.22)
1995	Tatabánya/4	2.22	(0.15)	1.63	(0.09)
1995	Wood Kamara	1.90	(0.10)	3.77	(0.21)
	Average:	1.05		2.20	

Species and year	Site of gathering	Cr content (mg/kg D.M.)		Ni content	
		$\overline{x}$	±s	$\overline{x}$	±s
Clitocybe nebularis (Fr.) Harmaja					
1993	Mt. Bakony	9.51	(0.35)	4.82	(0.27)
1993	Mt. Börzsöny	1.55	(0.15)	1.05	(0.08)
1993	Mt. Bakony	5.04	(0.32)	2.48	(0.06)
1994	Mt. Bükk	1.59	(0.16)	4.76	(0.10)
1995	Miskolc	2.23	(0.10)	1.90	(0.11)
	Average:	3.98		3.00	
Clitocybe odora (Bull.: Fr.) Kummer					
1993	Tatabánya/1	0.05	(0.01)	0.05	(0.01)
1993	Miskolc/1	0.05	(0.01)	1.67	(0.04)
1993	Tatabánya/2	0.05	(0.01)	0.05	(0.02)
1994	Miskolc/2	0.67	(0.04)	1.62	(0.10)
1994	Tatabánya/2	1.82	(0.08)	2.59	(0.13)
	Average:	0.52		1.19	
Hypholoma capnoides (Fr.): Fr. Kummer					
1993	Mt. Pilis	0.05	(0.01)	0.72	(0.03)
1993	Miskolc/3	0.05	(0.01)	0.21	(0.01)
1994	Mt. Pilis	0.65	(0.07)	1.50	(0.05)
	Average:	0.25		0.81	
Lactarius deliciosus Fr.					
1993	Miskolc/3	5.14	(0.23)	2.92	(0.12)
1995	Miskolc/3	3.88	(0.20)	2.58	(0.15)
	Average:	4.51		2.75	
Laccaria laccata (Scop.: Fr.) BK et Br.					
1995	Miskolc/3	4.06	(0.19)	7.08	(0.20)
1995	Őrség (County Vas)	2.05	(0.20)	5.64	(0.16)
	Average:	3.05	(0.14)	3.36	
Laccaria amethystina					
1995	Őrség (County Vas)	4.51	(0.15)	4.05	(0.19)
1995	Loipersdorf	1.31	(0.09)	10.49	(0.32)
	Average:	2.91		7.27	
Lepista gilva (Pers.: Fr.) Roze					
1994	Miskolc/1	0.98	(0.09)	3.91	(0.14)
1994	Miskolc/1	7.13	(0.21)	3.56	(0.15)
1995	Miskolc/2	5.04	(0.26)	2.50	(0.10)
1995	Loipersdorf	1.40	(0.08)	1.62	(0.09)
	Average:	3.63		2.89	

Species and year	Site of gathering	Cr content (mg/kg D.M.)		Ni content	
		$\overline{x}$	±s	$\overline{x}$	±s
Lepista nuda (Bull.: Fr.) Cke.					
1993	Tatabánya/2	0		0	
1993	Mt. Börzsöny	0		0.7	(0.07)
1993	Bakony/1	0		1.01	(0.06)
1993	Bakony/3	0.75	(0.06)	1.71	(0.12)
1995	Őrség (County Zala)	2.17	(0.04)	3.66	(0.17)
	Average:	0.61		1.41	
Lepista luscina (Fr.) Sing.					
1993	Wood Kamara	0.17	(0.02)	1.99	(0.13)
1993	Mt. Börzsöny	0.10	(0.01)	1.35	(0.10)
1993	Mt. Börzsöny	4.40	(0.24)	2.23	(0.11)
	Average:	1.55		1.85	
Lepista inversa (Scop.: Fr.) Pat					
1993	Wood Halmi	0.23	(0.02)	0.8	(0.05)
1993	Miskolc/1	0		0.35	(0.03)
1993	Mt. Bakony	0.38	(0.04)	0.82	(0.03)
1993	Mt. Pilis	0.19	(0.03)	1.23	(0.10)
1995	Tatabánya/1	2.66	(0.02)	3.22	(0.09)
	Average:	0.69		1.29	
Leucopaxillus giganteus (Fr.) Sing.					
1994	Tatabánya/1	1.05	(0.03)	2.28	(0.15)
1993	Tatabánya/4	0		0.28	(0.03)
	Average:	0.55		1.28	
Macrolepiota rhacodes (Vitt.) Sing					
1993	Mt. Pilis	0.66	(0.05)	1.59	(0.10)
1993	Mt. Pilis	0.28	(0.01)	0.70	(0.02)
1993	Miskolc/3	3.76	(0.14)	1.40	(0.02)
1994	Miskolc/2	1.00	(0.06)	1.90	(0.08)
1995	Miskolc/1	2.16	(0.11)	5.23	(0.27)
1995	Miskolc/2	2.15	(0.12)	14.3	(0.44)
	Average:	1.66		4.18	
Macrolepiota procera (Scop.: Fr.) Sing					
1993	Mt. Bakony	2.46	(0.15)	2.40	(0.05)
1994	Wood Kamara	0.79	(0.09)	2.15	(0.10)
1994	Miskolc/1	0.60	(0.04)	2.76	(0.08)
1995	Miskolc/1	2.67	(0.13)	7.19	(0.30)
	Average:	1.63		3.62	

Species and year	Site of gathering	Cr content (mg/kg D.M.)		Ni content	
		x̄	±s	x̄	±s
Stropharia aeruginosa (Curt.: Fr.) Quél.					
1993	Miskolc/3	0.05	(0.01)	0.05	(0.02)
1993	Miskolc/1	0.60	(0.03)	2.23	(0.09)
1994	Mt. Bükk	13.7	(0.23)	13.63	(0.17)
	Average:	4.78		5.30	
Tricholoma imbricatum (Fr.: Fr.) Kummer					
1993	Miskolc/3	0.59	(0.02)	1.91	(0.03)
1994	Miskolc/3	0.63	(0.02)	1.28	(0.07)
1995	Miskolc/3	1.95	(0.06)	3.80	(0.12)
	Average:	1.05		2.33	
Tricholoma scalpturatum (Fr.) Quél.					
1993	SBK	0.05	(0.01)	0.57	(0.04)
1993	Tatabánya/1	0.05	–	0.05	(0.01)
1993	Tatabánya/4	0.05	(0.01)	0.80	(0.03)
	Average:	0.05		0.47	
Tricholoma terreum (Schff.: Fr.) Kummer					
1993	SBK	2.31	(0.17)	1.31	(0.06)
1995	SBK	0.87	(0.06)	18.5	(0.81)
	Average:	1.59		9.9	
Xerocomus subtomentosus (L.: Fr.) Quél.					
1993	Wood Halmi	0		3.63	(0.21)
Xerocomus chrysentheron (Bull.: St. Amans) Quél.					
1993	Wood Halmi	0		3.63	(0.18)
1993	Mt. Börzsöny	0.56	(0.02)	2.81	(0.11)
1994	Miskolc/2	0.59	(0.03)	5.38	(0.24)
1995	Miskolc/1	1.75	(0.08)	6.33	(0.13)
	Average:	0.72	(0.04)	4.53	
Xerocomus porosporus Imler					
1993	Mt. Börzsöny	1.20	(0.09)	1.86	(0.08)
1993	Mt. Börzsöny	3.80	(0.17)	3.75	(0.15)
	Average:	2.50		2.80	

Species and year	Site of gathering	Cr content (mg/kg D.M.)		Ni content	
		$\bar{x}$	±s	$\bar{x}$	±s
Lycoperdon perlatum					
1993	Mt. Pilis	0.32	(0.01)	1.61	(0.07)
1993	Miskolc/3	0		1.03	(0.05)
1993	Tatabánya/1	0		0	–
1994	Miskolc/1	8.45	(0.36)	5.73	(0.12)
1994	Miskolc/1	1.90	(0.09)	3.99	(0.07)
1994	Miskolc/1	0.60	(0.03)	2.09	(0.14)
1994	Miskolc/2	0.50	(0.02)	1.03	(0.06)
1995	Miskolc/1	0.98	(0.04)	4.24	(0.22)
	Average:	1.60		2.47	
Hericium chlatrhoides					
1993	Mt. Bükk	0		2.17	(0.04)
1994	Tatabánya/2	0.21	(0.01)	1.97	(0.10)
1994	Mt. Budai	0.76	(0.02)	1.26	(0.09)
	Average:	0.34		1.8	

Abbreviations: SBK: Botanical Garden of Soroksár; Mt: mountain; the different number at the names (e.g.: Miskolc/1) means different sites of gathering in the region of this town

The average Cr-contents (on dry mass basis) changed between 0.05 and 4.91 mg/kg D.M. i.e. the differences are significant. The smallest Cr-contents were measured in *Tricholoma scalpturatum* (0.05 mg/kg D.M.), in *Xerocomus subtomentosus* (0.05 mg/kg D.M.), in *Hypholoma capnoides* (0.32 mg/kg D.M.). The highest concentrations were found in fruit bodies of *Lactarius deliciosus* (4.51 mg/kg D.M.) and of *Clitocybe nebularis* (3.98 mg/kg D.M.). It is interesting that the mushroom *Stropharia aeruginosa* – although its average Cr-concentration is 4.78 mg/kg D.M. – had considerable variability of the data for samples from different habitats (in two samples low, but in the third sample very high concentrations were measured). The Cr-content of the majority of mushrooms is between 0.5 and 3 mg/kg D.M. On the basis of this analyses it can be stated that the most common, edible species of wild mushrooms do not accumulate chromium.

The variability of Cr-concentration, compared to other metals in fungi (SANTOPRETE & INNOCENTI, 1984; TYLER, 1982a, 1982b; SOLOMKO et al., 1986) is not essential. It seems that the contamination of environment has not a definite role in the level of Cr in mushrooms. The toxicity of higher Cr-concentrations is well known but fortunately the Cr-concentrations of fresh fruit bodies (in consequence of the high water contents of mushrooms) is not dangerous from toxicological point of view. Our analysis did not indicate accumulation of Cr-ions in the fungi.

The nickel concentration of the mushroom species changed between 0.81 and 9.9 mg/kg D.M. The lowest concentrations were found in *Hypholoma capnoides* (0.81 mg/kg D.M.), *Leucopaxillus giganteus* (1.29 mg/kg D.M.) and *Lepista inversa* (1.29 mg/kg D.M.); higher concentrations were measured in *Laccaria amethystina* (7.27 mg/kg D.M.) and *Tricholoma terreum* (9.9 mg/kg D.M.) but these data mean no bioaccumulation. Which factors influence the Ni-content of the mushrooms? The taxonomical position of the species has a role; for example the two *Laccaria* species have similar and relatively high concentrations (6.36 and 7.27 mg/kg D.M.), the two *Macrolepiota* species (*M. rhacodes* and *M. procera*) have similar (to each other) but in absolute value lower concentrations (3.62 and 4.18 mg/kg D.M.), the four *Lepista* species have lower contents (1.29–2.89 mg/kg D.M.). The effect of habitats is relatively unimportant, although these sites of four mushrooms were very different.

The mineral composition of the edible mushrooms is today a worldwide investigated question. The accumulation of some toxic elements (cadmium, arsenic, copper, mercury) has food-chemical and toxicological aspects. The chromium and nickel levels of the mushrooms are relatively stable, balanced. In our investigations the bioaccumulation of these elements was not demonstrated the uptake of Cr and Ni is connected primarily with the taxonomical position and with the complex of ecological factors (habitat, character of substrate etc.).

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EFFECT OF IRRADIATION AND STORAGE ON CELL WALL STRUCTURE OF GOLDEN DELICIOUS AND EMPIRE APPLES

E. KOVÁCS[a], J. P. VAN BUREN[b], L. A. PITIFER[b], H. C. HOCH[c] and B. T. TERHUNE[c]

[a] Department of Microbiology Central Food Research Institute, H–1022 Budapest, Herman Ottó út 15. Hungary
[b,c] Cornell University, Ithaca, NY 14853. USA
[b] Department of Food Science and Technology, [c] Department of Plant Pathology

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Apples (Golden Delicious and Empire) were irradiated by a Co-60 radiation source (0, 1 and 2.5 kGy doses) then stored for 6 weeks at 16 °C.

The rate of autolysis of apple cortex of cell wall materials (Golden Delicious, Empire) increased significantly ($P \leq 0.05$) as a function of increasing radiation dose (1–2.5 kGy) and storage time. The amount of neutral carbohydrates released from the insoluble cell wall fraction increased significantly ($P \leq 0.05$) higher with 2.5 kGy irradiation. The activity of polygalacturonase enzyme increased and that of β-galactosidase decreased as a function of increasing radiation dose and storage time. Irradiation caused significant ($P \leq 0.05$) softening of Golden Delicious and Empire apple. Empire apple cultivar was more sensitive to irradiation and less storable than Golden Delicious cultivar.

The ultrastructure of stored apple cortex tissues was different in the investigated cultivars as observed by scanning (SEM) and transmission electron microscopy (TEM). While the cells in cortex tissues of the stored Empire apple shrunk and collapsed, the cortex tissue cell of stored Golden Delicious cultivar did not differ from those of the fresh one. The cells in the irradiated (1 and 2.5 kGy) apples shrunk and collapsed. The striation pattern on the cell wall of apple cortex tissues with 2.5 kGy irradiation also changed from the parallel to branch-like indicating the decomposition of cell wall materials.

Keywords: apple, irradiation, storage, pectin, β-galactosidase, polygalacturonase, cell wall, ultrastructure

The apple cell wall is comprised of cellulose microfibrils loosely woven together and embedded in an amorphous matrix of polysaccharides including pectic substances. Chemical changes in the pectic substances and middle lamellae are closely related to softening and changes in apple texture (ESCHER & LAPSLEY, 1990; ILKER & SZCZESNIAK, 1990; LAPSLEY et al., 1992).

Softening and loss of cell cohesion of fruit tissues is based on an increase in water-soluble pectin accompanied by a loss of protopectin (MASSEY et al., 1964; KNEE

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et al., 1977). The major constituents of pectic substances are rhamnogalacturonans in which the backbones consist of chains of a α-(1→4)-linked D-galactosyluronic acid residues interspersed with (1→2)- and (1→2,4)-linked L-rhamnosyl residues. Attached to the main chain there are oligosaccharide side chains, containing mainly D-galactose and L-arabinose residues, which are attached to position 4 of (1→2,4)-linked L-rhamnosyl residues. Complete or partial methyl esterification of the galacturonic acid residues gives pectins, or pectinates, which usually exist as calcium salt within the cell wall (CHAPMAN et al., 1987). An increase in water-soluble pectin is usually ascribed to the action of polygalacturonase which requires deesterified residues (PRESSEY et al., 1971; PRESSEY & AVANTS, 1976; PRESSEY, 1983). Exo-polygalacturonases remove uronic acid residues from the non-reducing end of the pectin molecules. Endo-polygalacturonases act randomly on 1,4-glycosidic linkages along the uronide chain. The action of the exo-polygalacturonase may be terminated by rhamnose units. Exo- and endo-polygalacturonases are bound to the cell walls and carry out autolysis of cell wall pectin (WALLNER & BLOOM, 1977; RUSHING & HUBER, 1990). Apples contain very low levels of polygalacturonase, and thus far, only the exo-polygalacturonase has been identified (BARTLEY & KNEE, 1982).

There is a great deal of variation in the softening of different apple cultivars during storage. Irradiation, by increasing activity of polygalacturonase and pectin methyl esterase, results in significant degradation of pectin (SOMOGYI & ROMANI, 1964; ROMANI et al., 1971; JARVIS, 1984; SJÖBERG, 1987; AYYAD et al., 1990) and textural changes (KERTÉSZ et al., 1964; YASIA et al., 1987). Calcium reduced the radiation induced softening in carrots (MASSEY & WOODAMS, 1973), and in apples and pears (KOVÁCS et al., 1985; 1988). Calcium plays a special role in maintaining cell wall structure in fruits and other storage organs by interacting with the pectic acids in the cell wall to form calcium pectate (VAN BUREN, 1984; 1991). Thus, fruits treated with calcium are generally firmer than controls (POOVAIAH, 1986). Light and transmission electron micrographs of the cells of apple fruits showed calcium ion to be effective in preventing cell wall structure changes and in preserving cell to cell adhesion (GLENN et al., 1987; GLENN & POOVAIAH, 1990).

The present paper describes changes in the pectin fractions, solubilized neutral carbohydrates released from insoluble cell wall, polygalacturonase and β-galactosidase activities in irradiated and stored Golden Delicious and Empire apple cultivars, and compares ultrastructural changes of cell walls in different parts of the apple. The aim of the study was to find correlation between structure of apple tissue and softening.

1. Materials and methods

1.1 Raw material and treatments

Golden Delicious and Empire apples were harvested in the orchard of Cornell University (Ithaca). Apples were harvested 146 (Golden Delicious), and 142 days (Empire) following full bloom. After 4 weeks in cold storage (1 °C), apples were irradiated at 1 and 2.5 kGy by a Co-60 radiation source (Food Research and Development Centre, Saint-Hyacinthe, Quebec, Canada). The dose rate was 1 kGy h^{-1}. After irradiation, apples were stored at 16 °C. Zero storage time values were obtained on the day of irradiation.

1.2 Cell wall preparation

Cell wall preparation was modified from the method of ROE AND BRUEMMER (1981). Fifty grams of peeled apple tissue was ground with 50 ml cold solution of 12% polyethylene glycol (molecular weight, 6,000–8,000) and 2% sodium bisulfite in a Waring blender for two minutes at full speed (powerstat at 140). The homogenate was filtered through a milk filter (Agway, Premium milk filter, 300, size 8 inches or ~20 cm). The residue was slightly squeezed to remove remaining liquid. The residue was washed twice by homogenization in 100 ml cold, distilled water followed by filtration. The final residue was weighed and brought upto 50 g with 0.05 mol l^{-1} NaCl. The suspension was stirred for one hour at 4 °C then filtered through a filter paper (Whatman 1) and designated as A (filtrate) and B (residue). Filtrate (A) was centrifuged for 20 min at 15,000 r.p.m. using Sorvall 5534 rotor (Sorvall RC-SB, DuPont, USA). The supernatant was filtered and used for assay of β-galactosidase and polygalacturonase enzyme activities.

For the autolysis measurements the saved, weighed residue (B) was resuspended to 50 g with 50 mmol l^{-1} NaOAc/0.2 mol l^{-1} NaCl, pH=2.5. Five hundred μl of 1% Geneticin (Sigma) solution was added to the suspension to prevent microbial growth. The suspension was incubated at 30 °C for 72 h. These suspensions were analyzed for total pectin. Seven ml of suspensions were taken and centrifuged (International Clinical Centrifuge Equipment Co., USA) at full speed (2,000 r.p.m.). Supernatants were pipetted in tubes and stored frozen until analyzed for solubilized pectin and carbohydrates.

1.3. Assays

The assays were carried out before irradiation (zero time), then 3 and 6 weeks after irradiation.

β-Galactosidase (EC. 3.2.1.23) assay was based on the hydrolysis of nitro-phenyl galactopyranoside (BARTLEY, 1974); and polygalacturonase assay (EC. 3.2.1.67) was based on an acid Cu reducing end group assay (SAJJAANANTAKUL et al., 1989). Total pectin was determined as galacturonic acid, using m-phenyl phenol reagent (BLUMENKRANTZ & ASBOE-HANSEN, 1973; KINTNER & VAN BUREN, 1982).

Solubilized carbohydrates were determined by the anthrone-sulfuric acid method (DREYWOOD, 1946; WHISTLER & WOLFROM, 1962). Each measurement was carried out in six parallels, and the results were statistically evaluated (see 1.7.).

1.4. Transmission electron microscopy (TEM)

Three plugs (50 mm in diameter) were removed from each of three apples by using a cork borer. One-two mm^3 of skin and cortex tissues were cut from plugs and prepared for TEM by chemical fixation. Fixation was carried out in 3% glutaraldehyde, which was buffered in 50 mmol l^{-1} potassium phosphate, (pH 6.8), at 4 °C for 2 h. Tissues were rinsed 4 times 15 min each in buffer followed by two rinses in distilled water for 15 min each. Tissues were postfixed for 2 h with 1% OsO_4 (aqueous weight/volume) at 20 °C, rinsed 6 times with distilled water for 15 min each, and dehydrated in an acetone series (10, 20, 40, 60, 80, 90 100%) for 20 min, each step followed by 3 changes of 100% acetone for 17 h each. Tissues were embedded in an Epon-Araldite resin mixture and polymerized. Sections were made with a Porter-Blum ultramicrotome equipped with an LKB glass knife, and after contrast staining for 15 min with uranyl acetate and for 3–5 min with 0.5% lead citrate, sections were examined in a JEOL 1005X TEM operated at 100 kV.

1.5. Scanning electron microscopy (SEM)

Apple cortex tissue were selected, chemically fixed, and dehydrated as described for TEM. Dehydrated samples were critical point dried through liquid CO_2 in a Balzers CPD 020 critical point drying apparatus. The tissues were then mounted on specimen stubs, and sputter-coated with gold-palladium in a SCD-040 vacuum evaporator. Skin and cortex tissues were examined with a Hitachi S-530 SEM operated at an accelerating voltage of 25 kV.

1.6. Texture

The texture of the apples was determined by a Magness-Taylor Pressure tester (D. Ballauf Mfg. Co., Washington DC, USA). For determination of softening 3–5 peeled apples were used, each apple was measured on both sites (BLANPIED et al., 1978). The softness value was expressed as penetration force in pounds.

1.7. Statistical analysis

General Linear Model procedure was used from the Statistical Analysis System (SAS) package (ANON, 1989). The six data per measurements served as the base for statistical analysis. The mean values (together with the standard deviations not indicated separately) were compared in the General Linear Model Procedure, and the missing values were also considered by the program. The contrast function was used to evaluate the P values for differences between columns, that is between radiation doses, considering the whole storage period. Columns labelled with different letters are significantly different at $P \leq 0.05$.

2. Results and discussion

2.1. Chemical analysis

Results of the autolysis of cell wall materials of apple tissues indicate differences among cultivars, treatments and storage as shown in Tables 1 and 2. The total pectin in the control apple cortex tissues of Golden Delicious and Empire cultivars decreased, while the soluble pectin increased, as a function storage time. The total pectin content significantly decreased as an effect of irradiation. Six weeks after irradiation about 40% of the original amount was found in the irradiated apple tissues (control 70%).

The soluble pectin fraction of polyethylene glycol insoluble cell wall materials of control and 1 kGy treated apple tissues of Golden Delicious cultivar increased significantly ($P \leq 0.05$) during autolysis in NaOAc/NaCl. The rate of release of soluble pectin in apple irradiated with 2.5 kGy dose did not change as a function of storage time. GLENN and POOVAIAH (1990) observed that the soluble pectin content increased in Golden Delicious apple tissue during the first 3.5 months of cold storage (2 °C), but no significant increase was observed during subsequent storage. As our results show, the degradation of pectin is not linear neither as a function of storage time nor as that of radiation dose. MASSEY and co-workers (1964) observed that, 6–12 h after irradiation and in the stored irradiated Rome Beauty apples, the water soluble pectin increased but the HCl soluble pectin decreased with the increasing irradiation dose. KERTÉSZ and co-workers (1964) observed that the increased doses of irradiation caused a progressive increase in the specific viscosity of water soluble extract and a decrease of the HCl soluble extract of pectin in McIntosh apple tissues. The changes in soluble pectin of Empire apple as a function of radiation dose did not differ from each other during storage.

Table 1

Results of autolysis of polyethylene glycol insoluble cell wall materials of apple tissues in Golden Delicious cultivar

Storage time (week)	Total pectin (μmol cm^{-3})			Soluble pectin released from insoluble cell wall nmol h^{-1} g^{-1} fresh weight			Solubilized neutral carbohydrates released from insoluble cell wall nmol h^{-1} g^{-1} fresh weight		
				Doses (kGy)					
	0	1	2.5	0	1	2.5	0	1	2.5
0	14.28[a]	(14.28)[b]	(14.28)[b]	30.00[a]	(30.00)[a,b]	(30.00)[b]	8.16[a]	(8.16)[a]	(8.16)[b]
3	12.55	7.32	7.32	39.00	32.90	30.10	7.68	9.60	21.60
6	10.98	6.02	7.43	59.50	43.40	28.70	14.88	23.04	52.80

( ) before irradiation

n = 6 (number of measurements)

Columns labelled with different letters are significantly different at $P \leq 0.05$ as a function of storage time among irradiation doses

Table 2

Results of autholysis of polyethylene glycol insoluble cell wall materials of apple tissues in Empire cultivar

Storage time (week)	Total pectin (μmol cm^{-3})			Soluble pectin released from insoluble cell wall nmol h^{-1} g^{-1} fresh weight			Solubilized neutral carbohydrates released from insoluble cell wall nmol h^{-1} g^{-1} fresh weight		
				Doses (kGy)					
	0	1	2.5	0	1	2.5	0	1	2.5
0	10.45[a]	(10.45)[b]	(10.45)	10.20[a]	(10.20)[a]	(10.20)[a]	3.84[a]	(3.84)[a]	(3.84)[b]
3	6.80	3.92	2.88	7.00	9.80	9.45	5.76	4.80	11.52
6	7.32	4.19	–	11.20	14.70	–	4.80	10.56	–

( ) before irradiation

n = 6 (number of measurements)

– no data

Columns labelled with different letters are significantly different at P≤0.05 as a function of storage time among irradiation doses

In Tables 1 and 2, the solubilized neutral carbohydrates released from polyethylene glycol insoluble cell wall fraction are also presented. In both cultivars the solubilized carbohydrates increased significantly ($P \leq 0.05$) with 2.5 kGy irradiation dose, indicating that the irradiation stimulates the autolysis of polysaccharides. Based on the results of GLENN and POOVAIAH (1990), it might be supposed that the solubilized carbohydrates mostly consist of glucose, which derived from cellulose or hemicellulose. The neutral sugar content cell wall in Golden Delicious apples consists of arabinose, galactose (1.3–1.45 mg g^{-1} fresh weight), glucose, xylose (0.34–0.58 mg g^{-1} fresh weight), glucose from cellulose (4.76 mg g^{-1} fresh weight) (GLENN & POOVAIAH, 1990). It was established that the irradiation of cellulose waste material (rice straw, chaff, sawdust) increased the efficiency of subsequent enzymic or acid hydrolysis (ELIAS & COHEN, 1983). Our results (Table 1 and 2) are in agreement with those of ELIAS and COHEN (1983) that irradiation increases the rate of autolysis of carbohydrates released from polyethylene glycol insoluble residue of cell wall of apple tissues. KERTÉSZ and co-workers (1964) investigated the changes in cellulose of irradiated McIntosh apples, carrots and beets; their results indicated progressive loss of viscosity with increased dose in apple (but not to a marked extent) and in carrot tissues. With beets, however, no consistent trend was obtained. HOWARD and BUESCHER (1989) observed that irradiation with 1 kGy dose caused only minor changes in the neutral sugar composition of cell walls from mesocarp tissues of refrigerated cucumber pickles. Decomposed starch could also modify the amount of the solubilized carbohydrates. Pectin appeared more susceptible to degradation by ionizing radiation than cellulose (KERTÉSZ et al., 1964). Our results indirectly support this observation. The increased solubilized carbohydrates should derive also from less radiosensitive cell wall fractions than pectin.

Most enzymes, which play role in the storage, processing, and preservation of food are rather resistant to ionizing radiations. Pectic enzymes in fruits were found to be very resistant to radiation damage in situ. Most of the published literature dealt with in vitro and in situ radioresistance of pectin methyl esterase and cellulase. The physiological significance of these changes in enzyme level is largely unknown (ELIAS & COHEN, 1983).

In Table 3 an increase with increasing storage time of β-galactosidase metabolic activity can be observed for Golden Delicious and Empire apple tissues. The β-galactosidase activity was very low in each treatment, and it decreased significantly ($P \leq 0.05$) as a function of increasing radiation dose in both cultivars. RAO and VAKIL (1983) also found dose-dependency in irradiated beans, but only upto 1 kGy.

The polygalacturonase (PG) activity of apple tissues was 3.5–4 times greater in the Golden Delicious apples, than in Empire apples. The changes in PG activity of control and 2.5 kGy treated Golden Delicious apples run the same way as a function of

a storage time (no significant difference between the two columns). The activity of PG increased significantly in apples irradiated with 1 kGy in Golden Delicious (Table 4). However, there was no difference between treatments in cv. Empire.

Table 3

Effect of irradiation of the β-galactosidase activity of Golden Delicious and Empire apple tissue

Cultivars	Storage time (week)	Doses (kGy)		
		0	1	2.5
		mmol min^{-1} g^{-1} fresh weight		
Golden Delicious	0	1.21[a]	(1.21)[b]	(1.21)[c]
	3	2.97	1.87	1.65
	6	3.70	2.68	1.45
Empire	0	0.33[a]	(0.33)[b]	(0.33)[c]
	3	1.65	0.66	1.32
	6	2.86	0.88	–

( ) before irradiation
n = 6
– no data
Columns labelled with different letters are significantly different at $P \leq 0.05$ as a function of storage time among irradiation doses

Table 4

Effect of irradiation on the polygalacturonase activity of Golden Delicious and Empire apple tissue

Cultivars	Storage time (week)	Doses (kGy)		
		0	1	2.5
		mmol h^{-1} g^{-1} fresh weight		
Golden Delicious	0	2.44[a]	(2.44)[b]	(2.44)[c]
	3	5.80	9.32	5.19
	6	6.57	12.84	5.19
Empire	0	0.61[a]	(0.61)[b]	(0.61)[c]
	3	0.46	0.61	1.22
	6	0.76	2.75	–

( ) before irradiation
n = 6
– no data
Columns labelled with different letters are significantly different at $P \leq 0.05$ as a function of storage time among irradiation doses

There were no significant differences between firmness of the cultivars at the beginning of storage. One week after irradiation the Golden Delicious and Empire apples were softer than the control. Irradiation with 1 kGy caused significant ($P \leq 0.05$) softening of Golden Delicious apples, but there were no significant differences between apples irradiated with 1 and 2.5 kGy (Table 5). The softening of Empire cultivars increased as a function of increasing radiation dose. The more intensive softening of Empire apples are in connection with the reduced pectin content (Table 2) and the composition of carbohydrates in cell wall. Not only the pectin and/or cellulose degradation is responsible for softening, but also the changes in cell turgidity. Irradiation induces electrolyte leakage, increases the permeability of membranes, these changes influence the cell turgidity of plant tissues (KERTÉSZ et al., 1964).

Table 5

Effect of irradiation on the texture of Golden Delicious and Empire apples

Cultivars	Storage time (week)	Doses (kGy) 0	1	2.5
		lbs		
Golden Delicious	0	15.10[a]	(15.10)[b]	(15.10)[c]
	1	12.00	6.50	6.00
	6	9.30	5.70	4.80
Empire	0	14.20[a]	(14.20)[b]	(14.20)[c]
	1	8.50	7.70	4.20
	6	5.50	1.00	–

( ) before irradiation
n = 6
– no data
Columns labelled with different letters are significantly different at $P \leq 0.05$ as a function of storage time among irradiation doses

2.2. Scanning electron microscopy and transmission electron microscopy

The ultrastructure of apple tissue was studied as a function of irradiation dose and storage time to observe relationship between macro- and microstructural changes. Figures 1a–d show the scanning electron micrographs of fresh and stored (6 wks) control and irradiated Golden Delicious apple. The shape of unirradiated cells in the fresh (1a) and stored (1b) apples did not differ from each other. In comparison, the cortex cells in the apple irradiated with 1 kGy (1c) and 2.5 kGy (1d) shrunk and collapsed. Large number of cortex cells separated, or were only slightly bound to the

neighboring cortex cells in the apple, due to the loss of pectin (as established by chemical analysis, Tables 1 and 2).

A characteristic cortex cell with broken wall of the fresh control apple was selected by SEM (Fig. 2a). On the outer side a smooth, continuous cell wall with an organized striation pattern was observed (Fig. 2b). On the inner wall, cytoplasmic residues were visible (Fig. 2c). Broken cells were not observed (see Fig. 1d) in the stored irradiated with 2.5 kGy apples. The outer side of cell walls (Figs. 3a and 3b) was fragmented and peeled, with an irregular, short branching networks.

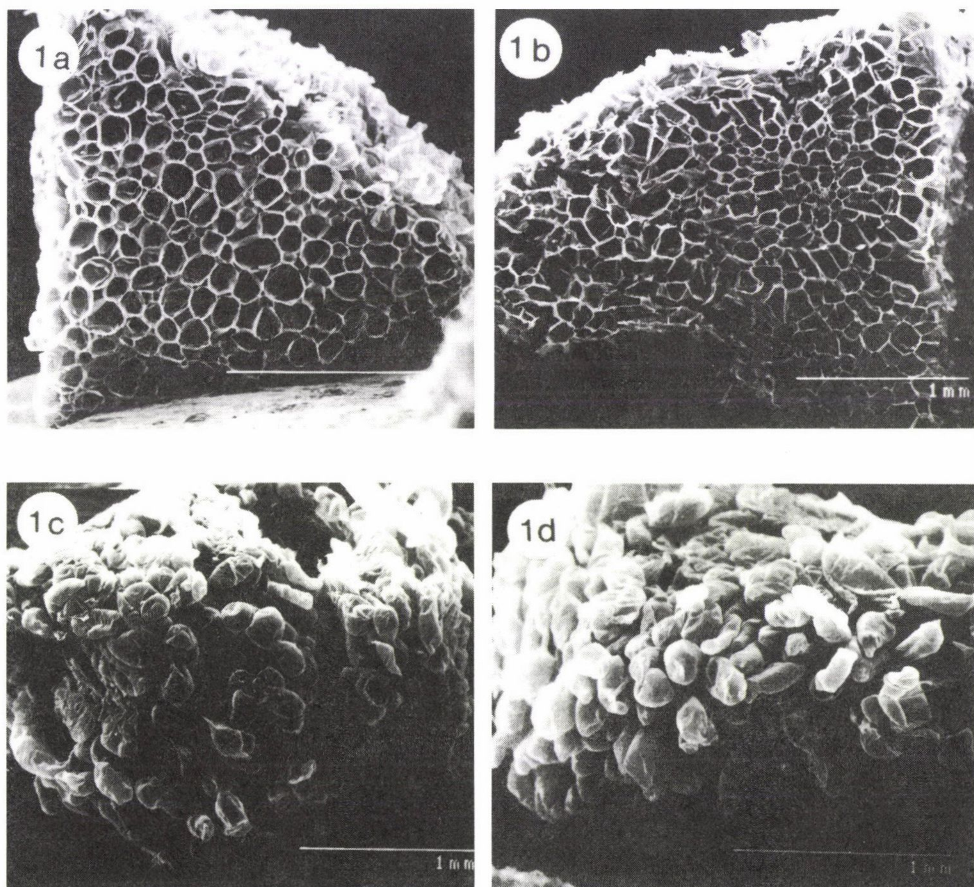

Fig. 1. Ultrastructure of Golden Delicious apple cortex (SEM). a: fresh control; b: stored for 6 weeks, control; c: stored for 6 weeks, 1 kGy; d: stored for 6 weeks, 2.5 kGy. Bars = 1 mm

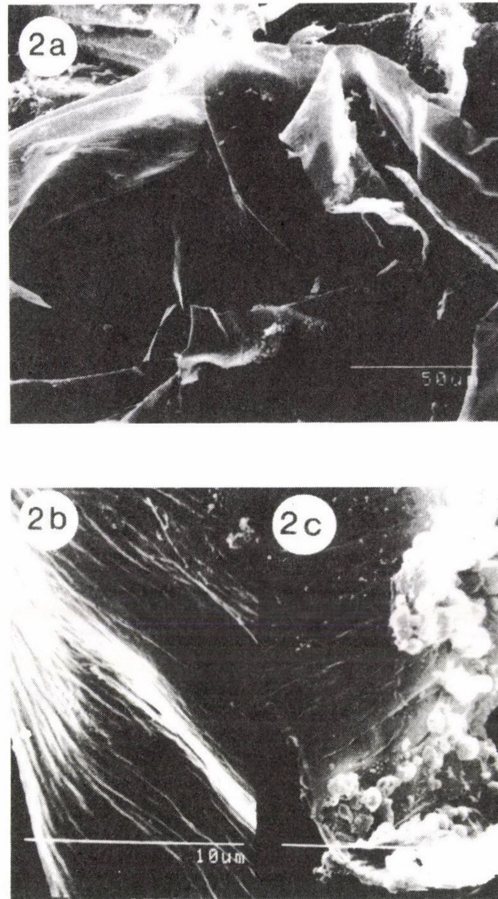

Fig. 2. Ultrastructure of surface of the cell wall of fresh Golden Delicious apple cortex (SEM). a: cells with broken wall; b: outer side of cell wall; c: inner side of cell wall. Bars = 50 μm (a); 10 μm (b, c)

In Figs. 4a–4d, the incipient breakdown of middle lamellae was visible in skin and flesh of stored control Golden Delicious apples (Figs. 4a and 4c) and, to a greater extent, in the Golden Delicious apples irradiated with 2.5 kGy (Figs. 4b and 4d). The middle lamellae was dense and relatively intact in the control, and less dense in the irradiated Golden Delicious apples. Similar changes were also reported for irradiated Gloster and Mutsu apples (KOVÁCS et al., 1985; 1988).

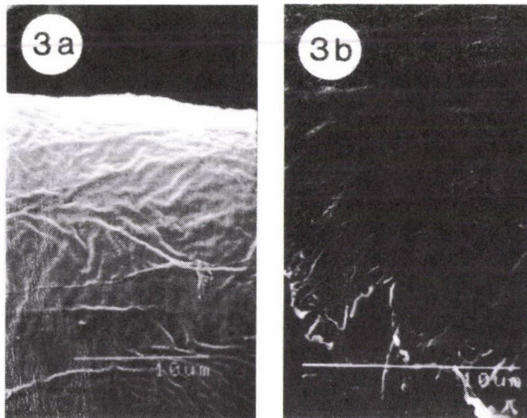

Fig. 3. Ultrastructure of the cell wall surface of stored Golden Delicious apple cortex (SEM). a: stored for 6 weeks, control; b: stored for 6 weeks, 2.5 kGy. Bars = 10 µm (a, b)

Visible degradation of the middle lamella has been observed by GLENN and POOVAIAH (1990) in the cortex of control Golden Delicious apple. In some cells, regions of the middle lamella were fenestrated, suggesting that extensive degradation occurred, while other regions showed only minor breakdown. A change in microfibril orientation was visible in the cell wall region of control apple: the middle lamella of calcium treated fruit stained dark, indicating the presence of intercellular material. Microfibril orientation in the cell wall structure of calcium treated apple was parallel with the long axis of the cell wall. According to ROLAND and co-workers (1987), the geometry of the cell wall at the supramolecular level consists of a multidimensional system, with microfibrils in a cell organized shape. The organization was changed by treatments such as auxin, ethylene, gibberellin, etc.

The ultrastructure of Empire apples was also studied (Figs. 5a–5c). As the chemical assays showed (Tables 1 to 5), Empire apples differ from Golden Delicious apples in many respects. Scanning electron micrographs of the cortex illustrate a degenerated middle lamella completely broken down in the stored control Empire apple; the cohesion between cells ceased to exist (Fig. 5b) and the apples were mealy. The same contrast was observed between Granny Smith and Rubinette apples (LAPSLEY et al., 1992). Granny Smith tissue has more fractures through the cells. Rubinette is very soft and mealy. Rubinette tissue had more intact cells with fractures around cells rather than through them, indicating that cell separation rather than

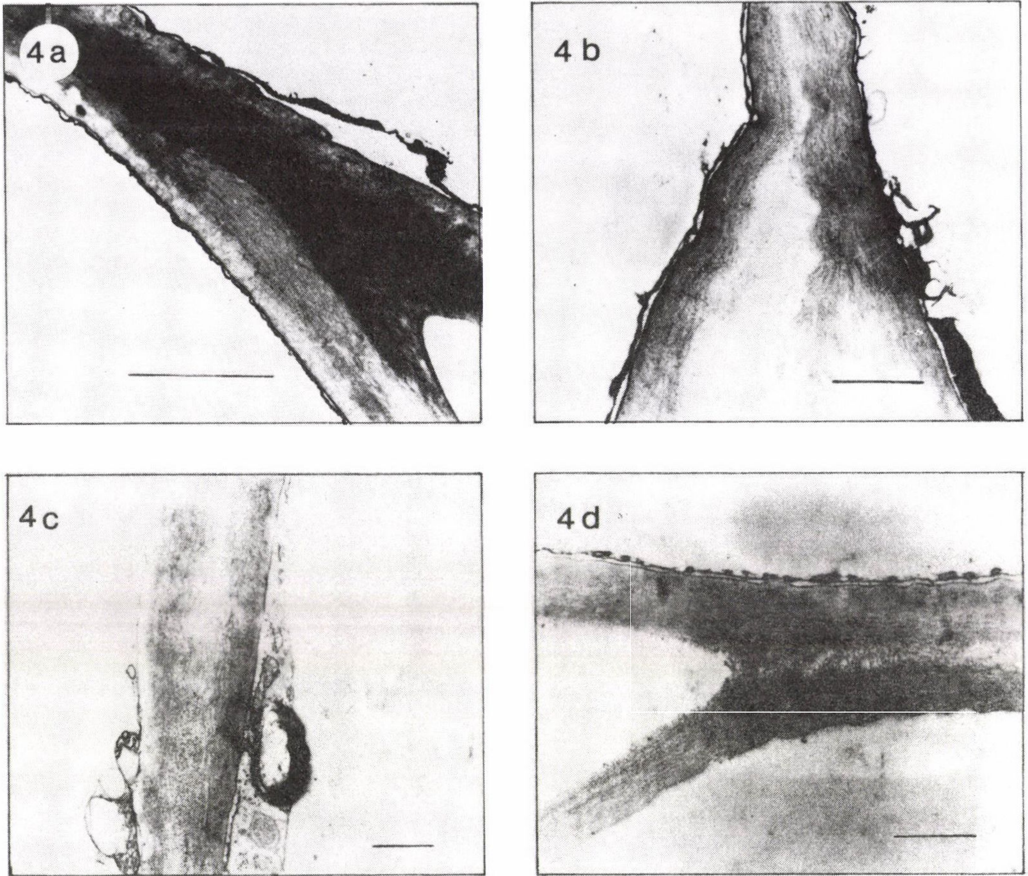

Fig. 4. Effect of irradiation on the middle lamellae of Golden Delicious apple skin and cortex (TEM). a: stored for 6 weeks, control skin; b: stored for 6 weeks, 2.5 kGy skin; c: stored for 6 weeks, control cortex; d: stored for 6 weeks, 2.5 kGy cortex. Bars = 40 μm (a); 80 μm (b, c); 200 μm (d)

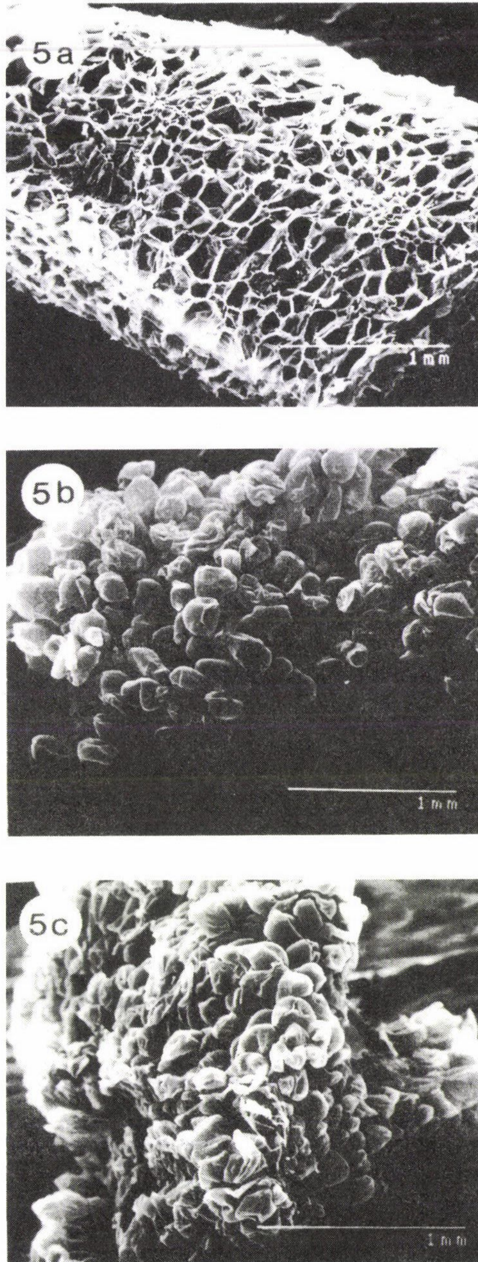

Fig. 5. Ultrastructure of Empire apple cortex (SEM). a: fresh control; b: stored for 6 weeks, control; c: stored for 6 weeks, 1 kGy. Bars = 1 mm

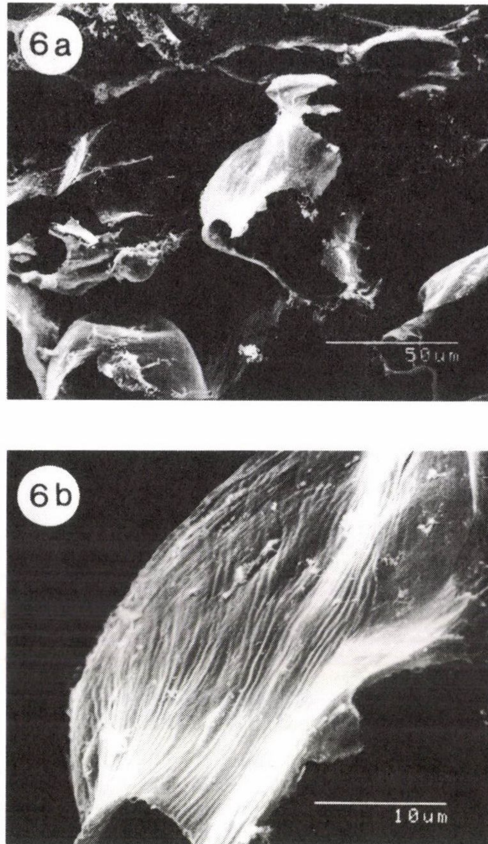

Fig. 6. Ultrastructure of surface of the cell wall of fresh control Empire apple cortex (SEM). Bars = 50 μm (a); 10 μm (b)

fracture of cell wall had taken place upon breaking apart the apple cylinder. The mealy Rubinette tissue is an independently compiled group of cells with decreased cell to cell adhesion (LAPSLEY et al., 1992). The Empire apples are generally considered susceptible to softening in storage. A relatively low radiation dose (1 kGy) initiated breakdown of the microstructure of Empire apples (Fig. 5c) greater than was observed in Golden Delicious apples by a treatment of 2.5 kGy. The degree of radiosensitivity is based on the differences of cultivars and the structural differences in cell wall materials in apples.

Structures similar to those in Empire and Rubinette apples are observed in watermelon treated with ethylene (ELKASHIF & HUBER, 1988). The activity of PG enzyme increased 1 day after ethylene treatment. Maximum activity coincided with the time at which the influence of ethylene on leakage was first noted. The increased leakage in ethylene-treated fruit would therefore appear to involve, in part, wall weakening brought about by PG action. A consequence of the event would be an enhanced tendency for membrane rupture.

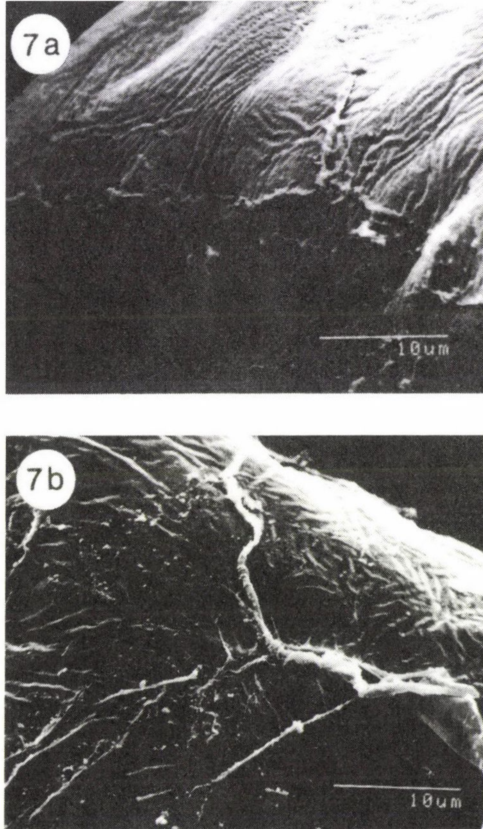

Fig. 7. Ultrastructure of surface of stored for 6 weeks Empire apple cortex cells (SEM). a: stored for 6 weeks, control and b: stored for 6 weeks, 1 kGy. Bars = 10 μm

The fine structure of fresh Empire apple is presented in Figs. 6a and 6b. A parallel striation pattern was observed in cell wall of fresh Empire (Fig. 6b). The stored control apple (Fig. 7a) was similar to the stored irradiated Golden Delicious apple (Fig. 3b). In Empire apples irradiated with 1 kGy, then stored tissues, collapsed and were more shrunk than in the stored control apple tissues (Fig. 7b). The striation pattern became shorter and showed a branch-like structure, and the surface of the cell wall was peeling. This branch-like structure may reflect chemical changes in cell wall materials, maybe in cellulose fibrils, related to irradiation. Further investigations are needed to prove this hypothesis. GLENN and POOVAIAH (1990) assumed that the disrupting parallel striation pattern of fibrils was probably based on the dissolution of matrix material in the cell wall and in the intercellular spaces.

3. Conclusion

Irradiation with increasing doses induced degradation of pectin. The total pectin content decreased, while the soluble pectin content increased. In the cell wall of stored irradiated (2.5 kGy) apples, there was less soluble pectin than in the control of 1 kGy irradiated apples. The amount of solubilized neutral carbohydrates of cell wall increased with the increasing radiation dose and as a function of storage time. Further investigation and separation of solubilized carbohydrates are needed to establish the effect of irradiation. The activity of the β-galactosidase enzyme decreased and the activity of the polygalacturonase enzyme increased as a function of increasing radiation dose. Irradiation induced softening in both cultivars (Golden Delicious, Empire), but Golden Delicious apples softened less than Empire apples. SEM and TEM results show a good correlation with the results of chemical analysis. On the basis of these results it is established, that the tissue structure, size of cells influence softening and storability. Further investigations are needed, what other cell wall compounds and structural properties are also responsible for the softening of irradiated apples.

*

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Acta Alimentaria, Vol. 26 (2), pp. 191–192 (1997)

Book reviews

Microorganisms in foods 5

Microbial specifications of food pathogens

International Commission on Microbiological Specifications for Foods (ICMSF)

Blackie Academic & Professional, London,
an imprint of Chapman and Hall, London, 1996, 513 pages

The control of food safety in modern food processing relies upon HACCP and other systems that identify hazards and define process to control them. These demand a thorough understanding of the properties of microbial pathogens under all the conditions that could be found in foods and food processing environment.

This book is directed toward persons with some technical background, including food industry personnel in quality assurance, technical support or research and development, and food inspection authorities at the local, state, regional or national levels. The book can serve as a relatively quick reference manual, a condensation of technical literature. Each chapter (26 chapters) summarizes the existing knowledge concerning a foodborne pathogen or group of pathogens, collecting the most reliable and useful information. The book is concerned solely with foodborne pathogens and does not discuss spoilage organisms.

The first part of each chapter provides a general survey of foodborne pathogen, with appropriate referencing to authoritative review material. The history and the occurrence of the organism in nature, as well as its taxonomy, are briefly reviewed. The symptoms but not the treatment of the relevant foodborne disease syndrome(s) are discussed, as well as the mechanism of pathogenicity. Consideration is given to the available methods for the enumeration and identification of the organism, as well as possible alternative methods. Finally, the epidemiology of the foodborne disease and its importance are reviewed.

Each chapter then concerns itself with the specific parameters that influence growth, survival or death of the microorganism. Included is information on temperature, water activity, pH, irradiation, preservatives, gases, disinfectants and, where information is available, on interactions between these parameters. The relevant data are presented as a brief text, followed by tables. References cited appear at the end of each chapter; these are supplemented by an Additional Reading section at the back of the book.

This compilation of high quality, reliable data on the behavior of food poisoning organisms is an essential source of information for those who wish to employ the HACCP system for the control of microbial hazards and for anyone who must verify the adequacy of an existing process, or is developing a new food process. This book is also intended to serve as a handbook for those involved in epidemiological investigations, responsible for identifying the source or cause of contamination, or who are recommending corrective actions or solutions to problems.

The very useful information has been prepared by the International Commission on Microbiological Specifications for Foods (ICMSF). The ICMSF was formed in response to the need for internationally acceptable and authoritative decisions on microbiological limits for foods moving in international commerce. The staff consists of eighteen food microbiologists from eleven countries, drawn from governmental laboratories in public health, agriculture and food technology, from universities and from the food industry.

CS. MOHÁCSI-FARKAS

Processing fruits: Science and technology I, II

L. P. SOMOGYI, D. M. BARRETT, H. RAMASWAMY and Y. H. HUI (Eds.)

Technomic Publishing Co., Lancaster, Pennsylvania, 1996, 510+558 pages

A new book has been published in 1996 by the Technomic for high level appliers in the field of food science and technology. The book consists of two volumes bound in hardback imitation leather and covers almost 1100 pages.

Volume 1 with sub-title *Biology, principles and applications* consists of 15 chapters:

Classification, composition of fruits and post harvest maintenance of quality; Thermal processing of fruits; Juice processing; Enzymes in the fruit juice and wine industry; Fruit preserves an jams; Refrigerated and controlled modified atmosphere storage; Fruit freezing; Drying of fruits; Ionizing radiation for preservation of fruits; Microbiology of fruit products; Direct food additives in fruit processing; Quality assurance, quality control, inspection and sanitation; Packaging of fruits and vegetables; Grades, standards and food labelling; Residual management in fruit processing plants.

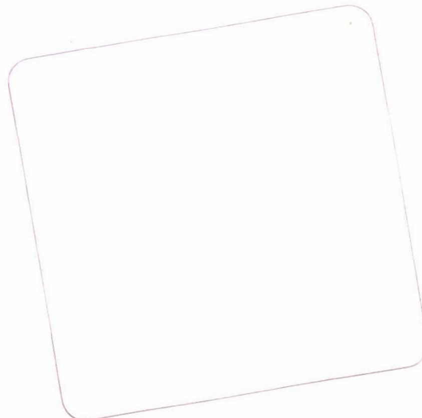
Volume 2 with sub-title *Major processed products* consists of 17 chapters and discusses the processing technologies of the following fruits (and nuts): Apples, apricots, peaches, cherries and sour cherries, plums and prunes, grapes and six other berries, five fruits inside the citrus genus, pineapples, bananas, seven tropical fruits, coconuts, avocados, olives and nuts. A separate chapter deals with raisin production.

Altogether 43 authors from 6 countries (Canada, USA, Germany, the UK, Taiwan, the Philippines) contributed to the text, of whom 4 undertook editorial work as well (L. P. Somogyi, D. M. Barrett, Y. H. Hui, H. S. Ramaswamy). All authors have excellent practice and qualification either in research, or industry, or in both.

Both volumes deserve the appreciation of experts, summing up high level application of food science achievements in a concise manner, covering all important fruit preservation methods. The book can be recommended for qualified industrial appliers (processing, plant design, engineering), and can be successfully used in the education at university level. The inclusion of fruits from outside the temperate climate can be rated as special feature. Chapters are completed by appropriate reference lists, volumes by subject indexes.

However, the reviewer has to criticize some parts: The thermal process parts are superficial, the formula method is of second importance in the light of modern measurement and calculation means (or should be?). Calculation methods and concepts for flow-through type pasteurization are almost completely lacking, quality attribute kinetics too. Neither the concept of residence time distribution, nor the expression fundamentals (on conceptual level) have been included. There is also some inconsequency in the use of the SI system. °F and °C are sometimes mixed, casually gallons are used (which gallon?, quite alien to continental Europeans).

I. KÖRMENDY



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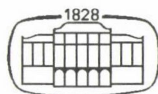

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LOW-TEMPERATURE THERMOLUMINESCENCE STUDIES ON MILK PROTEIN CONCENTRATE POWDER

L. I. KISS and J. KISPÉTER

College of Food Industry, H-6701 Szeged, P.O. Box 433, Hungary

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The thermoluminescence responses of the aromatic amino acids in a milk protein concentrate powder were investigated between 100 and 200 K. The structure was taken as that of an organic semiconductor, and the thermoluminescence curve was analysed in an appropriate band model to characterize the electron trap responsible for the phenomenon. The second-order activation energy of the processes producing the low-temperature thermoluminescence was determined by curve-fitting.

Keywords: thermoluminescence, activation energy, curve-fitting

Thermoluminescence (TL) is one of the most useful physical methods to demonstrate the previous irradiation treatment of many foodstuffs (BÖGL & HEIDE, 1991). Through the interpretation of this phenomenon in band models, TL also serves as a tool to describe the structure of special materials (CHEN & KIRSH, 1981), in our case a food-industrial product being considered as an organic semiconductor (KISS & KISPÉTER, 1995). Since a portion of the excitation energy is stored in deficiencies called electron traps (SZALAY & DAMJANOVICH, 1983 and SWALLOW, 1991), the characteristic parameters of these traps can be determined from TL measurements at different temperatures.

It has been found in low-temperature TL measurements (SZALAY & DAMJANOVICH, 1983) that aromatic amino acids emit light between 100 and 150 K (hence, the TL of peptides and proteins originates from their aromatic amino acid moieties), and that the TL yield of aromatic amino acids is at least twice as high as that of other amino acids. The first triplet state in aromatic amino acids has been presumed to be the excited phosphorescence state, so TL may arise not only from the recombination of trapped charge carriers, but also from the triplet–triplet interaction, as revealed by studies on nucleic acids (DESAI et al., 1977).

A recent paper (KISS & KISPÉTER, 1995) provided information on trap parameters obtained from TL curves at temperatures above room temperature. Here, as a complement to that work, low-temperature measurements are detailed, and the kinetic

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order and activation energy of the processes resulting in the TL of milk protein concentrate powder (MPCP) are determined. Such basic research studies are of value in modelling the structure of the material under investigation (by placing electron traps in the forbidden band or the gap), and thus they may assist in the "dosimetric" demonstration of irradiation treatment.

1. Materials and methods

Milk protein concentrate powder (MPCP), a model material, that had been carefully studied previously, was chosen for low-temperature TL measurements (KISPÉTER & KISS, 1993; KISPÉTER et al., 1994 and KISS & KISPÉTER, 1995). As earlier, the powder in the form of pressed disc was treated with ionizing γ-radiation of 0, 2, 5,10 and 20 kGy.

Low-temperature TL measurements between 100 and 200 K were carried out with a home-built set-up (outlined in Fig. 1) at linear heating rates of 0.46, 0.75, 0.98 and 1.15 K s^{-1}. The disc sample was fastened to a copper sample-holder in a metal cryostat. Energy states in the sample cooled down by liquid nitrogen were excited with a Xe arc-lamp of 150 W at about 90 K (at a pressure of ca. 100 Pa).

To attain a linear heating rate, the measured temperature was compared with a datum referring to the given heating rate, and a signal was then generated for the heating control unit through a D/A converter to govern the DC power supply of a heater spiral in the sample holder. In the considered interval of 100 K, the maximum temperature difference between the thermocouple (type E: NiCr–CuNi) and the sample

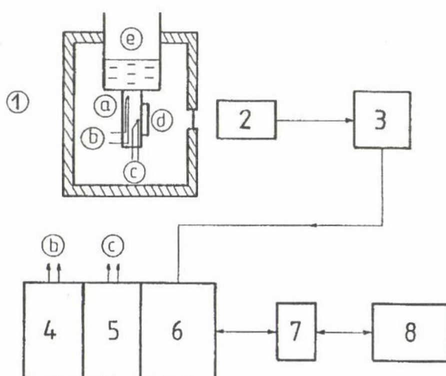

Fig. 1. Block diagram of the measuring system. 1. Cryostat: (a) sample holder, (b) heater spiral, (c) thermocouple, (d) sample, (e) liquid nitrogen; 2. photomultiplier; 3. DC electrometer; 4. heating control unit; 5. temperature block; 6. analogue unit; 7. interface and converters; 8. personal computer

was 1 K. An AD595 amplifier and compensator IC unit was used for the temperature measurement, and a card type PCL 711 was built in to achieve the connection between the units and the personal computer.

Light was detected with an EMI 6097S photomultiplier, and the current or voltage (proportional to the intensity of the emitted light) was amplified with a Keithley 610C DC-meter. The resulting TL curves could be both drawn with a recorder and processed in the computer.

Calculations to determine the main parameters of the electron traps responsible for the phenomenon were performed with SigmaPlot 4.1 software. On the basis of the mathematical formula for TL intensity for second-order kinetics (CHEN & KIRSH, 1981), a numerical curve-fitting technique was used with the least-squares method to minimize the sums of squared differences between the observed quantities and those evaluated from the model. The calculation procedure was detailed in a previous publication (KISS & KISPÉTER, 1995).

2. Results and discussion

The low-temperature TL of MPCP samples irradiated with different γ-doses was investigated at heating rates of less than 1.5 K s^{-1}. The single peak in each TL curve can be characterized as follows from the results of the present and former (KISPÉTER & KISS, 1993) studies.

1. At different γ-doses, the peak temperature shifted towards higher values (from 130 to 145 K in the indicated interval), while the intensity and the integrated intensity, i.e. the area under the curve, increased with increasing heating rate. (They nearly doubled in case of unirradiated sample.)
2. At different heating rates, the intensities decreased with increasing γ-dose to about 70% of the relative peak intensity of the unirradiated sample for 20 kGy.

Similar findings have been reported for inorganic and other organic materials (cf. monographs by CHEN & KIRSH, 1981 and SZALAY & DAMJANOVICH, 1983). Although the decrease of TL intensity was proportional to the absorbed γ-dose, its degree of about 30% up to 20 kGy might not make the detection of irradiation possible to the extent required in practice. This may be compared with the result of measurements above room temperature (KISPÉTER & KISS, 1993 and KISPÉTER et al. 1994), where the change was an increase in intensity values, the differences there being enough to prove the fact of irradiation after several months as well.

The single peak allowed determination of the features of the processes accounting for this phenomenon in several ways. On the basis of certain points of the TL curve, such as peak and half-intensity temperatures, the symmetry of the peak indicating the kinetic order of the processes, and also the activation energy could be

determined (CHEN & KIRSH, 1981). The value of the symmetry factor did not reflect pure first- or second-order kinetics, and the activation energy obtained via these certain points depended slightly on the applied heating rate, which is inconsistent with theory. Since second-order kinetics was suggested by the shape of the curve, the temperatures and relative intensities of the peaks obtained at different linear heating rates could be used for the appropriate Arrhenius plot (CHEN & KIRSH, 1981). The second-order activation energy was evaluated from the slope of the regression line, but its value could not be accepted for the relatively inaccurate determination of the peak data at the lowest and highest heating rates. The reliability of the calculated parameter values was enhanced by using many more measurement points in the mathematical procedure.

Dozens of measured values were utilized in the method of curve-fitting to decide the kinetic order of the processes and to determine their activation energy. Only these two main parameters could be evaluated from the TL measurements alone, as previously (KISS & KISPÉTER, 1995). Of the numerous analysed TL curves of MPCP at different γ-doses and linear heating rates, a characteristic one is presented in Fig. 2; the continuous line is the fitted curve, while the measured values are indicated by black circles.

In the curve-fitting procedure for the investigated MPCP, it was concluded that

- the low-temperature TL peak reflected second-order kinetics,
  the measured curve comprising a single second-order curve;
- the calculated activation energy was 0.07±0.005 eV, independently of the absorbed γ-dose and the applied linear heating rate.

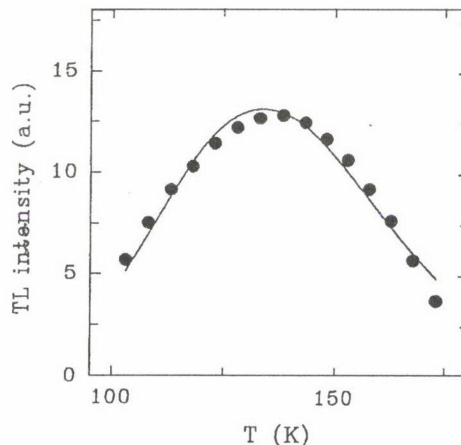

Fig. 2. Curve-fitting of low-temperature TL measurement for an MPCP sample treated with a 10 kGy γ-dose at a linear heating rate of 0.98 K s^{-1}. Standard deviations less than 10%; a.u.: arbitrary units

3. Conclusions

In an appropriate band model of semiconductors, the low-temperature one-component TL curves obtained for MPCP can be described on the theory of second-order kinetics. Assuming second-order kinetics, the activation energy of the processes resulting in the TL MPCP was found by numerical curve-fitting to be about 0.08 eV, i.e. the level of the electron trap from which the stored electrons escape and produce TL by recombination with the holes is about 0.08 eV, lower than the conduction band.

The low-temperature TL of MPCP most probably originates from the aromatic amino acids (tryptophane, phenylalanine, etc.). The activation energy calculated for tryptophane was 0.1±0.05 eV (SZALAY & DAMJANOVICH, 1983), so our result of 0.08 eV is acceptable, as the investigated product contained only 10–15% aromatic amino acids (BALATONI & KETTING, 1981). Though one shallow electron trap seemed to be responsible for the TL at low temperatures, it could not be an isolated trap, because the effect of the γ-dose was measurable. Hence, the irradiation energy is stored in the deep traps for a long time, and the dose-dependence of the TL intensity points to a connection between the shallow and deep traps. This connection should be clarified for a more correct interpretation, since a thermally isolated trap system is presumed in the interpretation of the TL phenomenon in a solid-state physical band model.

The interpretation of this phenomenon in this work was based on the recombination of trapped charge carriers, but the explanation involving a triplet–triplet interaction may be important from the aspect of basic research. Via measurements of the low-temperature TL curve of one component (samples of any pure aromatic amino acid), the results relating to these two interpretations can be compared to validate one or other of them. The behaviour of the TL characteristics (e.g. the TL intensity) during storage might be of interest to reveal the probable connection between the traps in MPCP as well.

*

Thanks are due to G. KISS for developing the home-built low-temperature TL set-up, and to É. HIDEG for curve-fitting and valuable suggestions.

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FOOD CONSUMER TYPES AND BEHAVIOR IN HUNGARY: A SURVEY OF FOOD CONSUMERS' ATTITUDES AND PRACTICAL BEHAVIOR

J. PAPP[a], Z. LAKNER[b], N. KOMÁROMI[a] and J. LEHOTA[a]

[a] Department of Agricultural Marketing; University of Agricultural Sciences, H–2100 Gödöllő, Páter Károly u. 1. Hungary
[b] Department of Food Economy; University of Horticulture and Food Industry, H–1118 Budapest, Villányi út 35-43. Hungary

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A direct-question representative survey was carried out in Hungary to determine the basic value system of Hungarian consumers to estimate the respondents' level of recognition of the importance of diet to health, and to characterize the consumers attitudes towards issue food consumption and food safety. Using a combination of factor and cluster analysis four main consumer groups could be separated: the "conservative, modest common man", "the well-balanced, staid, middle class", "skeptical (lower) middle class members" consumer groups and the cluster of "younger, material-values oriented consumers". On the base of these values in most cases characteristic differences could be detected between the attitudes and behavior of consumers. Results of segmentation on the base of "classic" consumer group segmentation (age, place of living, sex etc....) as well as on the base of combination of factor and cluster analysis have been compared. The results of the survey underline, that for the explanation and forecasting of consumer's behavior grouping of the consumers on the base of cluster-analysis is more accurate and sensitive, than the grouping on the base of generally accepted socio- demographic values. Importance of health as a value is overall accepted among Hungarian consumers, but in practice the consumers behavior is often not in accordance with theoretically accepted principles. Results of the survey mirror and underline contradicting character of Hungarian food buyers at the age of decreasing aggregate demand, sharpening social differences and economic transition.

Keywords: food consumption, consumer behavior, market segmentation

Hungarian food industry plays a determining role in satisfaction of home-demands and is a very important net foreign-exchange importer. The efficiency of food export is better than that of the majority of other products of the Hungarian economy, that's why the food industrial export is of vital importance from the viewpoint of the external balance of the national economy (SZERDAHELYI, 1994). The rush and dynamic changes in foreign markets of Hungarian food industry (collapse of the COMECON, emergence of the Single European Market) mean a new impulse to re-consider the

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competitiveness of Hungarian agrofood sector (UDOVECZ, 1994). In decades of intensive export-subsidy and safe COMECON-market the food economy concentrated mainly on export, because the positions on home market were considered as safe. The import-liberalization and the increasing competition on foreign market underline the importance of home market.

The analysis of some international competitive branches of food economy in the developed European states (Fig. 1) underlines, that the security of internal market is a necessary precondition from point of view of export success. It can be seen from the figure, that for example the French wine industry has a leading position on world market, but more than two-third of the wine products are sold on home market.

The bad health condition of Hungarian citizens is another fact (Fig. 2), which underlines the importance of studies, concerning with the buying habits and nutrition of Hungarian consumers. The tight relation between nutrition and cardiovascular diseases are well known, but in the latest literature there are numerous studies, concerning the relationship between nutrition and cancer (Fig. 3) as well.

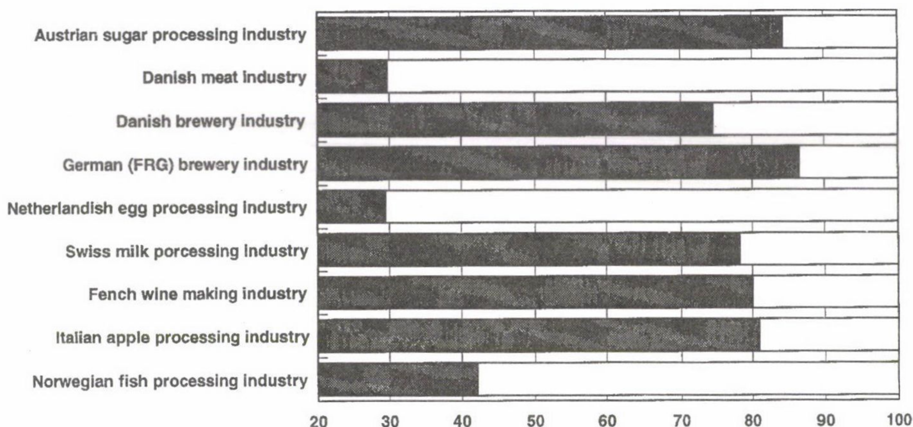

Fig. 1. The share of home consumption in some highly competitive branches of some member-states of EU. Source: FAO (1992). □: export, ■: domestic-consumption

As a conclusion it can be stated, that the survey of food consumption is interesting both from economic and healthy aspects.

The first modern, correct and representative consumer value analysis in Europe has been conducted by WINDHORST (1983).

The survey of consumers attitudes towards food consumption is well documented, but the sample often represented a well determined, narrow consumer group only (e.g. PHILLIPS, 1986 used a sample size of 100 for the representation of consumers attitudes in Reading) or the investigation concentrated only on a more

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specific issue (e. g. food safety evaluation of radurised products by LINDE, 1994). The scientific analysis of food consumption has considerable traditions in Hungary (ÚJLAKY-NAGY, 1937). Recently BÍRÓ (1996) has analyzed in detail the relationship between food consumption and health condition.

The aim of the article is

– to analyze the system of values and preferences of Hungarian consumers concerning the food consumption;

– to determine the segmentation possibilities of adult Hungarian consumers according to preferences and behavior their food consumption;

– to outline most effective ways and channels of communication with consumers in the field of food consumption.

The results of survey can be effectively utilized to determine the ways of communication with the population in order to disseminate the principles of healthy diet, and in planning various marketing activities (product development, price, distribution and communication policy) at food industrial enterprises.

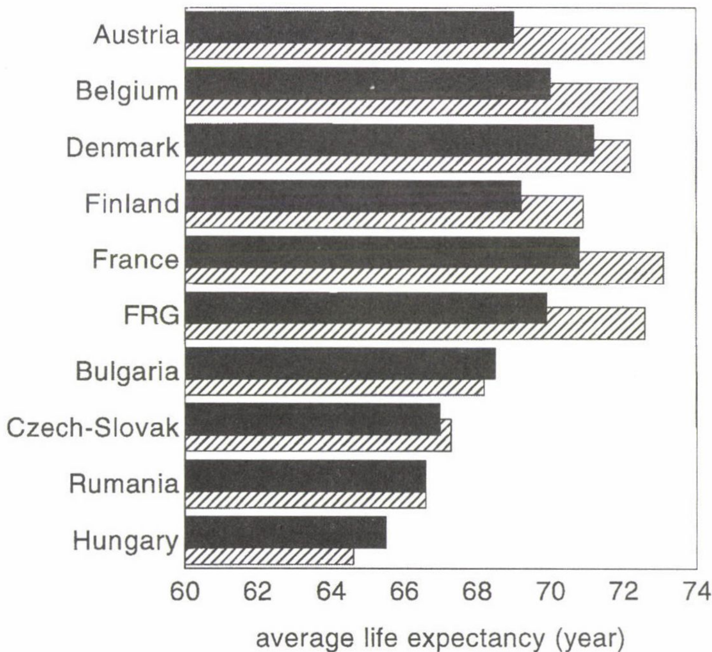

Fig. 2. The average birth expectancy at birth in some European countries at male population (1980–1990). Source: WHO (1994). ■: 1980, ▨: 1990

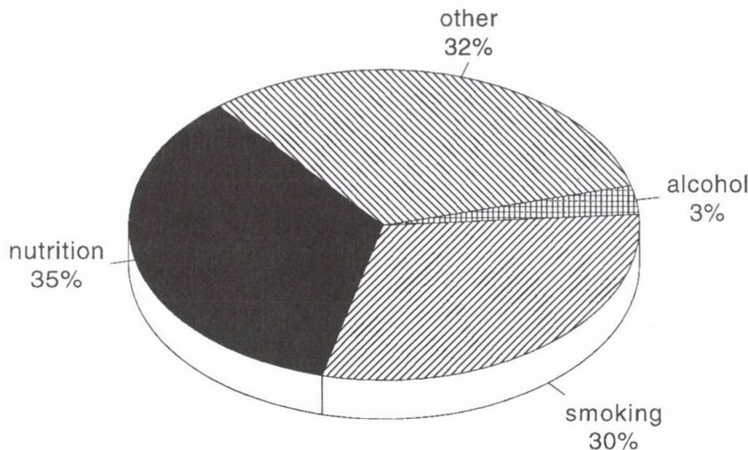

Fig. 3. The causes of cancer according to DOLL & PECO (1981)

1. Methods of investigations

The survey was conducted in 1993 among nearly one thousand respondents in Hungary. The sample was broadly representative of the Hungarian population. The most important characteristic features of the sample are shown in Table 1.

The survey was carried out by anketeurs, so the misunderstandings of the questions could be avoided. The flow chart of survey is shown in Fig. 4.

The results of survey were processed by SPSS™ for Windows® integrated statistical program package. To achieve a better overview of results the explorative data analysis was extensively used. The box- and- whisker plots display summary statistics for the distribution. It plots the median, the 25th percentile, the 75th percentile. The "whiskers" show the largest and the smallest observed values that are not extreme ones – outliners – (NORUSIS, 1993).

The factor analysis was carried out by "varimax" method, and the cluster analysis by centroid method, using Euclidean distances.

The analysis of variance (ANOVA) was carried out on the base of Scaffee-test.

Table 1

Basic characteristic features of the representative sample

Age	
Young (20–35)	31%
Middle-age (36–55)	42%
Elderly (56–)	27%
Sex	
Female	56%
Male	44%
Education	
Elementary school	46%
High school	36%
Coll. & univ.	18%
Residence	
Budapest	19%
Town with more than 100.000 inhabitants	30%
Town with inhabit. 1001–100.000 inhabitants	18%
Village with 100–1000 inhabitants	24%
Smaller village or farm	9%
Activity	
Active	54.2%
Leave on baby-care fee	1.8%
Retired	34.4%
Unemployed	6.5%
Not-working (family member)	1.3%
Student	1.5%
Other	0.3%

2. Results

2.1. Typology of Hungarian consumers

In the first stage of survey the basic value system of consumers was determined. For this purpose the respondents were asked to evaluate the importance of some statements (fit to scan the relevant value system from point of view of survey) on a 1 to 5 scale. The results of answers – analyzed by explorative data analysis (box and whisker plot) is shown in Fig. 5.

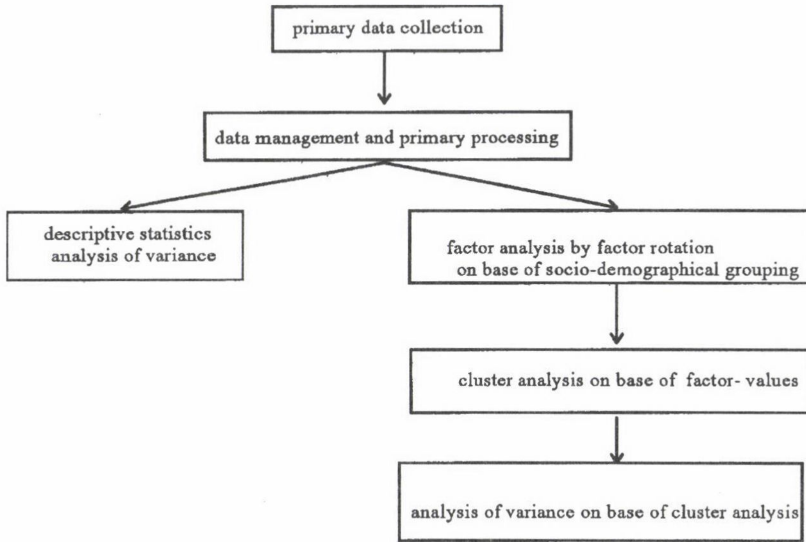

Fig. 4. Flow chart of investigations

The results were analyzed by one way analysis of variance (ANOVA), according to various socio-economic factors (Table 2).

Determination of the structure of investigated values was carried out by factor analysis. After varimax-rotation three main factors could be separated on the base of eigenvalues (Table 3).

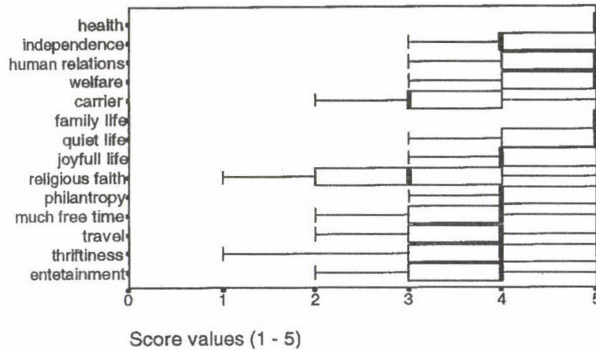

Fig. 5. Boxplot-analysis of various values according to their score. The lower boundary of the box is the 25th percentile and the upper boundary is the 75th percentile. The vertical line inside the box represents the median. Fifty percent of the cases have values within the box. The "whiskers" represent the largest and smallest values

Table 2

The ANOVA of estimated importance of some general social according to various socio-economic factors

	Health	Independence	Human relations	Material Welfare	Carrier	Family life	Quiet	Foyfull life	Religious faith	Philantropy	Much free time	Travel	Thriftiness	Pleasureful life
Age-group														
Young	4.81	4.30	4.48	4.44	3.78	4.67	4.53	4.31	2.74	4.06	3.83	3.93	3.56	4.04
Middle-aged	4.82	4.14	4.47	4.41	3.43	4.67	4.57	4.11	3.05	4.14	3.58	3.51	3.74	3.74
Elderly	4.86	3.81	4.36	4.33	2.87	4.63	4.58	3.79	3.73	4.16	3.01	2.88	3.88	3.52
Place of living														
Great town	4.78	3.98	4.39	4.38	3.33	4.6	4.46	3.92	3.05	4.08	3.48	3.61	3.43	3.83
Small town	4.88	4.16	4.44	4.36	3.46	4.71	4.59	4.09	3.05	4.08	3.56	3.49	3.75	3.69
Village, farm	4.82	4.1	4.48	4.44	3.3	4.68	4.64	4.15	3.42	4.2	3.38	3.23	4.00	3.78
Marital status														
Married	4.83	4.05	4.41	4.46	3.43	4.75	4.6	4.06	3.11	4.07	3.53	3.44	3.78	3.74
Single	4.83	4.11	4.46	4.33	3.29	4.57	4.52	4.07	3.24	4.17	3.43	3.44	3.68	3.79
Sex														
Male	4.81	4.17	4.49	4.42	3.47	4.63	4.51	4.14	2.96	4.01	3.52	3.5	3.65	3.81
Female	4.86	3.99	4.38	4.37	3.25	4.72	4.61	4.01	3.39	4.23	3.43	3.39	3.81	3.72
Education level														
Elementary	4.84	3.91	4.36	4.43	3.01	4.67	4.6	4.1	3.43	4.12	3.32	3.02	3.97	3.62
High school	4.84	4.17	4.49	4.44	3.59	4.75	4.61	4.18	2.99	4.14	3.66	3.71	3.72	3.92
College, univ.	4.88	4.41	4.54	4.4	3.91	4.78	4.64	4.12	2.99	4.12	3.64	3.80	3.65	3.69

] Significant difference at 95% probability level
Critical t value at 95% probability level: 1.96

Table 3

Rotated factor matrix

Basic values	Factor loading		
	Factor 1	Factor 2	Factor 3
Health	0.37626	0.26192	–0.35354
Independence	0.55111	–0.37164	0.18849
Human relations	0.57138	0.07415	0.04664
Material welfare	0.60816	0.03470	–0.11342
Career	0.62484	–0.40002	0.11279
Family life	0.046473	0.56136	–0.37326
Quiet life	0.48728	0.59134	–0.25725
Joyful life	0.70275	–0.01157	–0.17611
Religion	0.01431	–0.56315	0.64652
Philanthropy	0.44499	0.52085	0.38390
Much free time	0.65749	–0.40482	0.17702
Travel	0.67587	–0.41047	0.08022
Thriftiness	0.41813	0.49071	0.20992
Entertainment	0.64302	–0.23267	–0.02294

On the base of factor scores the respondents can be divided into groups by cluster analysis. The results of cluster analysis were summarized with simple descriptive statistical methods. By this way the most important characteristic features of the various consumer's groups can be estimated (Table 4).

2.2. Social values and consumer's behavior

In stage two the respondents were asked to express their concordance with some statements concerning food and eating habits on a five-score scale.

The results of ANOVA on the base of socio-economic grouping can be seen on Table 5.

Results of the combined method of cluster- and factor analysis for grouping consumers are shown on Table 6.

The box-plot analysis of consumer's attitudes towards various issues joining to eating habits and preferences is shown on Fig. 6.

Table 4

Typology of Hungarian food consumers

	"Modest common man"	"Well-balanced, staid upper middle class"	"Sceptical middle class members" consumers	"Younger material values oriented"
Typical job	unskilled worker	leading position, entrepreneur	skilled worker	leading positioning entrepreneur
Marital status	married (64%) or widow (28%)	married (72%) or single (12%)	married (66%) divorced (11%)	married (74%) or single (18%)
Age	rather old (average: 56 year)	middle (45)	middle (47)	younger (30)
Income	low	high	middle	middle or higher
Family size	"empty nest"	adult children	small family	middle
Religion	religious 44% lives in accordance with traditions of a church	16% follows some church	16% follows some church	only 3% follows some church
Less important values	career, travel, free time, joyful life	religious faith travel, career, free time	thriftiness, career, travel	religious faith career, thriftiness
Most important values	family life, quiet life, philanthropy	family life, quiet life, human relations	human relations, independence	family life, material welfare

Table 5

The ANOVA of scores, concerning consumers behavior according to various socio-economic factors

	It is worth... speeding more money on healthy food	...eating in restaurants to save time	...buying food at action	...trying in several shops	...writing down the expenditures on food	...looking at the product prices	...producing food at home	...entering into a bargain on the market	Eating is comparatively less important in life	Eating habits can be changed	Eating is a pleasure	Eating and dealing with having meals take too much time	The most important thing is not what to eat, but to be satisfied	Old-type foods have a better taste	Our income is not enough for food for the whole month
Age-group															
Young	4.28	2.27	3.21	3.59	2.05	3.72	2.79	3.69	3.3	3.74	3.51	2.00	1.86	1.86	3.36
Middle-aged	4.26	1.94	3.59	3.89	2.26	4.07	3.31	3.97	3.25	3.53	3.65	2.24	2.32	2.32	3.53
Elderly	4.07	1.64	3.64	3.95	2.65	4.22	3.44	4.10	3.31	3.48	3.75	2.16	2.64	2.64	3.72
Place of living															
Great town	4.03	2.06	3.38	3.64	2.39	4.09	2.68	3.73	2.94	3.53	3.86	2.04	2.27	2.27	3.43
Small town	4.59	1.86	3.54	3.90	2.30	3.80	2.90	4.00	3.24	3.47	3.46	2.45	1.83	1.83	3.89
Village, farm	4.32	1.91	3.52	3.88	2.27	4.06	3.97	4.02	3.42	3.74	3.59	2.21	2.44	2.44	3.59
Marital status															
Married	4.24	1.93	3.44	3.81	2.33	3.87	3.23	3.87	3.2	3.63	3.71	2.18	2.27	2.27	3.56
Single	4.02	1.95	3.53	3.8	2.32	4.13	3.14	3.97	3.32	3.53	3.56	2.09	2.35	2.35	3.5
Sex															
Male	4.05	2.03	3.47	3.76	2.27	3.97	3.16	4.00	3.18	3.45	3.72	2.11	2.34	2.34	3.46
Female	4.2	1.89	3.5	3.85	2.40	4.03	3.2	3.85	3.34	3.71	3.55	2.18	2.25	2.25	3.61
Education level															
Elementary school	4.09	1.67	3.65	3.95	2.59	4.06	3.66	4.14	3.24	3.44	2.11	2.21	2.53	2.53	3.74
High school	4.32	2.09	3.47	3.84	2.20	3.91	3.07	3.85	3.23	3.73	2.17	2.11	2.15	2.15	3.49
University, college	4.35	2.23	3.17	3.67	2.20	3.72	2.83	3.63	3.48	3.89	2.17	2.09	1.96	1.96	3.13

] Significant difference at 95% probability level

Critical t value at 95% probability level: 1.96

Table 6

Results of combined factor and cluster analysis on the base of consumers behavior

Statements	Clusters A	B	C	D	Significant differences between groups at 95% probability level
It is worth… …spending more money on healthy food	3.95	4.54	3.84	4.24	B–A;C;D D–A;C;B
…eating in restaurants to save time	1.37	1.96	2.01	2.12	A–B;C;D
…buying food at actions	3.78	3.44	3.48	3.40	A–B;C;D
…trying several shops	4.08	4.12	3.46	3.72	A;B–C;D D–A;B;C
…planning monthly food expenses	3.63	3.53	2.71	2.86	A;B–C;D
…writing down the expenditures on food	2.65	2.73	2.17	2.07	A;B–C;D
…looking at the product prices	4.28	4.09	3.62	3.80	A;B–C;D
…producing food at home	3.81	3.44	3.08	2.96	A–B;C;D B–A;C;D
…entering into a bargain on the market	4.35	4.10	3.57	3.73	A;B-C;D
…following the religion rules	2.29	2.23	2.25	1.93	
…following the old eating customs and traditions	3.38	3.58	2.96	3.50	
Eating is comparatively less important in life	3.27	3.27	3.40	3.21	
Eating habits can be changed	3.41	3.96	3.22	3.63	A–C
Eating is a pleasure	3.56	3.82	3.40	3.62	C–A;B;D
Eating and dealing with having meals take too much time	1.93	2.26	2.46	2.02	
The most important thing is not what to eat, but to be satisfied	2.56	2.35	2.30	2.08	A–B;C;D
Old-type foods have better taste	3.15	2.80	2.98	2.44	
Our income is not enough for food for the whole month	3.66	3.63	3.56	3.37	D–A;B;C

Table 7

The evaluation of various quality attributes of food consumption by combined method of cluster and factor analysis

Quality attributes of food consumption	Clusters				Significant differences between groups at 95% probability level
	A	B	C	D	
Appetizing look of food	3.87	4.48	3.74	4.25	B–A;C;D
Freshness	4.84	4.94	4.59	4.87	B–A;C;D
Good taste	4.82	4.92	4.47	4.79	B;C–A;D
High energy content	4.33	4.34	4.01	4.19	C–A;B;D
High vitamin and mineral content	4.51	4.73	4.15	4.52	A;D–B;C B–A;C;D C–A;B;D
High protein content	3.98	4.17	3.62	4.06	A;D–B;C B–A;C;D
Peculiarity of food	3.91	4.21	3.50	3.87	A;D–B;C B–A;C;D C–A;B;D
Setting	4.09	4.53	3.68	4.12	A;D–B;C B–A;C;D C–A;B;D
Variousness of nutrition	4.02	4.46	3.80	4.15	A;D–B;C B–A;C;D C–A;B;D
More dishes at meals	2.47	3.09	2.91	2.90	A–B;C;D
Ready-to-eat foods	3.57	3.86	3.44	3.67	B–A;C;D
Price	4.44	4.36	3.90	4.01	
Keeping familiar eating traditions	3.74	3.79	3.16	3.48	A;B–C;D C–A;B;D D–A;B;C
Tasting new foods	3.19	3.72	3.18	3.51	A;B;C–D

Table 8

The ANOVA of scores, given to various statements concerning risks of food consumption and way of life according to various socio-economic factors

	Environmental pollution	Lack of excercises	Obesity	Alcohol consumption	Smoking	Using of too much salt	Smoked foods	Sugar consumption	Fat consumption	Butter consumption	Pesticide residuals	Artifical preservers	Food irradiation
Age-group													
Young	3.57	3.09	3.06	3.41	3.61	2.78	2.80	2.65	3.18	1.64	3.27	2.55	2.82
Middle-aged	3.46	3.06	3.06	3.38	3.69	2.68	2.70	2.55	3.14	1.55	3.29	2.65	2.73
Eldery	3.38	3	3.01	3.37	3.66	2.67	2.68	2.63	3.04	1.61	3.14	2.55	2.60
Place of living													
Great town	3.62	3.1	3.1	3.35	3.79	2.7	2.75	2.62	3.09	1.55	2.95	2.61	2.67
Small town	3.41	3.01	3.01	3.38	3.65	2.64	2.72	2.71	3.15	1.65	3.19	2.41	2.71
Village, farm	3.38	3.3	3.03	3.4	3.56	2.79	2.71	2.5	3.02	1.6	3.57	2.73	2.76
Marital status													
Married	3.47	3.05	3.03	3.62	3.63	2.74	2.83	2.65	3.18	1.59	3.25	2.6	2.79
Single	3.47	3.05	3.05	3.40	3.68	2.68	2.63	2.57	3.07	1.61	3.23	2.57	2.64
Sex													
Male	3.51	3.02	3.08	3.32	3.73	2.86	2.83	2.7	3.25	1.67	3.26	2.64	2.78
Female	3.43	3.08	3.02	3.44	3.58	2.60	2.62	2.52	3.01	1.54	3.21	2.53	2.65
Education level													
Elementary school	3.33	2.90	3.28	3.54	3.59	2.62	2.62	2.49	3.05	1.51	3.23	2.58	2.72
High school	3.47	3.11	3.44	3.62	3.63	2.82	2.91	2.70	3.25	1.66	3.36	2.55	2.75
University, college	3.68	3.30	3.56	3.68	3.76	2.86	2.90	2.80	3.32	1.76	3.41	2.74	2.87

] Significant difference at 95% probability level
Critical t value at 95% probability level: 1.96

Table 9

The estimation of relative importance of various information-resources from point of view of dietary advice

Risks	Clusters				Significant differences between groups at 95% probability level
	A	B	C	D	
Environmental pollution	3.46	3.64	3.15	3.41	A;D–B;C B–A;C;D C–A;B;C
Lack of exercises	2.94	3.26	3.01		B–A;C;D
Obesity	3.28	3.53	3.26	3.36	B–A;C;D
Alcohol consumption	3.64	3.72	3.38	3.54	C–A;B;D
Smoking	3.64	3.80	3.46	3.54	B–A;C;D
Using too much salt	2.57	3.19	2.47	2.60	B–A;C;D
Smoked foods	2.50	3.17	2.50	2.70	B–A;C;D
Sugar consumption	2.54	2.89	2.43	2.55	B–A;C;D
Fat consumption	2.96	3.45	3.04	3.06	B–A;C;D
Butter consumption	1.52	1.76	1.54	1.59	B–A;C;D
Pesticide residues	3.33	3.61	2.82	3.27	A;D–B;C B–A;C;D C–A;B;C
Artificial preservatives	2.49	3.08	2.41	2.38	B–A;C;D
Food irradiation	2.69	3.20	2.47	2.57	B–A;C;D

Table 10

The evaluation of various information resources on an ordinary scale

Physician	1.90
Family members	2.76
Books, newspapers	3.80
TV	3.84
Friends, acquaintances	3.93
Radio broadcasts	4.73

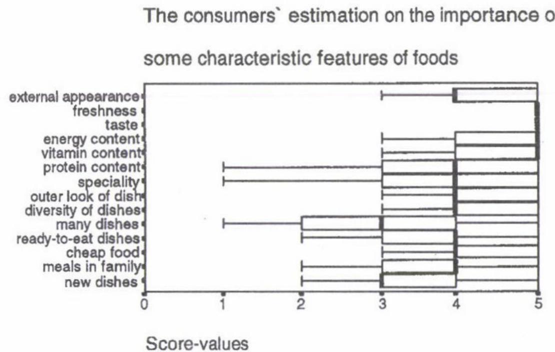

Fig. 6. The consumers estimation on the importance of some characteristic features of foods

The segmentation of respondents by combined method of factor- and cluster analysis (Table 7) underlines, that this approach is fit to differentiate the consumer's attitudes towards the quality of food consumption and nutrition.

The ANOVA of various risk factors according to social-economic grouping factors is shown in Table 8, on the base of combination of factor and cluster-analysis it is presented in Table 9.

Consumers attitudes toward food safety issues and nutrition-related risk factors according to four-cluster grouping.

It is a generally accepted fact, that the eating habits of the Hungarian population are out of accordance with the guidelines of up-to-date nutritional science, that is why the changing of nutritional culture would be highly effect of various information sources the respondents were asked to evaluate the relative importance of some information sources on an ordinary scale (Table 10).

3. Discussion and conclusions

It can be seen from Fig. 5, that the acceptance of health and family life as basic values are practically uniform, but the estimation of importance of the other values shows a greater dispersion. These phenomena can be explained not only by plurality of values in the society, but also by the fact, that during the last six decades (only two generation !) the half feudal-capital, military, Stalinistical, egalitarian, liberal, classical capitalistic and social-democratic values quickly followed each other or existed parallel.

Analyzing the results of survey on the base of "classic" grouping of consumers by one-way analysis of variance (ANOVA) it can be stated, that the most significant

differences occurred between sexes (Table 2). It is important to underline, that the various consumer groups do not correlate with the place of living. A possible explanation of this phenomenon could be the fact, that during the last five decades (1945–1995) a rapid and intensive migration was in Hungary, that's why there is not significant difference between the value system of people who are living in a city or in a town. The other possible answer is the effect of mass-communication. There is a considerable difference between value-system of people with different age. The cause of this phenomenon could be the difference in education and social experiences.

The woman respondents estimated the values joining to health and quiet, family-oriented life higher than the men. Of course, values joining to dynamic, joyful life are more important for younger consumers.

According to the well-known theory of MASLOW (1954), values joining to independence and quality of life are higher evaluated at younger cohorts, and intellectuals.

Three main groups of values can be determined using factor analysis on the base of factor loadings (Table 3):

- values joining to short-run pleasure and hedonistic way of thinking
- values connected with inner peace and well-balanced, quiet way of life
- values allied with religious faith and philoantrophy.

It can be seen from Table 4, that using combination of factor and cluster analysis four characteristic consumer groups can be separated. Members of first cluster value high the quiet life and in formation of their attitudes the classic values of religion play a rather important role. Members of second cluster are recruited from upper middle class. For members of this group the religion, as a guiding idea plays a less important role, but for them the quiet family life and human relations are basic values, too. The third cluster consists mainly of high-school graduated skilled workers. This group comparatively highly evaluated the independence, as a basic value. This could be a reason of the fact, that in this cluster the number of divorced persons is the highest. The values, joining to the pleasures of life are evaluated by lower marks, too. Members of fourth group are characteristically different from the above three consumer groups. They are younger, have better social conditions, the religion is less important for them, and the material welfare play a more significant role in their value system.

The Table 5 shows, that the high nutritional value of food was more important for the elder generation, and the criteria, joining to variousness of nutrition was highly estimated by the younger population.

It can be seen from the Table 6, that the material value oriented, and/or upper class consumers evaluated much higher the healthy foods, than the members of clusters, consisting of modest and/or skeptical consumers. To eat in restaurants in Hungary remains the habit of a relative small consumer's group. Cluster D consists of younger

consumers, so to go to restaurants is much more a popular habit for the member of this cluster, than for members of another clusters.

The food price is an important factor even for members of cluster B, consisting mainly of members of the upper class. The less sensitive for food price conditions are members of cluster C ("modest common man"). At first sight this might be a contradiction, but in practice it should be taken into consideration, that the members of this cluster in numerous case live single, relatively undervalue the importance of values joining to the "sunny side of life", so the disposable income for food consumption is relatively high. This fact underlines, that there is not direct relationship in all cases between income level and price sensitivity.

It is worth underlining, that eating habits seem to be changed according to the opinion of members of cluster C. The possible cause is, that this class is the most open to the world, has the most information about new trends in nutrition and eating habits of another nations.

The teachings of various religions are considered moderately important, and from this point of view only the members of cluster D (younger, material value oriented consumers) show an extremely low average score.

It can be seen on the Fig 6, that the Hungarian consumers evaluate the importance of freshness and taste of food extremely and unanimously high. It is interesting to detect, that the estimation of importance of energy and vitamin content are practically the same. This fact underlines the long-lasting effect of classical Hungarian eating habits, with a sharp preference for energy rich foods. The estimation of importance of variousness and diversity has showed a considerable variation. These phenomena can be explained by the increasing segregation of Hungarian society according to the income level.

According to our previous expectations, the highest demand for quality of food and nutrition could be detected in members of cluster B. It is worth underlining, that there are not significant differences among clusters from point of view of cheapness of foods. This fact underlines that purchasing power is rather low, so there is only a limited possibility to influence food consumers behavior, and however there is a demand for ready-to-eat, high-quality foods, this demand is not supported by a considerable purchasing power.

The ANOVA of awareness of various risk factors (Table 8) emphasizes, that Hungarian consumers are to a less extent aware of risk factors, than consumers in industrialized states. In some cases (e.g. evaluation of food irradiation and butter consumption) this phenomenon is not an adverse one. Generally it can be stated, that estimation of importance of various risk factors has been higher in case of consumers with higher qualification and female respondents.

The results of ANOVA according to cluster grouping at evaluation of various risk factors show, that the members of cluster B (material value oriented, well balanced upper middle class) are characteristically more sensitive to the various risk sources than members of other clusters. This phenomenon can be explained by two possible arguments:

– this consumer group has the most information about problems of environmental pollution and unhealthy eating habits;

– this group has soon some bad personal experiences concerning healthy problems, joined to bad eating habits and civilization diseases.

Members of cluster C considered the adverse effect of various danger factors comparatively low. This group, – however theoretically accepts the importance of health, as a basic value – in everyday practice lives less in accordance with principles of healthy way of life.

From Table 10 it can be seen, that the physicians are considered as the most important carriers of dietary advice. The influence of family members is on the second rank. There was no significant difference between the estimation of importance of written materials and TV programs. The role of friends and acquaintances is slightly less important. It is worth expressing, that effect of broadcast station is much more less evaluated than the effect of other information sources. There were only slight differences among various groups (both from social and psychrographical point of view) according to estimation of relative importance of various information sources.

Evaluating the methods of investigations it can be stated, that the four groups, formed on the base of values, show some characteristic differences from point of view of food consumption. The grouping of consumers gives an effective tool for differentiated marketing communication strategy, because there could be often significant differences detected between the four groups, revealing new, more deeper relations between various clusters, than using conventional, socio-economic clusters (Table 11).

Table 11

The significant differences detected at various methods of segmentation

Issue	Age	Place of living	Family status	Sex	Education level	Clusters
Basic social values	9	2	5	11	8	12
Consumers behavior	8	6				5
Nutritional quality	5	3	3	8	5	9
Risk factors	0	2	1	11	8	12

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CHEMICAL CONTAMINATION POTENTIAL OF BOTTLE MATERIALS

M. S. TAWFIK[a,c] F. DEVLIEGHERE[a], W. STEURBAUT[b] and A. HUYGHEBAERT[a]

[a] Department of Food Technology and Nutrition,
[b] Department of Crop Protection, Faculty of Agricultural and Applied Biological Sciences,
Coupure links 653, B–9000 Ghent. Belgium

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Bottles made of polycarbonate (PC), polyethylene terephthalate (PET), high density polyethylene (HDPE), polypropylene (PP), and polyvinylchloride (PVC) bottles were treated with thirteen common household chemical solutions, including eight pesticides, in order to simulate, consumer abuse of plastic bottles. Five days after immersion in the contaminant, the plastics were detergent washed, filled with water, and stored at room temperature for another six days. The water was analyzed for residual contaminant concentrations. Sensory testing and visual contamination was performed on the contaminated plastics. The tested chemical substances showed to be absorbed and remigrated into the beverage simulant at detectable levels. Contaminants with high octanol-water partition coefficients (Kow) resulted in high residual contamination levels in the beverage simulant. The initial concentration of the active ingredient did not always influence the residual contamination level. The plastics which are commercially used for the production of refillable bottles (PET, PC) showed in general the lowest residual contamination. Toxicological evaluation of the analytical results of contaminant residue remigration give no risk to public health concerns. However, in order to prevent negative effects on product quality such as odour and taste, good manufacturing procedures, including visual and electronic inspection systems, and improvement of the plastic bottles cleaning procedures are required to eliminate abused bottles.

Keywords: chemical contamination, pesticides, refillable or recyclable plastic bottles, safety assessment, toxicological evaluation, public health

Food packaging materials nowadays have to meet – next to traditional properties concerning communication, convenience, protection and containment – certain environmental demands. The destiny of the packaging material after consumption contributes more and more to the image of the packaged food stuff.

Plastic share in waste in W-Europe in 1993 was 7% in weight and 25% in volume where plastics "as waste" reach more than 15 million tons in W-Europe. The European Union recently promulgated the directive 94/62/EC where three possible

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[c] Corresponding author. Present address: Development of Food Science and Technology, Alexandria University, Alexandria. Egypt

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solutions are proposed to decrease the waste volume: prevention, use of multiple-use systems and recycling. In the same directive, quota for recycling of waste are imposed. In five years, a minimum of 25% and a maximum of 40% in weight of the packaging waste has to be recycled and in total minimum 50% and maximum 65% in weight of the packaging waste has to be regained. Each specific packaging material must meet a recycling rate of 15%. In 1993 however, only 5.6% of total plastic discard in the EC was mechanically recycled, 75.7% was still landfilled (SOFRES CONSEIL, 1995).

Next to the specific problems for obtaining a pure recycled plastic, a substantial and reliable market has to be found for the recycled material. The recycled plastic can be used in long life products as garbage cans or crates but more and more the use of recycled polymers for food packaging is considered. However, due to possible commercial and consumer misuse as containers for chemicals, special care has to be taken to protect the consumer.

Plastic refillable bottles are more and more used in the food industry. PET refillable are already widely spread over the world and are mainly used for carbonated lemonades and water. General Electric Plastics developed in 1990 a refillable polycarbonate bottle for fresh milk and juices. Beginning of March 1996, Melkunie N. V. introduced refillable PC bottles for pasteurized milk in the Netherlands. Because of their infrangibility, light weight, recyclability and processability the advantages of the glass bottle and the one way plastic bottle are combined when a returnable plastic bottle is considered.

Refilling and recycling of plastic containers meet an environmental demand. However, they also create several problems not only for the consumer but also for the industry. Indeed, the industry has only limited control on what the consumer has stored in the container before it is returned for recycling or refilling nor does the consumer have any way of knowing how a container may have been misused before being returned to the filling plant. Such misuse can result in contamination of containers that could produce off-flavour when these containers are used later for packaging of products like water, milk, or juice. Hence, in the current study the sensory evaluation of the exposed plastics and waters were determined by trained judges.

BODYFELT and co-workers (1976) and LANDSBERG and co-workers (1977) reported that sensory evaluation was more effective than the contaminant detector ("snifter") for identifying the off-odour in washed containers. The human nose is very discriminating detection device. In conjunction with the taste mechanism it determines the suitability of a product before consumption or at the first drink. The taste and smell mechanisms are the final test before consumption and operate independently from any fail safe devices that might be used before filling a washed container. Furthermore, GASAWAY (1978c) confirmed also the ability of the human nose as the best detection device.

However, earlier studies have proved (FERON et al., 1994; JETTEN et al., 1994; GASAWAY, 1978a; LANDSBERG et al., 1977) that certain chemical substances (pesticides, household chemicals) are absorbed by the plastic when bottles were misused by the consumer. Part of the chemical is retained by the plastic even after normal caustic washing and can migrate in the contents of a refilled bottle. To decrease contamination risks filling lines for returnable plastic bottles are equipped with a "sniffer" device, checking each container before it is filled.

The aim of this study is to compare different materials (polycarbonate, polyethylene terephthalate, high density polyethylene, polypropylene and polyvinylchloride), which can be used for bottle production, in their potential to absorb and desorb pesticides and household chemicals, after simple detergent washing. An estimation of the risks for adults and children is made when beverages would be packed in chemical contaminated bottles.

1. Materials and methods

1.1. Test substances

Thirteen chemical substances (Table 1) were selected to be contacted with plastics. Selection was based on the octanol/water partition coefficient (Kow), availability to the consumer and toxicity. The selected chemicals included eight pesticides, four household chemicals and one beverage. The selected pesticides included systemic, non systemic and selective fungicides and herbicides. The active ingredients and the concentrations of tested substances used throughout the study are presented in Table 1.

1.2. Plastic materials preparation

Brand new polycarbonate (PC) sheets (thickness=19 μm), polyethylene terephthalate (PET=26 μm), high density polyethylene (HDPE=59 μm), polypropylene (PP=54 μm) and polyvinylchloride (PVC=34 μm) bottles were used. The plastics were cut into 4 strips of 5 cm×4 cm and linked with glass rods to keep them separated during immersion (four strips in glass jar). The plastic strips were soaked in the chemical substance for five days in dark conditions at room temperature. A glass jar without plastic for each chemical substance was used as a blank. After immersion, the chemicals were removed and plastic materials and glass jars were rinsed with distilled water until visually clear. The glass jars containing the plastic strips were filled 2 times with a 1% of detergent solution at room temperature and shaked vigorously for 1 min. The cleaning solutions were discarded and the containers were rinsed again three times by shaking for 1 min with distilled water. They were then filled with water (300 ml/jar) and stored at room temperature. After six days, the plastic was removed and the water was immediately analysed for residues.

Table 1

Selected chemical compounds for plastics treatment

Compound, usage and manufacturer	Active ingredients	Kow[a]
Pesticides:		
Tilt 250 EC, Systemic fungicide (Ciba-Geigy)	propiconozole ($250\ g\ l^{-1}$)	3.7
Ronilan (SC), Contact fungicide, (BASF)	vinclozolin ($500\ g\ l^{-1}$)	3.0
Ympact (EC), Contact and systemic fungicide, (Zenica)	flutriafol ($125\ g\ l^{-1}$)	2.3
Decis (EC) Insecticide, (Hoechst)	deltamethrin ($25\ g\ l^{-1}$)	4.6
Sportak (EC) Contact fungicide, (Schering)	prochloraz ($450\ g\ l^{-1}$)	4.4
Racer (SC) Herbicide (Zenica)	flurochloridane ($250\ g\ l^{-1}$)	3.4
Javelin (SC) Herbicide mixture (Rhone-Poulenc)	isoproturon ($500\ g\ l^{-1}$)	2.5
Gesaprium (SC) Herbicide (Ciba-Geigy)	atrazin ($500\ g\ l^{-1}$)	2.5
Household chemicals:		
Bleaching agent: La croix, disinfects, Colgate-Palmolive Belgium.	Chlorine (<5.0)	NA
Furtilizer: Substral, nutrients for indoor plants, S. A. Henkel Belgium.	Nitrite and Nitrate (6.8)	NA
Disinfectant: Dettol, S. A. Peckitt & Colman N. V.	Chloroxyphenol (4.9)	NA
Detergent: Dreft, n.v. Procter & Gamble Benelux N.V.	Sodium lauryle sulfate (0.6)	NA
Beverages:		
Orange juice, Granini, Bielefeld Germany.	Limonene (0.01)	NA

[a] log (partition coefficient K of the substance between octanol and water)

NA: not available.

EC: Emulsifiable concentrate

SC: Suspension concentrate

Table 2

Tested residual substances used as contaminants of plastics remigration and analytical methodology

Test substance	Extraction method	Detection method	Detection limit (ppb)
Pesticides:			
Propiconozole	n-hexan extr.	GLC/electron capture detector	< 3
Vinclozolin	n-hexan extr.	GLC/electron capture detector	< 1
Flutriafol	Dichloromethan extr./evap./Aceton extr./evap.	GLC/	< 3
Deltamethrin	n-hexan extr.	GLC/electron capture detector	< 3
Prochloraz	n-hexan extr.	GLC/electron capture detector	< 200
Flurochloridone	Dichloromethan extr./evap./Aceton extr./evap.	GLC/thermionische detector	< 20
Isoproturon	Dichloromethan extr./evap./Aceton extr./evap.	GLC/electron capture detector	< 0.7
Atrazine	Dichloromethan extr./evap./Aceton extr./evap.	GLC/electron capture detector	< 3
Household chemicals:			
Chlorine	N,N-diethylphenylene-1,4 diamine method	Spectophotometry at 510 nm (INTERNATIONAL STANDARD, 1985)	< 1000
Nitrite and Nitrate	Evap>water	HPLC/UV at 220 nm	< 100
Chloroxyphenol	4-aminoantipyrine/potassium cyanoferrate/chloroform extr.	Spectrophotometry at 544 nm	< 100
		Spectrophotometry at 637 nm	< 50
Sodium lauryle sulfate	H_2SO_4/Azure A/chloroform extr.	(A.O.A.C., 1990)	
Beverages:			
Limonene	Isopropanol distillation	Bromide-bromate titration (SCOTT & VELDHUIS, 1966)	10000

Extr.=Extraction Evap.=Evaporation

1.3. Organoleptic method

Plastics and water samples were evaluated for odour by five trained judges. Samples for sensory evaluation were coded and judged for odour differences against a reference. Untreated coded samples were provided as internal controls.

1.4. Analytical methods for residual substances

The analytical conditions for the residue analysis of tested substances and the corresponding limits of the detection are summarized in Table 2.

2. Results and discussion

2.1. Organoleptic method

All five types of treated plastics and water samples were judged and the degrees of off-odour and plastic appearances are presented in Table 3. After five days of storage of purchased chemicals, followed by washing and filling of the containers with water, there were positive sensory responses in water for off-odours for the eight pesticides (pronounced off-odour with Decis, Sportak 45, and Racer 25) and two household chemicals (slight off-odour with dettol and orange juice) with all kinds of plastic. The high amount of off-odour responses for the pesticides can be explained by the use of undiluted chemicals in combination with a mild washing process (detergent washing at room temperature). GASAWAY (1978b) reported for PC containers 12 (14%) positive sensory responses for off-odour out of 85 pesticide cases tested after 10 days of storage of dilute pesticide and washing the containers. After 3 days of storage in HDPE plastic containers he reported 35 (43%) positive sensory responses out of 82 products tested. LANDSBERG and co-workers (1977) reported off-odour in milk stored in nine of the fifteen treated polyethylene containers. They concluded that both the HDPE and PC containers showed to absorb contaminants and to impart off-odours into the product. In a report of JETTEN and co-workers (1994) the organoleptic quality (compounds able to cause an off-flavour) and flavour carry-over (flavour applied in beverages) in relation to polycarbonate (PC) bottles was studied. 10 of the 15 tested flavours showed to be significantly different from the blank water samples after 30 days of storage at 30 °C and filled after caustic washing. The presented study also shows that from the investigated plastics only the PC and PVC were changed in colour and appearance after exposure to some pesticides (Decis, Sportak 45, and Racer 25).

2.2. Analysis of test substance residues in water

The levels of remigrated test substances found in the water samples are shown in Table 4. The data were presented as μg dm^{-2} of plastic. For all investigated chemicals, except for deltamethrin, detectable levels of residues were found in the water after 6 days of storage. Also GASAWAY (1978 a,b) showed that pesticides were migrating into liquids filled in HDPE and PC returnable bottles at detectable levels. FERON and co-workers (1994) also concluded that chemicals could be absorbed by the wall of PET refillable (PRB's) if misused. Storage of simulated beverages in the exposed bottles showed remigration of various chemicals into the beverage. JETTEN and co-workers (1994) reported also detectable migration of contaminants into PC returnable after exposure. The low residual concentrations of deltamethrin in the water samples can be explained by the low initial concentration of the pesticide (2%).

The effect of the concentration of the active ingredient can be derived from the comparison between the remigration of flutriafol and atrazin which are having a similar Kow value. In all cases atrazin levels were significantly higher compared to the remigration level of flutriafol. However, when the results of flutriafol (active ingredient=12%, logKow=2.3) and Isoproturon (active ingredient 45,5%, logKow=2.5) are compared, an inverse effect of the initial concentration could be noticed. This contradiction was also mentioned by FERON and co-workers (1994) who reported that dilution strength appeared to affect some chemicals, but not others and the dilution of the commercial preparations to user strengths did not always result in comparable decreases in migration.

Prochloraz and isoproturon showed to give complete different levels of residue migration although having similar initial concentrations. This can probably be explained by the difference in Kow value. Prochloraz, showing higher residue migration levels has a high Kow value (logKow=4.4) compared to Isoproturon (logKow=2.5). In general, high Kow values resulted in high residue migration levels in the water samples.

The plastic type also clearly influences the amount of residue migration but is dependent on the contaminant type. FERON and co-workers (1994) stated that the behavior of other substances or other packaging materials than PRB's could not be precisely predicted. The variation of responses between the plastics with different kind of chemical materials could be explained by the difference between those plastics in absorption, retention, and remigration to the water of such active ingredients and these phenomena occur according to the crystallinity, composition, polymerization type, thickness, polarity and surface structure of each plastic. The plastics commercially applied for refillable (PET, PC) showed in general the lowest residue levels in the water samples.

Table 3

Organoleptic test of chemical treated plastics and water after storage and detergent washing[a]

Product	PC			PET			HDPE		
	Odour evaluation of water	Odour evaluation of plastic	plastic app.	Odour evaluation of water	Odour evaluation of plastic	plastic app.	Odour evaluation of water	Odour evaluation of plastic	plastic app.
Tilt250EC	++	+	destroyed	++	+	–	++	+	–
Ronilan FL	+	–	–	+	–	–	+	–	–
Flutriafol	+	–	–	+	–	–	+	–	–
Decis	+++	+	destroyed opaque	+++	–	–	+++	–	–
Sportak 45	+++	++	destroyed opaque	+++	–	–	+++	+	–
Racer 25 CS	+++	++	opaque	+++	–	–	+++	++	–
Javelin	++	–	–	++	–	–	++	–	–
Gesaprium	+	–	–	+	–	–	+	–	–
La Croix	–	–	–	–	–	–	–	–	–
Substral	–	–	–	–	–	–	–	–	–
Dettol	+	–	–	+	–	–	+	–	–
Dreft	–	–	–	–	–	–	–	–	–
Orange juice	+	+	–	+	+	–	+	+	–

Table 3

(Cont.)

Product	PP			PVC		
	Odour evaluation of water	Odour evaluation of plastic	plastic app.	Odour evaluation of water	Odour evaluation of plastic	plastic app.
Tilt250EC	++	+	–	++	+	–
Ronilan FL	+	–	–	+	–	–
Flutriafol	+	–	–	+	–	–
Decis	+++	–	–	+++	–	destroyed
Sportak 45	+++	–	–	+++	++	destroyed soft
Racer 25 CS	+++	++	–	+++	–	–
Javelin	++	–	–	++	–	–
Gesaprium	+	–	–	+	–	–
La Croix	–	–	–	–	–	–
Substral	–	–	–	–	–	–
Dettol	+	–	–	+	–	–
Dreft	–	–	–	–	–	–
Orange juice	+	+	–	+	+	–

[a] The response degrees are given as (+), (++), (+++), and (–)
+ = Slight off-odour in water
++ = Definite off-odour in water
+++ = Pronounced off-odour in water
– = No off-odour in plastic, nor clear change in appearance
app. = appearance

Table 4

Residual contamination levels observed in water after exposure (undiluted, 5 days at room temperature) *and detergent washing of different plastics followed by storage* (water, 6 days at room temperature)

Test substance	Plastic containers				
	PC (μg dm^{-2})	PET (μg dm^{-2})	HDPE (μg dm^{-2})	PP (μg dm^{-2})	PVC (μg dm^{-2})
Pesticides:					
Propiconozole	10.46	10.02	126.13	77.95	63.79
Vinclozolin	4.50	4.79	15.41	19.06	5.32
Flutriafol	1.74	0.00	5.10	2.57	1.05
Deltamethrin	0.00	0.00	0.00	0.00	1.35
Prochloraz	4475.35	1689.98	2096.53	2556.19	3706.51
Flurochloridane	37.54	37.63	74.21	85.27	54.28
Isoproturon	0.49	1.45	1.76	1.52	0.67
Atrazin	3.36	5.66	22.65	7.41	12.95
Household chemicals:					
Chlorine	1.56	1.56	1.49	1.55	1.21
Nitrite, Nitrate	11.41	57.10	30.61	6.81	8.61
Chloroxyphenol	89.13	552.90	1537.00	460.18	57.62
Sodium lauryle sulfate	0.93	0.09	3.57	2.95	1.29
Beverage:					
Limonene	55.28	34.25	76.99	78.64	8.62

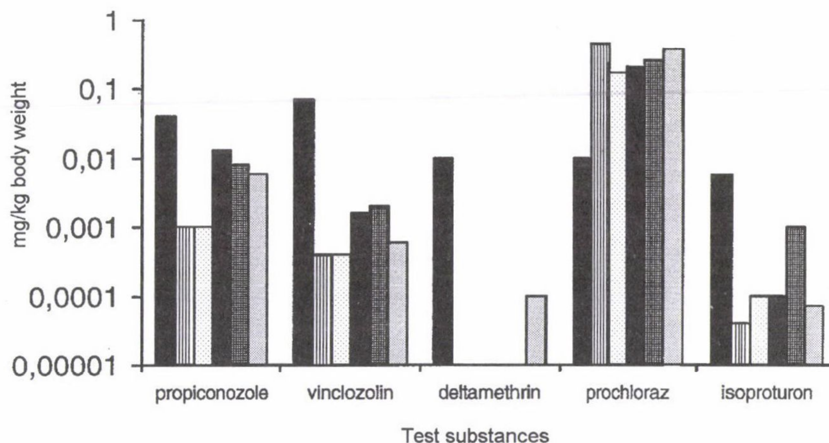

Fig. 1. Evaluation of maximum potential exposure of migrated pesticides and household products observed in water after storage with different plastic types (PC, PET, HDPE, PP, PVC) using ADI value as a health assessment reference. ADI= Acceptable Daily Intake based on WHO/FAO recommendations, MPE= Maximum Potential Exposure for a 70 kg person using the worst case exposure data from the studies conducted (i.e. highest mg/1.5 l bottle value)

2.3. Toxicological evaluation

Pesticides are toxic, and small quantities possess high potential risks for the consumer. Most of the household chemicals may cause an off-taste but no significant health hazard. To estimate the significance of the measured levels of contaminants in water with regard to human health, the highest concentration of each test substance detected in water was used to calculate a "maximum potential exposure" or "MPE" for that chemical for each type of plastics (Fig. l). The calculation assumes that a 70 kg person (GASAWAY, 1978) would drink daily the entire content of a contaminated 1.5 l bottle at one time. This means that for example for prochloraz in a PET bottle (11.83 mg/1.5 l)/70 kg b/w.=0.169 mg/kg/day. In this worst case scenario it is unlikely that a consumer would ingest the full contents of a 1.5 l bottle at one time or for that matter, over a period of a day. When the concentration fell below the detection limit, an estimation of MPE was calculated based on this concentration (FERON et al. ,1994).

MPE values were compared with estimated standards when available. One set of standards used were Acceptable Daily Intakes (ADI's) set by the Joint FAO/WHO Committee on Pesticide Residues. The ADI, based on long-term animal exposure data, is the daily intake (expressed as mg/kg bodyweight/day) of a compound which, if ingested by a human over life time, including childhood, poses no appreciable risk. In addition to ADI's of FAO/WHO, other ADI references were established also by the

Safe Drinking Water Committee. The relative ratio between calculated MPE to reference ADI, was used as a safety indicator. When the resulted ratio is below one no risk is occurring. Only five test substances had an ADI for comparison to the calculated MPE for each kind of plastics. The comparison between MPE values and ADI values is shown in Fig. 1. All MPE values were less than 1 (i.e. , exposure was below the ADI), except for prochloraz which had higher MPE values than the ADI value. FERON and co-workers (1994) showed that most of the MPE/ADI ratios for twenty-seven test substances contacted with PRB's were less than 1 but for some of the tested substances (alachlor, anilazine, lindane.....) the MPN/ADI ratio was greater than 1. Furthermore GASAWAY (1978) also compared the ADI values with the amount of pesticide for Lexan resin (PC) and found that most of pesticide residues were lower than the ADI values.

Another toxicological value named "Estimated non-toxic doses (ENTD)" (LAYTON et al., 1987; VENMAN & FLAGA, 1985, MC NAMARA, 1971) was calculated to compare the acute toxicity data in animal and humans to maximum potential exposure (MPE) from contaminated bottles for an assessment of hazard. Estimated non-toxic doses (ENTD) in humans were determined for test substances that did not have established ADI's (flutriafol, flurochloridone, atrazin, chlorine, and sodium lauryl sulfate) or for the substances for which the MPE/ADI ratio exceeded 1 (prochloraz). The ENTD values were estimated using LD 50 values (oral, rat) (Fig. 2). To calculate the ENTD, the LD 50 values were divided by a safety factor of 1000 to yield the ENTD level. The LD 50/1000 is a safety estimation of a non-toxic dose level for humans (FERON et al., 1994). The results from Fig. 2 also show the comparison between MPE and ENTD values. All of the MPE values were less than ENTD values under worst-case conditions of exposure (for adult 70 kg body weight – 1.5 l/day and for children 20 kg body weight – 0.5 l/day). In reality, daily consumption of carbonated soft drinks is much lower than 1.5 l/day, especially for children. However, for milk 0.5 l/day is a realistic quantity. Heavy users of soft drinks consume less than one liter of beverage per day (average consumption is a approximately 355 ml/day) based on consumption level in the USA, a country generally recognized as having the highest carbonated soft drink consumption per capita (unpublished data of the U. S. based National Soft drink Association, Washington D. C.). GASAWAY (1978) also made the assumption that a 70 kg adult would consume 3.78 l of milk at one time, an amount 15 times greater than the average daily consumption of milk according to the Milk Industry Foundation. Therefore, it is unlikely that the ENTD would be exceeded even under the most conservative actual use exposure conditions. Consequently, these chemicals are not considered to demonstrate a health risk for adults or children when stored in these plastic materials. The results reported here are in general agreement with those reported

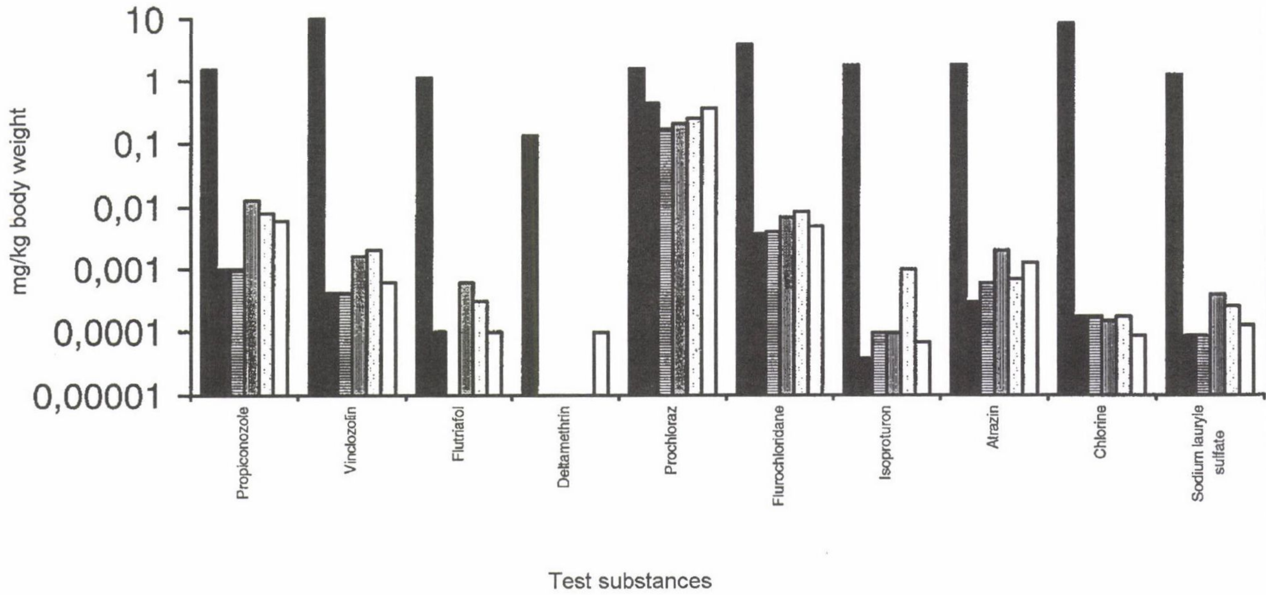

Fig. 2. Evaluation of maximum potential exposure for adult of migrated pesticides and household products observed in water after storage with different plastic types (■ PC, ▤ PET, ▥ PE, ▦ PP, □ PVC) using ■ ENTD value as a health assessment reference. ENTD= The single dose (or range) that a person could receive that would not produce acute toxicity

by FERON and co-workers (1994) who worked on refillable beverage containers of polyethylene terephthalate and concluded that none of the tested substances stored in PRB's was shown to migrate into the beverage at levels that would pose a public health concern. Only one pesticide (parathion) was remigrated into beverage at a level requiring a more detailed hazard assessment. This indicated no health hazard to adults or children. In the studies performed by GASAWAY (1978 a,b) on HDPE and PC returnable milk bottles, containers were exposed to diluted pesticide products, washed and subsequently filled with milk or water. Certain pesticides were shown to migrate back into the liquid at detectable levels. GASAWAY concluded however that there was little hazard associated with the use of returnable milk containers. JETTEN and co-workers (1994) and FERON and co-workers (1994) also concluded that none of the investigated toxic contaminants for PC returnable and PRB's respectively caused any concern for public health.

3. Conclusions

The objective of the present study was to investigate the potential of different plastic materials (PC, PET, HDPE, PP and PVC) to be contaminated with chemical compounds after detergent washing.

Sensory evaluations indicated some positive sensory responses for off-odour in water and plastics and change of plastic appearance for PC and PVC especially, in the case of pesticides contaminants. Chemical substances showed to be absorbed into the wall of these plastics, resulting in retention and remigration into water. The residual contamination level was influenced by the Kow value of the contaminant with high Kow values resulting in high residue contamination levels. The initial concentration of the active ingredient appeared to effect only the residual contamination level in some cases. The plastics commercially applied for returnable bottles (PC and PET) showed in general the lowest residual contamination. The chemical contamination level in the water (beverage simulant) showed to be low and none of the tested substances were shown to migrate into water at levels that would pose a public health concern. Although, the presence of the contaminants would not cause health risks, the applied detergent washing procedure is not able to prevent flavour carry-over that might result in quality problems. Development and optimization of processes to clean plastic materials designated for recycling or re-use will be of major importance in the future to ensure the safety of the consumer and to avoid flavour carry over.

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EFFECT ON FIRMNESS OF CANNED PEELED TOMATOES DIPPED IN CALCIUM SOLUTION AT NEUTRAL PH

G. VILLARI[a], F. DE SIO[a], R. LOIUDICE[a], D. CASTALDO[a,c], A. GIOVANE[b] and L. SERVILLO[b]

[a] Department of Tomato Products, Stazione Sperimentale Industria Conserve Alimentari, via Nazionale 121/123 Angri (SA) Italy
[b] Department of Biochemistry and Biophysics, II University of Naples, via Costantinopoli 16, Naples Italy

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The consistency of peeled tomato was increased by an experimental procedure consisting of immersion of tomatoes in a bath containing a solution of calcium ions at pH 7.5. The calcium ion concentration was in the range of 400–800 mg l^{-1} and the time of immersion spanned from 4 to 12 min. The samples were compared with those obtained by direct calcium addition in the range of 100–1000 mg/kg of product (tomato and juice). The results showed that samples immersed in the calcifying bath contained less calcium than those having the same consistency but obtained by direct calcium addition. The best result was obtained by treating the peeled tomatoes in a calcifying bath at calcium ion concentration of 800 mg l^{-1} for times between 8–12 min. After this treatment, the product consistency was found to increase of about 90% with respect to the untreated product.

Keywords: consistency, firmness maintenance, peeled tomato, canned tomato

The improvement of the mechanical characteristics of a very popular product like peeled tomato is of a relevant importance for the food industry. For this reason a lot of studies have as a target the development of new technologies in order to improve the fruit texture. As reported by BELLUCCI and co-workers (1975), although the peeled tomato integrity in the end products depends mainly on the consistency and the suitability of the starting fruits to the peeling, it has been often noted that the thermal treatments employed for the microbial stabilisation of the product, have an adverse effect on the fruit consistency and on the end product quality. The loss of fruit consistency is often observed in the processes that employ rotating box sterilizer to increase the heat penetration rate in the product. To counterbalance this effect, it is a common practice in the tomato industry to add calcium chloride to the product in order to increase its firmness. In fact, calcium ions react with free carboxyl groups of pectin

[c] To whom correspondence should be addressed E-mail: Giovane@ds.unina.it

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matrix producing a polymeric network of calcium pectates which contributes to strengthen the cell wall, thus increasing the product firmness (GRANT et al., 1973; VAN BUREN, 1968;1979). This technology has also the advantage to increase the drained weight and the fruit integrity (LOPEZ & SCHOENEMANN, 1971; BELLUCCI et al., 1975). Furthermore, it reduces the time of heat treatment due to the pH decrease of the product brought about by the calcium chloride addition (BELLUCCI et al., 1972; FLOROS et al., 1992). However, when the calcium ion concentration is higher than 450–500 mg/kg, adverse effects on the product taste can be observed. Recently, it has been found that the immersion of tomato pieces in a solution containing calcium ions at pH near the neutrality gives a product with a high firmness and a lower calcium ion content with respect to that obtained by direct addition of calcium (CASTALDO et al., 1995a, b; 1996). Furthermore, it was observed that the addition of the enzyme pectin methylesterase in the immersion bath containing calcium ions, increased the product consistency even more. On the basis of these results, we have investigated the application of this technology to the peeled tomatoes.

1. Materials and methods

1.1. Samples preparation

A hundred kg of tomatoes were washed and peeled by hand after 2 min immersion in water bath at 90 °C followed by immersion in cold water for the same time. Samples of 3 kg each were drained for 2 min and immersed in a calcifying solution for 4, 8 and 12 min at three different calcium concentration (400, 600 and 800 mg l^{-1}). Each experiment was made in duplicate.

The calcifying bath was prepared by mixing tomato serum (obtained after centrifugation at 9000×g for 20 min of tomato juice at 8 °Brix), water, sodium chloride (50 mg l^{-1}) and calcium ions (as calcium chloride). The final optical refractometric residue of the calcifying solution was 3. The experiments were conducted at room temperature in a 20 l reactor with a lid on the top to avoid the product floating during the immersion. The calcifying solution was maintained at pH 7.5 by addition of sodium hydroxide solution (25% w/w) using an automatic titrator (Crison TT2050) equipped with a pH-stat controller system. The solution was continuously mixed with a helix stirrer. The added volume of the sodium hydroxide solution as well as the pH were continuously recorded. The ratio between the product and the calcifying solution was 1:3 (weight:volume). After treatment, the product was drained for 1 min and immediately packaged in tin plate cans. Each package was prepared by adding 650 g±3 g of product with 150 g±3 g of juice (at 5 °Brix) previously brought to pH 3.9 with 1 mol l^{-1} citric acid solution.

The remaining part of the peeled tomatoes was employed to prepare samples with direct calcium addition. Six packages for each calcium addition were prepared by adding calcium to 650 g±3 g of product with 150 g±3 g of juice (at 5 °Brix) without pH correction. The calcium ion additions were of: 0, 100, 300, 500, 700 and 1000 mg/kg of product.

All the samples were thermally stabilised in a water bath for 75 min at 100 °C, cooled in running water for 20 min and finally stored at room temperature. Analyses were made after 45 days of storage.

1.2. Physico-chemical analyses

The following determinations were performed on each sample. The pH was measured employing a pH-meter Crison TT2050. The drained weight was determined weighting the product that remained after 30 s draining on sieve with holes of 2.8 mm× 2.8 mm according to the Italian official methods of analysis (MINISTERO DELL'AGRICOLTURA E DELLE FORESTE, 1989). The total acidity, expressed as g citric acid monohydrate per kg of product, was determined by titrating the slurry with 0.1 *N* NaOH to pH 8.1 with an automatic titrator Crison TT2050, according to the Italian official methods of analysis. The Ca^{2+} content in the final product (i.e. peeled tomato + juice) was determined by atomic absorption spectroscopy after wet ashing concentrated nitric and perchloric acids (A.O.A.C., 1980). Texture measurement of the peeled tomatoes was determined by measuring their firmness. The firmness (defined as maximum force recorded during compression) was measured with a universal traction-compression instrument (Instron 4500) with computerised data acquisition (software Instron series 9, version 5.23), equipped with a 5KN load static cell and a Kramer model CS-1 measuring cell (Food Technology Co., Reston, Virginia). Measures were made in triplicate on 200 g of drained product at compression rate of 25 mm min^{-1} with a sample thickness of 95 mm at a sampling rate of 50 points s^{-1}.

In order to make the measurements more reproducible, the peeled tomatoes were placed in the measurement cell in two layers. The first with the fruits having the fibro-vascular direction orthogonal to the cut direction, while the other fruit layer was parallel.

2. Results and discussion

Samples of peeled tomatoes, obtained with different concentrations of added calcium, were prepared and compared with the same product immersed in calcifying solutions at different calcium concentration and at various time.

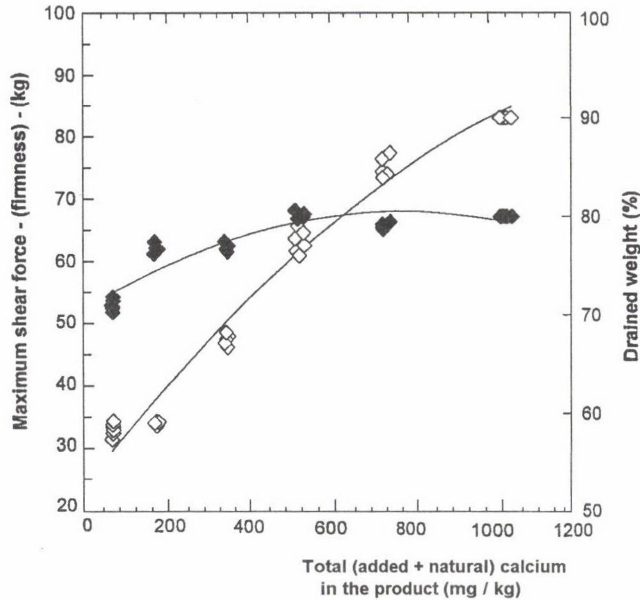

Fig. 1. Influence of direct calcium addition on the consistency (◊) and on the drained weight (♦) of peeled tomatoes

The calcium directly added to the product brings about an increase of the consistency, as shown in Fig. 1. The increase correlates, quite linearly, with the calcium concentration in the product in the range between 70–1000 mg of calcium ions per kg product (tomato and juice). Furthermore, also the tomato drained weight increases, although to less extent, when the calcium concentration increases. In fact, the drained weight increases by 10% in the range between 70–500 mg of calcium, whereas a further calcium increase did not produce any effect on the drained weight. This effect was also observed by BELLUCCI and co-workers (1975).

On the basis of previous works (CASTALDO et al., 1995a, b), we have investigated if the immersion of peeled tomato in a calcifying solution could replace the direct calcium addition giving a product with equivalent consistency but with less calcium content. For this purpose, tomatoes were immersed in a bath containing calcium ions at concentrations between 400 and 800 mg l^{-1} at pH 7.5. The immersion in calcifying solution at concentration higher than 1000 ppm did not cause significant consistency increase with respect to that obtained with calcium concentration of 800 ppm. Moreover, concentrations above 800 ppm noticeable increased calcium absorption by the product. The results, reported in Fig. 2, clearly show that the increase of calcium ion concentration in the bath produces a product consistency increase

between 65 and 94% with the immersion time of 12 min. Longer immersion time does not produce any further increase of the product firmness. The measured consistency of peeled tomatoes is lower than that observed for the diced tomatoes with the same treatment conditions (CASTALDO et al., 1995 a, b). This can be due to the different degree of subdivision of the two products. In fact, peeled tomatoes have a lower surface exposed to the calcifying solution with respect to that of diced tomatoes and this considerably reduces the interaction between the free carboxylic groups on the pectic matrix and calcium ions, which leads to a lower calcium pectate concentration and therefore to the lower consistency found in peeled tomato. Moreover, the product firmness could be influenced by the action of enzymes, like pectin methylesterase (PME) and polygalacturonase (PG), that respectively produce an increase in free carboxylic groups on pectin and its depolimerization. These enzymes are bound to the cell wall, thus their activities in the product depend on their degree of subdivision. In particular, two PG isoenzymes were found in tomato with an activity optimum at pH 4.6 (MOSHREFI & LUH, 1984), while the pH optimum of PME isoenzymes is around 8.5 (GIOVANE et al., 1994). Hence, the immersion of product in the calcifying bath at pH 7.5 inhibits PG while activates PME producing an increase of carboxylic groups and, as a consequence, the increase of calcium pectate adducts which cause the consistency increase. As far as a comparison between the products with added calcium and those obtained by immersion is concerned, in Tables 1 and 2 the results from the two sample series after 45 days of storage are reported. The data seem to confirm, as already reported for diced tomatoes (CASTALDO et al., 1996), a significant loss of sugars and acids from the products treated by immersion with respect to those treated by direct calcium addition. This loss is essentially due to the osmotic exchange between the product and calcifying solution during the process which also affects the sugars and acidity quotients. Instead, the drained weight of product treated by calcifying bath is higher than that obtained by direct calcium addition.

Table 1

Samples obtained by direct addition of calcium ions[a]

Calcium ions added (mg/kg)	Drained weight (%)	pH	Sugars (g%)	Acids (g%)	Sugars ratio	Acidity ratio
0	70.5	4.48	3.22	0.45	48.8	6.8
100	76.3	4.40	3.12	0.36	49.7	5.7
300	76.5	4.36	3.15	0.39	49.4	6.1
500	79.8	4.35	3.15	0.39	49.3	6.1
700	78.9	4.41	3.17	0.39	49.3	6.1
1000	80.0	4.36	3.17	0.41	48.7	6.3

[a] Each value represents the mean of six determinations

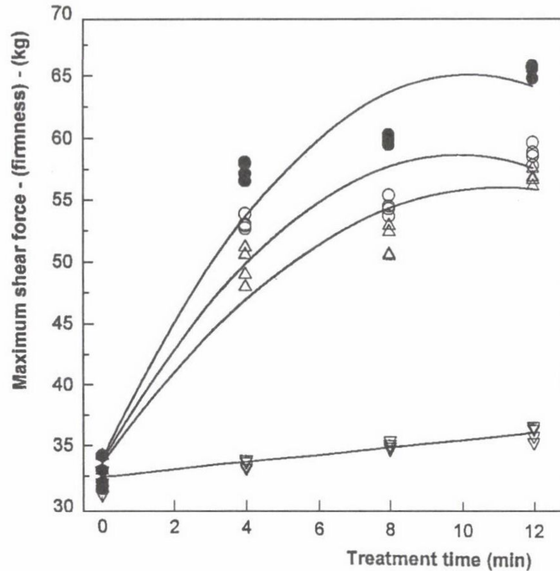

Fig. 2. Measurement of peeled tomato consistency as a function of treatment time in calcifying solutions containing no calcium added (∇), and with addition of 400 mg l^{-1} (Δ), 600 mg l^{-1} (O) and 800 mg l^{-1} (•) of calcium ions

Table 2

Samples obtained by immersion in calcifying solution[a]

Calcium ions (mg l^{-1})	Drained weight (%)	pH	Sugars (g%)	Acids (g%)	Sugars ratio	Acidity ratio	Immersion time (min)
	85.4	4.46	3.10	0.39	49.7	6.3	4
(Ca^{2+})=400	84.7	4.46	2.74	0.36	45.8	6.1	8
	83.7	4.45	2.64	0.35	47.0	6.1	12
	86.9	4.46	2.92	0.38	46.9	5.9	4
(Ca^{2+})=600	88.5	4.44	2.80	0.35	48.2	6.3	8
	85.3	4.42	2.59	0.35	46.2	6.1	12
	84.3	4.39	2.80	0.41	45.3	6.6	4
(Ca^{2+})=800	89.6	4.43	2.75	0.35	45.8	5.9	8
	87.4	4.43	2.68	0.34	46.3	5.9	12

[a] Each value represents the mean of four determinations

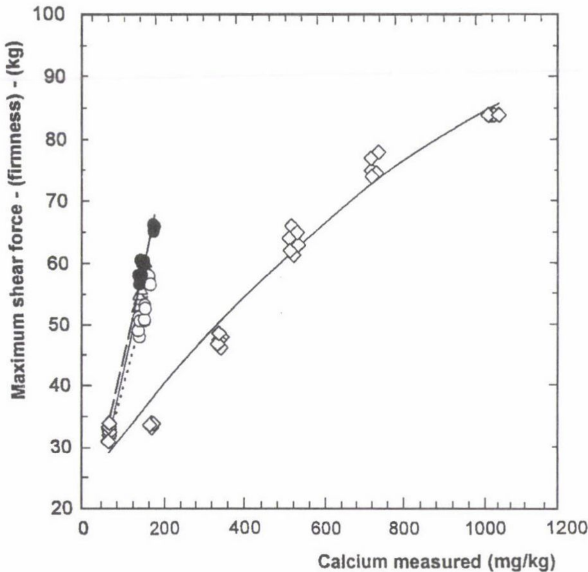

Fig. 3. Comparison of the consistency of products obtained by direct addition of calcium ions from 100 to 1000 mg/kg (◊) and that of samples obtained by immersion for 4 to 12 min in solutions of 400 (Δ), 600 (O) and 800 (•) mg l^{-1} of calcium ions

A significant result from a technological point of view arises from the comparison between the consistency values and the calcium concentration in the products as showed in Fig. 3. In fact, it appears that products obtained from the calcifying bath contained a lower calcium concentration with respect to those treated by direct calcium addition which showed the same consistency.

3. Conclusion

The immersion of peeled tomatoes in a bath containing calcium ions increases their firmness with a very little increase of their calcium content that, in any case, does not exceed 200 mg/kg of product. Instead, to reach the same product consistency by direct calcium addition, 500–600 mg/kg have to be added to the product. This calcium concentration is around the limit over which effects on the product taste can be observed. For these considerations, the calcifying bath process is to be preferred to the direct calcium addition.

*

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A CHEMICAL STUDY OF THE MINERAL FRACTION OF TARRAGONA HAZELNUTS (*CORYLUS AVELLANA* L.)

J. SERRA BONVEHI[a] and F. VENTURA COLL

Agricultural and Food Laboratory (Generalitat of Catalonia), 08348 Cabrils, Barcelona, Catalonia. Spain

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Mineral contents and phytic acid were determined in the main varieties of Tarragona hazelnuts (Negret, Pauetet, Gironell, Culplà, Morell and Grifoll). Macro- (K, Mg, Ca, and Na) and micro elements (Cu, Mn, Fe, Zn, and Cr) were quantified using atomic absorption spectrophotometry, on dry-ashed samples. N, P, and phytic acid were determined using Kjèldahl method, colorimetric vanadium-molybdate, and HPLC system. respectively. The analysis of variance showed significant difference ($P \leq 0.05$) in N, Mn and Na contents between farming methods. Only K content showed significant difference ($P \leq 0.05$) between varieties. The correlations between mineral contents were calculated, but only the Cu/Zn correlation was greater than the Pearson-Lee limit ($r=0.660$, $P \leq 0.05$). The percentage of phytic acid is low (0.17 g/100 g). The mineral intake that hazelnut consumption could represent was evaluated with regard to the Recommended Dietary Allowance (RDA).

Keywords: hazelnuts, mineral elements, phytic acid, nutrition

Since 1987, hazelnut consumption in Spain has grown by 12.7 per cent, mainly at homes (MAPA, 1991). Simultaneously, the importance of hazelnuts as a food ingredient has increased, i.e. elaboration of cocoa-derivative products, muesli, snacks, cookery, bakery, ice-creams, “turrones”, marzipan, etc. The use of nuts allows manufacturers to obtain many textures (crunchy, cream, etc.) and give a defined and pleasant flavor, at the same time improving the nutritional value of the product. Hazelnut paste possesses very estimable characteristics in these aspects (RIVELLA, 1984). Although Italy, Spain and Greece are hazelnut producers, the European Union is deficient in its dry fruit products and the majority is imported from Turkey. Tarragona produces some 27000 t, representing 92% of the total production of Spain (SERVEI AGRARI, 1989). The main varieties produced are Negret (80%) and Pauetet, Gironell, Culplà, Grifoll, Morell and others, in decreasing order of importance (GIL CARBAJO et al., 1985; SERRA BONVEHI & VENTURA COLL, 1992). For many years, intensive efforts including this study have been made, to characterize the main varieties cultivated in

[a] Author to whom correspondence should be addressed (fax +34–3–7532607)

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Tarragona. The aim is to determine their main qualities of industrial and nutritional interest and to define the stability parameters to control during product handling (SERRA BONVEHI & VENTURA COLL, 1992, 1993a,b,c; SERRA BONVEHI, 1995; SERRA BONVEHI & SERRANO ROSUA, 1996). In the present study, the results obtained from the mineral fraction analysis of the main varieties from Tarragona are reported. The nutritional importance of hazelnut as a source of minerals is discussed.

1. Materials and methods

1.1. Samples

Twenty-two samples of hazelnuts of the main Tarragona varieties cultivated using dry-farming, irrigation and mixed methods (in 1989 and 1991) were studied (Table 1). The samples were collected in the stores of the principal producers, specifying origin, farming method, number of cultivated fields, gathering method, drying and storage conditions. The samples were artificially dried at an air temperature of approximately 40 °C and air flow rate of 1.25 m s^{-1} until a shelled hazelnut moisture of less than 5 g/100 g was obtained. Drying was discontinuous, and reduced water content at a rate of 1 g/100 g per h. To complete the process, cold air (5–10 °C) was passed through for 60–90 min. The hazelnuts were then preserved at room temperature and in the dark. Among the six varieties considered in this study, two were grown using only irrigated farming (Pauetet and Gironell), and the other four were farmed dry or with irrigation (SERRA BONVEHI & VENTURA COLL, 1992). Analyses were all done in triplicate.

2.1. Physicochemical analyses

Ash. Ash percentage was measured by calculation, overnight at 500–550 °C in a furnace, to constant mass (A.O.A.C., 1995).

Mineral Elements. To determine mineral elements, 5 g of ground hazelnuts was reduced to ashes at 500–550 °C and dissolved in 2 ml 2 *N* HCl, made up to 50 ml with distilled water at 40 °C, and 5 ml 15 mM Cl_3La and 5 ml 3.8 mM ClCs were added (SANUI & PACE, 1968; DE RUIG, 1986). The K, Na, Mg, Ca, Zn, Fe, Cu, Cr and Mn contents were evaluated using an atomic absorption spectrophotometer Model 703 (Perkin-Elmer). The samples were diluted to provide concentration in the proper absorption range. The repeatability with variation values in the range of 2.2 of 10%, is acceptable, whereas the detection and quantification limits.

Phosphorus. The Mission reaction was used, reading the vanadium phosphomolybdate yellow-orange complex at 400 nm in 5 g of ground hazelnuts. The

standards were prepared using anhydrous KH_2PO_4 (CHAPMAN & PRATT, 1978). Repeatability (r=0.726+0.067.x; x: mean).

Nitrogen. Nitrogen was determined by conventional Kjeldahl digestion using a copper catalyst. The ammonia was distilled and collected in a solution of boric acid which was then titrated against standard acid. Digestion and distillation were carried out using the Kjeltec apparatus (Model 1002, Tecator, Sweden).

Inositol phosphates. Inositol tri- [IP3], tetra- [IP4], penta- [IP5], and hexaphosphate [IP6, phytic acid] were separated by HPLC, using the method of SANDBERG and AHDERINNE (1986) as modified by SEGUEILHA and co-workers (1993). Inositol phosphates extracted by agitation with 0.5 *N* HCl (20 ml/0.5 g) for 3 h at ambient temperature. The mixture was centrifuged at 12000 g for 15 min and the supernatant evaporated to dryness under vacuum at 40 °C. The dry extract obtained was recuperated in 15 ml of 0.025 *N* HCl and loaded on anion-exchange resin [AG1-X4 mesh 100–200 Cl^- form (500 mg; Bio-Rad, Richmond, CA)] that was then washed with the same volume of 0.025 *N* HCl (0.75 ml min^{-1}). Inositol phosphates were eluted from the resin using 25 ml of 2 *N* HCl (0.75 ml min^{-1}). The resulting extract was evaporated to dryness, resuspended in 2 ml of bidistilled water, and filtered through a 0.45 μm filter and injected into the chromatographic system. HPLC-RI was carried out on a HPLC system consisting of Model 590 Waters Associate pumping units, a Model 712 Rheodyne valve loop injector fitted with a 20 μl loop, and Waters Associate Differential Refractometer detector Model 410. For phytic acid analysis, a PRP–1 5 μm (150×4.1 mm) reversed-phase analytical column was used. Mobile phase was prepared by mixing 560 ml of methanol and 410 ml of 0.035 M formic acid. Ten milliliters of tetrabutylammonium hydroxide (TBA-OH) (40%, w/w, solution in water) was added, and the pH was adjusted to 4.3 by the addition of 72% (w/w) sulfuric acid. Solvent was pumped through a heated (40 °C) PRP–1 column at a rate of 1 ml min^{-1} (LEHRFELD, 1989). Inositol hexaphosphate (Sigma Chem. Co, St. Louis, MO) were dissolved in water an used as reference compound.

Statistical analyses. Analysis of variance was conducted considering the factors variety and farming method. Statistical package software Statgraphics (SAS, 1990) was used to make the calculations.

2. Results and discussion

The results of the analysis are summarized in Tables 1–5. Table 1 shows ash, N (total), mineral elements, variety, geographic origin and type of cultivation for each analysed sample. Ashing gave an average mineral content of 2.21 g/100 g, ranged from 1.96 to 2.37 g/100 g. The presence of ten chemical elements were determined with a net predominance of potassium [54% of the total, followed by P, Mg, and Ca (44.5%)].

The values obtained for mineral elements are of the same order of magnitude as the values published by other authors for hazelnuts (McCance & Widdowson, 1969; Hadorn et al., 1977 a,b, 1978; Diaz Marquina et al., 1980; Fincke, 1981; Ozdemir, 1985; Shrestha & Thompson, 1987; Souci et al., 1989; Mehlenbacher, 1991; Holland et al., 1992). Hazelnut mineral contents depend on environment [soil, cultural practices (fertilizers spreading, irrigation), climate, etc], genetics (ontogenesis, varieties) or can be altered because of physiological disorders (Shrestha & Thompson, 1987). However, excluding symptomatic cases, it is accepted that variations on plant mineral contents are influenced firstly by environment, within limits imposed by genetics (Saura Calixto et al., 1988; Kupka, 1992). Taking this consideration in mind, the statistical analyses were performed between varieties and farming methods (Table 2). ANOVA did not detect differences between varieties, except in K content ($P \leq 0.05$). Applying the same test to the farming methods, only differences in Na, Mn and N contents were detected ($P \leq 0.05$). The correlations between minerals were calculated, but it exceeded the Pearson-Lee limit only in the case of Cu and Zn ($r=0.660$, $P \leq 0.05$). Diaz Marquina and co-workers (1980) also found no correlations between different mineral contents working with hazelnuts from Tarragona. However, Saura Calixto and co-workers (1988) detected high correlations in almond. According to Student test, there were significant differences ($P \leq 0.05$) in ash, Mg, Na, Ca, Cu and Fe contents between our results and those obtained by other authors (Table 3). It should be pointed out that Diaz Marquina and co-workers (1980) detected higher Ca, Na and Mg contents in hazelnuts from Tarragona. The higher Na content detected in hazelnuts from dry-farmed hazelnuts could be due to: 1) a higher Na concentration in the soil, and 2) the effect of a higher osmotic potential in the radicular environment that diminishes mineral absorption except for Na and P (Marschner, 1986; Kupka, 1992). It should be remarked that during the last decade the average of irrigated hazelnut crops increased (Serra Bonvehi & Ventura Coll, 1992) and that is reflected in higher Na levels in hazelnut found by Diaz Marquina and co-workers (1980). Almond and hazelnut differences in correlations between minerals could be due to agronomic conditions of cultivation. The extent of fertilization and irrigation practices are much higher in hazelnut crops than in the case of almonds, a crop that usually is restricted to dry and poor soils. While mineral nutrient deficiency in almond cultivation could be a limiting factor of productivity, in hazelnut it is the excess of fertilization (Gil Carbajo et al., 1985). This point should be confirmed through foliar analysis and the evaluation of the results through the Diagnosis and Recommendation Integrated System (DRIS) to determine deficiences or excesses in inorganic elements and to establish optimal levels of fertilizers that will allow higher plant productivity and the desired mineral contents in hazelnut kernels (Alkoshab et al., 1988).

Table 1

Mineral contents of the hazelnuts samples studied

Sample no.	Variety	Geographical origin	Farming method	Ash	N(total)	K	Mg	Na	Ca	Cu	Mn	Zn	Fe	P	Cr
				[g/100 g d.m.]		[mg/100 g d.m.]									
1	Pauetet	Vila-Seca	I	2.26	2.40	574	62.4	4.86	33.9	1.66	1.27	2.14	1.87	319	0.06
2	Pauetet	Alcover	I	1.96	2.68	514	80.4	5.58	39.7	1.54	2.36	1.91	1.04	271	0.06
3	Pauetet	Riudoms	I	2.23	2.34	503	92.1	6.33	34.8	1.32	2.00	1.89	1.91	295	0.12
4	Negret	Riudecols	DF	2.26	2.11	495	85.8	12.18	53.0	5.09	2.62	5.42	2.27	292	0.12
5	Negret	Vilaplana	DF	2.07	2.47	546	97.6	10.66	65.3	1.90	1.21	2.09	1.93	365	0.09
6	Negret	Vilallonga	I	2.37	2.52	513	110.4	5.05	45.0	1.88	2.43	2.57	2.12	306	0.04
7	Negret	Vilaplana	I	2.05	2.35	492	80.3	4.62	52.7	1.58	1.52	1.93	1.61	427	0.10
8	Negret	Riudoms	I	2.30	2.48	515	81.1	8.46	62.6	1.97	1.73	2.63	1.98	312	0.08
9	Negret	El Morell	I	2.37	2.08	540	117.5	8.35	59.4	1.67	1.59	1.76	1.60	276	0.11
10	Negret	Reus	I	2.07	2.86	465	99.7	10.13	64.9	1.37	1.43	1.92	1.41	266	0.16
11	Morell	Falset	DF	2.34	2.04	532	84.6	11.30	50.4	1.75	1.16	1.61	1.94	279	0.19
12	Morell	Falset	I	2.25	2.41	568	99.4	5.86	48.0	2.16	1.66	1.98	2.05	283	0.13
13	Grifoll	Vilaplana	DF	2.22	2.12	488	100.6	6.22	44.1	1.52	0.83	2.03	1.69	335	0.04
14	Grifoll	Vilaplana	I	2.31	2.09	518	73.3	4.07	45.2	2.27	3.19	2.65	1.68	302	0.11
15	Gironell	Riudoms	I	2.29	2.60	526	83.7	12.02	87.5	1.46	3.74	2.01	1.39	274	0.07
16	Gironell	El Morell	I	2.33	3.00	524	123.1	7.92	33.8	2.10	3.97	1.80	1.39	320	0.11
17	Gironell	Vilallonga	I	2.16	2.41	553	92.5	5.81	45.2	2.39	2.11	2.30	2.00	300	0.09
18	Gironell	Vila-Seca	I	2.06	2.41	555	93.7	4.95	45.4	1.71	1.67	2.01	1.68	223	0.12
19	Gironell	Riudecols	DF	2.10	1.91	568	92.6	8.54	42.7	1.83	2.91	1.95	1.97	293	0.15
20	Culplà	Falset	DF	2.10	2.08	541	99.8	7.91	57.4	0.97	0.74	1.83	1.88	298	tr.
21	Culplà	Falset	DF	2.30	1.93	591	74.2	12.37	34.8	1.66	1.20	1.82	1.35	291	0.04
22	Culplà	Falset	I	2.20	2.21	570	74.9	6.96	37.4	1.39	1.07	1.91	1.98	250	0.17
x				2.21	2.34	531	90.9	7.73	49.2	1.87	1.93	2.19	1.76	299	0.10
SD				0.12	0.29	32	14.8	2.68	13.1	0.79	0.90	0.77	0.30	41	0.04
V_{min}				1.96	1.91	465	62.4	4.07	33.8	0.97	0.74	1.61	1.04	223	tr.
V_{max}				2.37	3.00	591	123.1	12.37	87.5	5.09	3.97	5.42	2.27	427	0.19
CV				5.4	12.4	6	16.3	34.7	27.2	42.2	46.6	35.2	17	13.7	40

d.m.: dry-matter; I: irrigated; DF: dry-farmed; tr.: traces

Table 2

Composition of the different varieties and influence of farming method on the mineral elements

Variety	Ash	N(total)	K	Mg	Na	Ca	Cu	Mn	Zn	Fe	P	Cr
	[g/100 g d.m.)		[mg/100 g d.m.]									
Pauetet	2.15	2.47	530	78.3	5.59	36.1	1.51	1.88	1.98	1.61	295	0.08
Negret	2.21	2.41	509	86.1	8.49	57.6	2.21	1.79	2.62	1.85	321	0.10
Negret[a]	2.17	2.29	521	91.7	11.42	59.2	3.50	1.92	3.76	2.10	329	0.11
Negret[b]	2.23	2.46	505	97.8	7.32	56.9	1.69	1.74	2.16	1.74	317	0.10
Morell	2.30	2.23	550	92	8.58	49.2	1.96	1.41	1.80	2	281	0.16
Morell[a]	2.34	2.04	532	84.6	11.30	50.4	1.75	1.16	1.61	1.94	271	0.19
Morell[b]	2.25	2.41	568	99.4	5.86	48	2.16	1.66	1.98	2.05	283	0.13
Grifoll	2.27	2.11	503	87	5.15	44.7	1.90	2.01	2.34	1.69	319	0.08
Grifoll[a]	2.22	2.12	488	100.6	6.22	44.1	1.52	0.83	2.03	1.69	335	0.04
Grifoll[b]	2.31	2.09	518	73.3	4.07	45.2	2.27	3.19	2.65	1.68	302	0.11
Gironell	2.16	2.47	545	97.1	7.85	50.9	1.90	2.88	2.01	1.69	282	0.11
Culplà	2.20	2.07	567	83	9.08	43.2	1.34	1	1.85	1.74	280	0.07
Culplà[a]	2.25	2	566	87	10.14	46.1	1.32	0.97	1.83	1.62	295	0.02
Culplà[b]	2.20	2.21	570	74.9	6.96	37.4	1.39	1.07	1.91	1.98	250	0.17

d.m.: dry-matter; [a]: dry-farmed; [b]: irrigated

Table 3

Mineral content of hazelnut

Literature cited	Variety/ origin	Ash	N(total)	K	Mg	Na	Ca	Cu	Mn	Zn	Fe	P	Cr
		[g/100 g d.m.]		[mg/100 g d.m.]									
Our results		2.21	2.34	531	91	7.73	49.2	1.87	1.93	2.19	1.76	299	0.10
SOUCI et al., (1989)		2.57	2.02	671	165	2.11	238	1.35	6.02	1.97	4.01	351	0.01
MCCANCE & WIDDOWSON (1969)	Barcelona		2.18	992	214	2.65	180	1.02			3.15	317	
MEHLENBACHER (1991)		2.14		471	302	3.06	199	1.60	2.13	2.54	3.46	329	
*	Tomboul			650	165	5.0	125		7.69	6.24	2.44	319	
*	Cakildak			920	190	3.41	155		7.99	5.55	2.67	296	
HADORN et al., (1977a,b, 1978)	Tarragona	2.45	2.52										
*	Italy	2.43	2.57										
*	Turkey	2.36	2.49										
FINCKE (1981)	Tarragona	2.26	2.85										
*	Italy	2.27	2.73										
*	Turkey	2.21	2.49										
*	Oregon	2.26	3.11										
	Average	2.32	2.53	706	188	4	158	1.46	5.15	3.70	2.92	318	
	SD	0.13	0.32	209	69	2.08	65.6	0.36	2.95	2.03	0.80	23	
	CV	5.6	12.6	29.6	36.7	52	41.5	24.7	57.3	54.9	25	7.8	
DÍAZ MARQUINA et al., (1980)[a]	Negret			333	278	26.4	190	1.85	1.05	2.68	2.66		
*	Morell			422	312	41.5	221	1.74	1.56	2.46	3.77		
*	Grifoll			417	286	34.3	234	1.53	0.96	2.47	3.73		
*	Gironell			393	356	37.3	180	1.98	1.25	2.77	3.93		
*	Culplà			506	266	41.0	168	1.52	0.67	2.56	3.85		
	Average 23 varieties			403	313	33.5	200	1.57	1.02	2.50	3.31		

[a] Expressed as edible matter; d.m.: dry-matter

With regard to human nutritional aspects, hazelnuts have significant mineral content. In accordance with the Recommended Dietary Allowances of the National Research Council (N.R.C., 1989), 100 g of hazelnuts represent an important supply of Cu, P, Mn, Mg and K, but have low amounts of Ca (Table 4). A high K/Na ratios make hazelnuts interesting for diets with a defined electrolytic balance (STAMLER, 1994). There are some compounds that interferes in the diffusive processes of minerals at the digestive tract. Phytic acid, dietary fiber, tannins and oxalic acid inhibit absorption of Fe, Ca, Mg, Mn and Zn, while proteins, sugars, ascorbic acid and citric acid enhance these (HAZELL, 1988). Phytates have been shown to reduce the bioavailability of minerals and to inhibit proteolytic and amyolytic enzymes (DESPHANDE & SALUNKHE, 1982; SERRAINO et al., 1985; NOLAN & DUFFIN, 1987). Phytic acid content determined by HPLC was 2.58±0.15 mmol kg^{-1} (0.17 g/100 g) (Table 5). The results are similar to those reported by HAZELL (1988). A series of samples of different foods containing 0.008–10.7 g/100 g phytic acid were analysed (LEHRFELD, 1994). According to these results we conclude that the percentage of phytic acid is low. Chromatographic analysis revealed small amounts of hexaphosphate. Tri-, tetra-, and pentaphosphate inositol were not detected. In mature plant seeds, the inositol phosphates occur mainly as hexaphosphate, but during food processes including prolonged heat treatment, it is likely that other inositol phosphates are formed. ELLIS et al., (1982) recorded phytate/zinc molar ratios of 3.3±1.4, 4.5±0.1, and 7.6±0.7 for regular, ovo-lacto vegetarian, and soy as meat substitutes in hospital diets. In the present study, the average phytate/zinc molar ratio was 8.06±1.48 (Table 5). The phytate × calcium/zinc molar ratio has been suggested by DAVIES and co-workers (1985) to be a more accurate predictor of the effect of other factors on zinc bioavailability. The ratios obtained (0.045–0.14) (x=0.10±0.031) are considered values equilibrated with respect to human zinc deficiency. According to HAZELL (1988), hazelnut have a higher percentage of diffusible Fe than other nuts. This fact and the notable mineral contents are two remarkable qualities that place hazelnut as a very important nutritional complement.

3. Conclusions

We did not detect significant differences between varieties of hazelnuts from Tarragona with respect to mineral content, except in the case of K. Na, Mn and N contents have significant differences between farming methods. Only the Cu/Zn correlation exceeded Pearson-Lee limit (r≥0.660, P≤0.05). The properties of hazelnuts as a source of minerals to the diet were reported.

Table 4

Recommended Dietary Allowances (RDA) of minerals

Mineral element	RDA[a] [mg/day]	Hazelnut [mg/100 g d.m.]	% 100 g hazelnut/day
K	2500	531	21
Mg	350[b]/300[c]	91	26[b]/30[c]
P	800	299	37
Ca	800	49.2	6
Cu	2[b]/3[c]	1.87	93[b]/62[c]
Mn	2.5[b]/5.0[c]	1.93	77[b]/32[c]
Zn	15	2.19	15
Fe	10[b]/18[c]	1.76	18[b]/10[c]

[a]: according NRC (1989); [b]: male, 23–50 years old; [c]: female, 23–50 years old; d.m.: dry-matter

Table 5

Concentration of different compounds that inhibit or enhance the bioavailability of mineral elements

Results	Protein[a]	Sugars[b]	NDF[b]	ADF[b]	Phytate	[Phy]/[Zn]	[Phy][Ca]/[Zn]
	[g/100 g deffated matter]				[mmol/kg]	[mmol/kg]	[mol/kg]
X	14.87	7.51	9.25	6.41	2.58	8.06	0.099
SD	1.91	1.6	1.18	0.71	0.15	1.48	0.031
V_{max}	18.8	10.14	10.9	7.5	2.84	10	0.14
V_{min}	12.1	3.6	7.5	5.1	2.33	3.38	0.045

according to [a]SERRA BONVEHI (1995); [b]SERRA BONVEHI & VENTURA COLL (1993a);
NDF: neutral detergent fibre; ADF: acid detergent fibre

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CHANGES IN HYGIENIC AND TECHNOLOGICAL WHEAT QUALITY CAUSED BY MOULD INFECTION

M. ŠARIĆ, M. ŠKRINJAR, G. DIMIĆ, N. FILIPOVIĆ and J. RASIĆ

University of Novi Sad, Faculty of Technology, 21000 Novi Sad, Bulevar Cara Lazara 1. Yugoslavia

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The consequences of field mould attack, especially of *Fusarium* spp. but also *Alternaria* spp. are yield losses and a significant decrease in technological wheat quality that can go so far as to render its useless for processing. The aim of this work was to highlight field moulds as one of the causes of decreases in technological quality in Yugoslavia in the last two years to experts and a scientific public. In some localities where harvest was investigated every year, a significant contamination by these microorganisms was registered. Beside visual evaluation that statement was confirmed by mycological and toxicological analyses as well. By comparing the results of infected samples with those at the localities where there were no severe mould infections, a significant decrease in total technological wheat quality was proved, which caused also the impossibility of bread production out of such kinds of wheat.

Keywords: wheat kernel, *Fusarium* spp., *Alternaria* spp., black point, gliadine, glutenine, proteins, mycotoxins, quality determination

Cereals, especially wheat and its products, can be infected by moulds in all phases of the production cycle: in the field, during harvest, storing, processing and delivering as well as during the period from production to consumption of food (CHRISTENSEN, 1964).

In the world generally, and locally, the problems of kernels infection by field moulds are given adequate attention in agriculture. The processing of wheat contaminated by field moulds into human food is not questioned in the majority of developed countries. Those kinds of wheat are not processed. Locally, however, such kind of wheat is often processed into industrial or wholemeal flours, no matter that a significant percentage of infected kernels are present on receipt (BECHTEL et al., 1985; KLING, 1988).

The consequences of kernel infection by field moulds, especially *Fusarium* spp. and *Alternaria* spp. are losses in wheat yield, and also a very significant decrease in the technological quality of infected kernels, being unsuitable for further processing into the human food. The problem is very complex because moulds are capable of

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producing numerous toxins (BAMBURG et al., 1969; ŠARIĆ et al., 1973; ŠARIĆ et al., 1980b; BOYACIOGLU & HETTIARACHCHY, 1995; KEYSERLUNG & KÖSTER, 1995).

Kernels infected by *Fusarium* species or those having "black point" show a characteristic appearance. They are more or less shrivelled, appear chalky or pinkish (Š ARIĆ et al., 1980 a,b; MILLER et al., 1988). According to their appearance these kernels are visually classified either as "little infected by Fusarium" or as "much infected by Fusarium".

Kernels "little infected by Fusarium" are less shrivelled and appear not so chalky or pinkish. Usually these kernels are infected during the period from latter seed filling up to full maturity. Mostly, during the cleaning process in the mill these kernels are not eliminated and enter further process of grinding. The greatest mould infection is in the aleurone layer (ŠARIĆ et al., 1973; SCHNÜRER, 1991).

Kernels "much infected by Fusarium" are very shrivelled, chalky or pinkish and light weight. Such kernels are infected by moulds in the earlier phases of the plant development (ŠARIĆ et al., 1973). It is supposed that during cleaning processes in the mill only a part of these kernels is eliminated, but the rest is further processed.

At receipt, the raw material usually is a mixture of wheat consisting of both fractions of kernels infected by *Fusarium* and sound grain. During the unloading process, samples are judged visually and according to the content of impurities their value is determined. Kernels infected by *Fusarium* are classified (or not) into a group of so called "black Besatz", i.e. sick wheat. According to YUGOSLAV STANDARD (1978), these kernels are defined as: "Sick wheat comprise decayed grains, mouldy grains, heat damaged grains and other grains which became unsuitable for human use".

"Black point" disease results when fungi attack the embryo regions and crease and these parts are darkened or discolored. In the extreme the whole grain area could be changed. These discolorations are associated with different fungi, so called "black fungi", *Alternaria* spp., and *Helmintho sporium* (MILLER et al., 1988; KEYSERLUNG & KÖSTER, 1995). According to YUGOSLAV STANDARD (1978), these kernels are not accounted into impurities.

Concerning these characteristics of fusarious kernels and black point, it is unknown, how during cleaning process in the mill they are arranged, i.e. which part of these kernels is further processed into flour and other products for human consumption. But, it is known that producing a wholemeal flour means that all grain is ground and processed into bread and small goods (SCHNÜRER, 1991).

Hygienic control of suspicious portions on wheat receipt in the mill, i.e. store house, is almost never done. In 1995, in most cases after visual wheat inspection and sorting, our mycological analyses confirmed the existence of *Fusarium* and *Alternaria* species.

The aim of this work was to indicate field moulds on some Yugoslavian localities as important causes of decreasing the wheat quality observed in the last two years.

1. Materials and methods

Ten wheat varieties (Rodna – belonging to the improvers, Proteinka, Pobeda, NS-rana 5, Milica, Danica, Slavija, Dićna and Studenica – belonging to bread varieties and Evropa 90 – belonging to a basic variety) were investigated. Samples were taken from localities, A, B and C. Samples from localities A and B on visualization exhibited severe infection by field moulds, particularly *Fusarium* spp. Samples from the locality C had no infected kernels and were used as a control.

1.1. Mycological analyses

In order to determine the number of moulds per kernel, ten g of sample was treated with 100 ml of sodium hypochlorite to eliminate saprophytic moulds on kernel surfaces. Erlenmayer-flasks (300 ml) with samples and sodium hypochlorite were shaken for 2 min on a rotating shaker. Then, each sample was washed out with sterile distilled water (2×100 ml). Under aseptic conditions twelve kernels were placed on each plate with Sabouraud maltose agar containing antibiotic (1 ml of 1% chloramphenicol and 1 ml of 1% oxytetracycline per 100 ml of isolation medium). Incubation was done at 25 °C for seven days. Duplicate analyses were done.

After mould number determination, colonies were inoculated on appropriate media. Identification of species was performed according to ELLIS (1971), PIDOLIČKO and MILJKO (1971), NELSON and co-workers (1983) and SAMSON and VAN REENEN-HOEKSTRA (1988).

1.2. Analyses of mycotoxins

Qualitative and quantitative determination of aflatoxin B_1, ochratoxin A and zearalenone in kernels was performed by TLC method as described by BALZER and co-workers (1978). Concentrations of the toxins were determined visually and densitometrically (Camag TLC Scaner Integrator SP 4290).

1.3. Technological quality

Sampling and technological quality including trade quality (test weight, 1000 kernel weight and protein content) was done according to YUGOSLAV REGULATIONS (1988), dough rheology (farinograph, extensigraph and amylograph) and baking quality (test baking) of all these samples from three localities were analyzed applying indirect

and direct methods for determining breadmaking quality (KALUDERSKI & FILIPOVIĆ, 1990). Data were statistically evaluated according to HADŽIVUKOVIĆ (1979).

2. Results

2.1. Microbiology and mycotoxicology of wheat

By visual inspection a significant proportion of fusarious kernels and black point was reported. Data from Table 1 show presence of moulds of *Fusarium* spp, as well as moulds belonging to the family *Dematiaceae* which comprise moulds of *Alternaria* spp, also present in all samples.

By the toxicology control, it is evident that in the majority of samples, field moulds did not produce mycotoxins; not even in traces. Only three varieties from the locality A exhibited a content of ochratoxin A above the limit value (of 10 μg/kg) specified in "Regulations about quantities of pesticides, metals and metalloids and other poisonous substances, chemotherapeutic, anabolics and other substances which could be present in food" (YUGOSLAV REGULATIONS, 1992).

The mycotoxin problem is very real in the world generally, as well as locally. Concerning kernels little infected by *Fusarium*, i.e. kernels that are not shrivelled and which are not removed in the cleaning process, it was stated that mould biomass is concentrated in the seed coat i.e. there is 97% of mould biomass present in bran pointing at the highest content of mycotoxin in the seed coat (SCHNÜRER, 1991).

The fact that makes the problem of mycotoxin very real is the new trend in human nutrition of consuming wheat or other cereals whole grain products – wholemeal flour and its products. From the nutritive point of view these products are very beneficial, but there is a big question concerning what to do with wheat infected by field moulds (BAMBURG et al., 1969; SCHNÜRER, 1991).

2.2. Technological quality of wheat and flour

2.2.1. Trade quality. Besides wheat infected by field moulds not being healthy and safe for human nutrition, its technological quality is decreased (ŠARIĆ et al., 1973; BOYACIOGLU & HETTIARACHCHY, 1995) and in some cases wheat becomes completely useless for further processing. Data presented in Tables 2, 3 and 4 give proof of these statements.

A high share of kernels infected by field moulds, particularly much-infected kernels, cause a significant decrease of test weight (Table 2) which is confirmed by high F-distribution value.

Table 1

Infection of investigated wheat samples by moulds and some mycotoxins

Locality	Wheat variety	No. of moulds per kernel	Moulds (genus/group)	Mycotoxins (µg/kg)		
				AB1	OA	F2
	Rodna		*Mucor* spp,	–	–	–
A		1.7	*Fusarium* spp.			
			Alternaria spp.			
A	Proteinka		*Fusarium* spp.	–	20–40	–
		3.0	*Dematiaceae*			
B		2.3	*Mucor* spp.	–	–	–
	Pobeda		*Mucor* spp.			
A			*Fusarium* spp.			
		2.3	*Dematiaceae*	–	–	–
			Aspergillus spp.			
	NS-rana5		*Mucor* spp.			
A		4.0	*Fusarium* spp.	–	–	–
A	Milica	2.7	*Fusarium* spp.	–	20–40	–
B		1.7	*Mucor* spp.	–	–	–
A	Danica		*Fusarium* spp.			
		2.3	*Penicillium* spp.	–	traces	traces
	Slavija		*Fusarium* spp.			
A		3.8	*Dematiaceae*	–	16.0	–
B		1.9	*Rhizopus* sp.	–	11.4	48
	Dicna		*Mucor* spp.	–	20–40	–
A		2.5	*Dematiaceae*			
	Studenica		*Mucor* spp.			
A		2.2	*Penicillium* spp.	–	–	48
			Dematiaceae			
B		1.9	*Aspergillus* spp.	–	11.4	48
	Evropa 90		*Mucor* spp.			
A		2.7	*Fusarium* spp.		8.5	

AB1: aflatoxin B1; OA: ochratoxin A; F2: zearalenone (F2)

Table 2

Trade quality of sound wheat samples and those infected by Fusarium

Wheat variety	Locality[a]	Test weight (kg/hl)	1000 kernel weight (g)	Protein (% d.m.)
	A	80.10	27.6	12.1
Rodna	B	79.10	30.2	12.2
	C	85.70	35.4	16.2
	A	79.70	33.7	14.5
Proteinka	B	78.25	36.2	13.2
	C	84.10	32.9	15.6
	A	78.70	28.6	14.0
Pobeda	B	77.25	35.8	11.7
	C	84.50	36.8	15.0
	A	75.45	27.1	13.2
NS-rana 5	B	75.85	31.9	11.9
	C	84.10	40.2	14.5
	A	78.70	30.7	12.8
Milica	B	77.85	39.4	12.8
	C	84.90	36.8	15.8
	A	79.30	30.3	12.9
Danica	B	77.85	32.2	12.8
	C	84.90	37.4	14.8
	A	77.05	28.0	13.4
Slavija	B	76.45	32.0	11.2
	C	84.50	34.3	13.9
	A	77.45	28.6	12.2
Dicna	B	75.85	31.6	12.3
	C	84.90	40.3	14.6
	A	78.90	30.3	10.5
Studenica	B	76.85	34.2	11.7
	C	83.30	33.4	14.5
	A	78.05	29.7	11.9
Evropa 90	B	76.65	32.0	11.8
	C	83.70	34.6	13.8
Statistics: Calculated F-distribution		129.3	18.95	26.00
F-distribution for the probability 0.05 and $r_1=2$; $r_2=27$ degree of freedom		3.36	3.36	3.36

[a] A and B localities with frequent occurrence of mouldy grains C locality producing sound grain

"1000 kernel weight", as an indicator of milling properties, depends on weather conditions and on infection by field moulds, too. The data in Table 2, clearly indicate, that a high share of kernels infected by moulds, present in an average sample, causes a decrease of 1000 kernel weight.

Almost all varieties from the localities A and B exhibited protein decreases between 0.5% and 4.1% in comparison with the locality C (Table 2) and concerning F-distribution it is confirmed to be significant.

According to BECHTEL et al., (1985) by microscopic inspection of kernels little infected by *Fusarium* the greatest number of mould hyphae is detected in the seed coat and in the aleurone layer but there is a considerably smaller number in the endosperm. The degradation of proteins from the aleurone layer leads to the protein decrease.

If moulds of *Fusarium* spp. infect wheat in earlier stages of development, earlier maturation is caused, kernels are not filled properly and much-infected kernels exhibit endosperm and aleurone layer degradation, i.e. deterioration of proteins and starch. On account of the carbohydrate components decrease (moulds are decomposing or using them), the protein share is increased in the grain. Higher protein content is influenced by an increased number of hyphae whose biomass comprise over 40% of proteins (BOYACIOGLU & HETTIARACHCHY, 1995).

2.2.2. Dough rheology. According to BOYACIOGLU & HETTIARACHCHY (1995) there is a difference in the protein content between sound, little- and much-infected wheat, as well as in the share of protein fractions. A distinct decrease of gluten share in the total proteins, of about 80%, is found. Also, it causes a considerable deterioration of the protein quality and flour, especially if it is taken into account that the glutenine fraction influences dough elasticity (ŠARIĆ & SEKULIĆ, 1981).

Predicted gluten quality decreases of wheat infected by *Fusarium* can be proved using apparatus which detect dough physical properties, i.e. farinograph and extensigraph.

Gluten damaged by the action of mould enzymes exert smaller absorption, from the localities A and B, which is confirmed by high F-distribution values (Table 3).

From localities A and B, quality according to the farinograph is in almost all samples, inferior in comparison to locality C (Table 3). The reason again is protein degradation (ŠARIĆ & SEKULIĆ, 1981).

Along with the increase in mould numbers in the kernel, i.e. along with a more severe infection by field moulds, the 15 min drop is increased (Fig. 1) and the farinograph curve is changed (Fig. 2) and therefore wheat variety is classified into an inferior group of quality (ŠARIĆ & SEKULIĆ, 1981; Table 3).

Extensigraph detects the quality deterioration too. Distinct differences in areas between sound (locality C) and infected wheat (localities A and B) are evident (Fig. 3, 4).

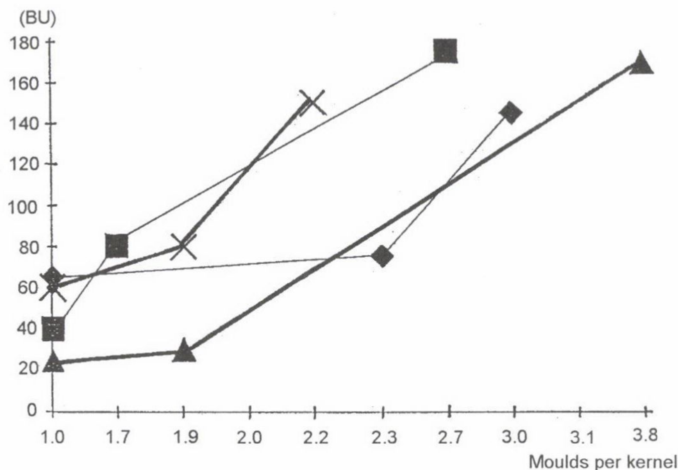

Fig. 1. Influence of the degree of wheat contamination by field moulds on farinographic relaxation value, ■: Proteinka; ♦: Milica; Δ: Slavija; X: Studenica

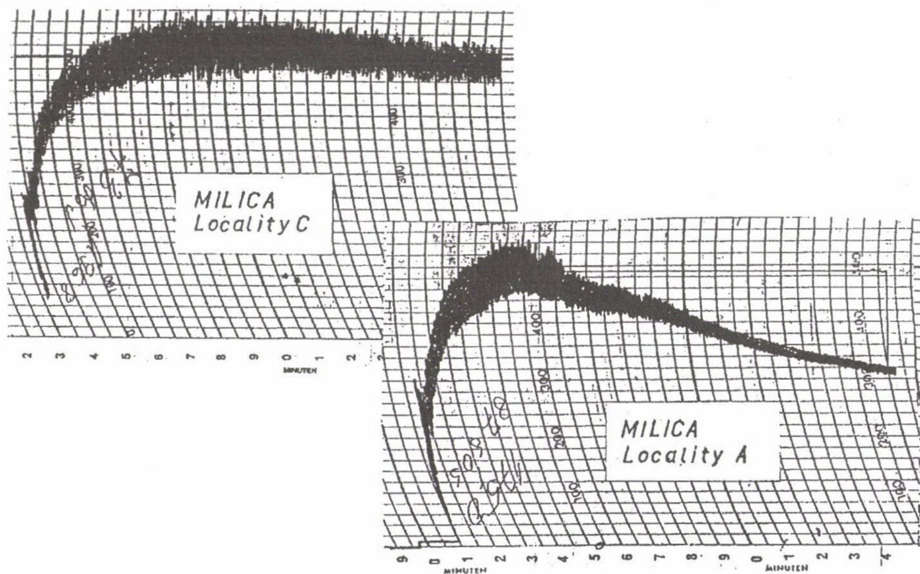

Fig. 2. Typical farinograms of sound (C) and infected wheat (A)

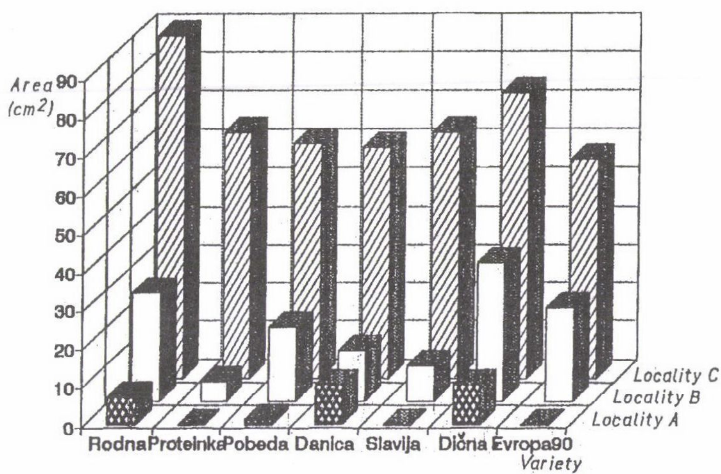

Fig. 3. Extensigraph area (cm^2) of sound (C) and infected wheat (A and B)

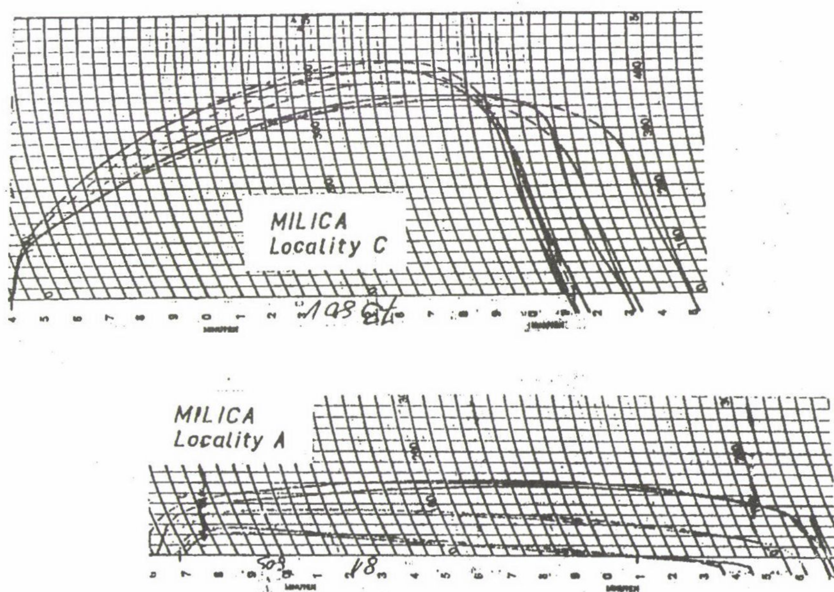

Fig. 4. Typical extensigrams of sound (C) and infected wheat (A)

Table 3

Farinograph data of sound and infected wheat

Wheat variety	Locality[a]	Absorption (% 13% m.b.)	Number of quality	Quality class
Rodna	A	57.7	70.5	A2
	B	58.3	80.8	A2
	C	66.3	88.0	A1
Proteinka	A	64.9	48.8	B2
	B	63.9	76.7	A2
	C	68.0	71.0	A2
Pobeda	A	62.4	74.0	A2
	B	60.4	77.7	A2
	C	66.3	81.7	A2
NS-rana 5	A	56.0	81.7	A2
	B	58.6	68.0	B1
	C	65.1	73.1	A2
Milica	A	58.8	40.2	C1
	B	60.1	68.8	B1
	C	65.5	77.7	A2
Danica	A	59.7	71.9	A2
	B	59.7	81.7	A2
	C	63.0	86.9	A1
Slavija	A	59.1	45.1	B2
	B	59.1	88.8	A1
	C	67.8	87.5	A1
Dicna	A	56.0	67.8	B1
	B	57.9	66.2	B1
	C	65.9	74.6	A2
Studenica	A	56.5	43.8	C1
	B	61.0	71.6	A2
	C	63.6	73.1	A2
Evropa 90	A	57.9	56.0	B1
	B	58.4	65.7	B1
	C	63.3	77.4	A2
Statistics: Calculated F-distribution		26.70	9.24	
F-distribution for the probability 0.05 and r_1=2; r_2=27 degree of freedom		3.36	3.36	

[a] A and B localities with frequent occurrence of mouldy grains, C locality producing sound grain

Table 4

Baking quality of sound (C) and infected wheat (A and B)

Wheat variety	Locality	Yield of bread (g)	Yield of volume (cm^3)	VBS*
	A	131.4	532	6.1
Rodna	B	133.4	477	4.5
	C	140.6	756	6.6
	A	137.0	373	0.5
Proteinka	B	–	–	–
	C	139.3	634	7.0
	A	135.6	460	2.1
Pobeda	B	135.2	443	4.5
	C	139.7	618	6.8
	A	132.3	455	2.0
NS-rana 5	B	134.4	491	3.4
	C	140.1	596	6.6
	A	132.6	356	0.4
Milica	B	–	–	–
	C	137.8	657	7.0
	A	134.3	476	4.1
Danica	B	135.4	448	2.3
	C	138.0	568	5.9
	A	–	–	–
Slavija	B	–	–	–
	C	140.2	646	6.6
	A	–	–	–
Studenica	B	–	–	–
	C	136.5	584	5.1
	A	131.0	460	3.5
Dicna	B	132.6	460	3.1
	C	140.3	586	6.5
	A	133.9	372	1.0
Evropa 90	B	133.1	401	1.1
	C	138.4	534	5.1
Statistics: Calculated F-distribution		6.57	11.77	31.73
F-distribution for the probability 0.05 and $r_1=2$; $r_2=27$ degree of freedom		3.44	3.44	3.44

VBS*: numerical values for crumb quality, grain score and crumb elasticity, maximum 7.0, minimum 0

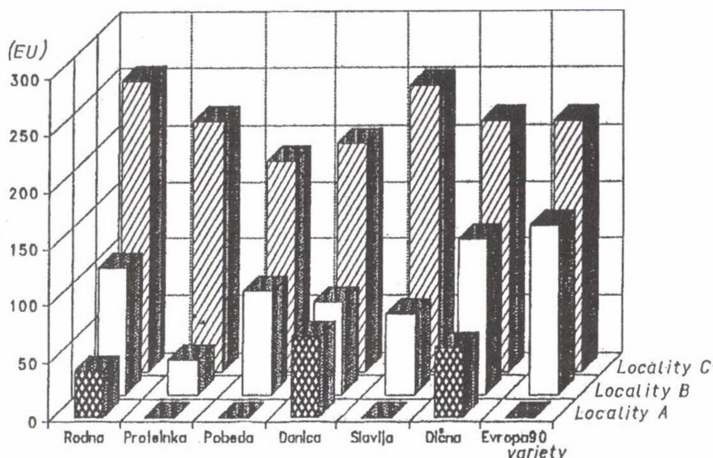

Fig. 5. Resistance to dough stretching of sound (C) and infected wheat (A and B)

Figure 5 indicates that a resistance to dough stretching is significantly smaller in the samples from localities A and B in comparison to locality C. Concerning locality A, resistance to dough stretching was not possible to be determined in 4 wheat varieties.

Rheological properties expressed through farinograph and extensigraph data indicate a gluten quality decrease as a result of the activity of mould proteolytic enzymes (ŠARIĆ & SEKULIĆ, 1981). But, bearing in mind that moulds use carbohydrates as sources of energy, they perform their activity through amylolytic enzymes degrading starch too (Fig. 6).

The amylolytic activity is intensified in both fractions of kernels either little- or much-infected by *Fusarium*, especially in much-infected wheat, as stated earlier (ŠARIĆ & SEKULIĆ, 1981).

2.2.3. Baking quality. Negative changes in wheat structure caused by field mould infection leads to a baking quality deterioration. Parallel to a decreased absorption, the yield of bread is also decreased in comparison to sound one (Table 4). Because of a degraded protein structure, i.e. gluten, and a smaller capacity of gas retention, the volume yield is inferior. Infected wheat exhibited poor bread quality because of a decreased elasticity and more coarse pore structure which is confirmed by high F-distribution values for baking data (Table 4). Those varieties like Proteinka, Milica, Slavija and Studenica, were fully damaged, so bread could not be baked at all.

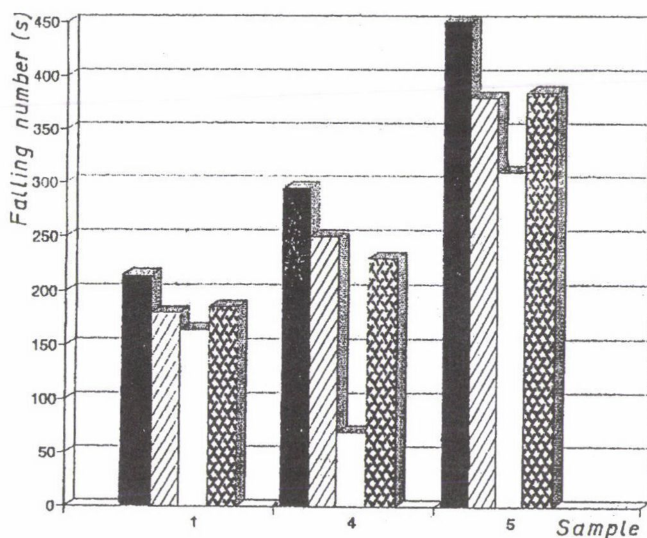

Fig. 6. Amylase activity of wheat depending on wheat contamination by *Fusarium*. ■: Sound kernels; ▨: little infected; □: much infected; ▩: average sample

3. Conclusions

Infection by field moulds, especially *Fusarium* spp. and *Alternaria* spp. more or less decreased wheat quality depending on the share of infected kernels and how big the infection was.

The category of kernels infected by moulds is an open question: whether to classify them as sick wheat or to put a new paragraph into regulations, which would limit the allowed quantity of these kernels.

Increased enzyme activity of moulds in those samples having a higher share of kernels much infected by moulds has negative impact on physical dough properties which may lead to a complete wheat uselessness for further processing.

Wheat portions with a high share of fusarious kernels should not be used as raw materials for processing, because of multiple harm consequences.

Concerning wheat, flour and bread, regulations should precisely define the question of sanitary safety, particularly for wholemeal flour and wholemeal products; it is necessary to establish limits for allowed quantities of microorganisms and their metabolites.

Systematic visual, technological, sanitary and toxic control at all receipts in the milling industry, as well as in store houses next to either agriculture organizations or private store houses, would be very important because of getting a complete insight into wheat usefulness as raw material for human food production.

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Short communications

PRODUCTION OF THE MYCELIUM OF SHIITAKE (*LENTINUS EDODES*) MUSHROOM AND INVESTIGATION OF ITS BIOACTIVE COMPOUNDS

L. LELIK[a], GY. VITÁNYI[a], J. LEFLER[a], J. HEGÓCZKY[b], M. NAGY-GASZTONYI[b] and G. VERECZKEY[b]

[a] University of Horticulture and Food Industry, Central Laboratory, H-1118, Budapest, Villányi út 35. Hungary
[b] Central Food Research Institute, Department of Bioengineering, H-1111, Budapest, Budafoki út 59. Hungary

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Mushroom cultivation embodies the principles of microbiology, environmental technology, and solid state fermentation in the conversion of domestic, agricultural and industrial organic waste materials into food for humans. Because of the large demand for edible mushrooms as a food and as a flavouring agent, there is research interest in attempting to produce flavour-rich mushroom mycelium in submerged fermentation. In addition to its use as food, *Lentinus edodes* (Shiitake) has been reported to have a number of beneficial health and medical effects.

The aim of this work was to find an economical method to produce Shiitake mycelium in submerged culture, and then to study whether the bioactive compound (eritadenine) and the flavour components (the main component lenthionine) were produced by *Lentinus* mycelium.

Lentinus edodes mycelium was successfully produced in submerged culture (biomass productivity was 1.0–1.2%) and a considerable reduction could be achieved in the time required for mushroom production as compared to the traditional technologies.

On the basis of the GC-MS measurements it was proved that eritadenine, the bioactive compound was produced in the Shiitake mycelium. Based on NBS library data lenthionine, trithiolane and other aroma compounds were successfully identified in the biomass and in the fruiting body as well.

Keywords: edible white-rot fungi, fermentation, bioactive compounds, volatile compounds, gas chromatography – mass spectrometry

Mushroom biology is a new discipline concerned with the scientific study of mushroom. It includes not only the principle and practice of mushroom cultivation, but

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also deals with other aspects of mushrooms, such as for example nutrition, medicinal and tonic attributes.

The consumption of edible fungi as food and drugs is closely related to the history of mankind. Shiitake, *Lentinus edodes* has been cultivated for 2000 years, first in China and later on in Japan. In addition to its use as food, *Lentinus edodes* has been reported to have a number of beneficial health and medical effects (antiviral and antitumor activities). Concentrated extracts of it are sold as a natural remedy – the hypocholesterolemic principle of Shiitake was proved (KIM et al., 1992) and this effect was accounted for the lentinacin (eritadenine) 2,3-dihydroxy-4-(9-adenyl)butyric acid content in the fruiting body.

The fresh mushroom exhibits only a slight odour, but upon drying and/or crushing, a characteristic sulphurous aroma gradually develops. CHEN and HO (1986) identified 18 sulphur-containing volatile compounds. One of them, lenthionine, a cyclic S compound is known to possess the characteristic aroma of this mushroom. Lenthionine was reported to be enzymatically produced from lenthinic acid in dry mushroom. It is also worthy of note that lenthionine shows fairly strong antibiotic activities against a number of microorganisms including bacteria and fungi (YASUMOTO et al., 1971).

Shiitake growing on artificial logs in the USA and Canada is almost independent of outside climatic conditions, with modern structures and equipment providing conditions most favourable to the mushrooms during the various phases of the growing cycle. Artificial media also have been used to obtain mushrooms, the substrate usually consists of a mixture of woodchips and cereal bran. The other way of mushroom production is the application of a liquid medium enriched with nutrients. Submerged cultures of edible fungi have two main applications, as starter cultures (the liquid spawn) for inoculating growth substrates and as food or flavour components (the biomass) in food products.

The aim of the present work was to prepare a well homogenized liquid spawn for inoculation and to develop an economical method to produce Shiitake mycelium in submerged liquid cultures. Authors also studied whether the bioactive compound – eritadenine – and the flavour components (first of all lenthionine) were produced by *Lentinus* mycelium.

1. Materials and methods

1.1. Strains

Lentinus edodes Le 3343, a stock culture belonging to DSM (Germany) and *Lentinus edodes* Le-4 a stock culture from Slovakia were used.

1.2. Culture conditions

For the inoculation solid spawn (150–150 g wheat, barley or millet was cooked, 0.1% yeast extract was added and filled in Erlenmeyer flasks) and liquid medium (components: yeast extract, glucose and minerals) were used. Shiitake mycelium was produced in shaken flasks and in fermenters – BIOSTAT, B. Braun and BIOFLO III-Batch/Continuous Fermenter. For the shaken fermentation the medium consisted of malt extract and yeast extract, the conditions were as follows: stirring 100–150 r.p.m., temperature 25 °C, incubation time two weeks. The pH was adjusted to 4.7 in all case at the beginning of the fermentation. The fermenter – BIOSTAT – was filled with 8 dm^3 medium, the BIOFLO III was filled with 2.5 dm^3 liquid medium, which consisted of malt extract and yeast extract in various concentrations. The temperature of the fermentation was 25 °C, stirring 150 r.p.m., aeration 0.5 $dm^3/dm^3/min$, cultivation time was 8–10 days.

Dry weight of mycelium: Broth from the shaken flasks and the fermenter was filtered and was dried at 105 °C overnight and weighed.

Glucose measurements: Glucose concentration was measured with the method of Somogyi-Nelson.

1.3. Sample preparation

Prior to measuring the eritadenine content of the samples the following procedure was performed – extraction with 80% ethanol, ion-exchange chromatography (Amberlite IR-45, Amberlite IR-120), production of derivative.

Prior to measuring the sulphur-containing compounds by means of GC-MS technique, the adequate extractive method was selected. The flavour components were extracted by a mixture of *n*-hexane-ethanol 9:1.

1.4. Analytical procedure

Instrumentation: Identification of the above mentioned compounds were conducted on a HP 5890 gas chromatograph (GC)-Hewlett Packard, Avondale, USA – coupled through a direct capillary interface to a VG TRIO-2 quadruple mass spectrometer (VG-Masslab, Altrincham, Great-Britain).

GC conditions for the eritadenine measurements: The GC was equipped with a fused silica capillary column HP Ultra-2, 5% Ph-Me Silicone- (25 m×0.32 mm× 0.52 μm) and with a split/splitless injector. The injector temperature was 270 °C and the temperature of the oven was programmed: the initial temperature was 200 °C, then at 8 °C min^{-1} to 310 °C and held for 5 min. The transfer line was 280 °C.

GC conditions for the sulphur containing compounds: The GC was equipped with a fused silica capillary column HP-1, (25 m×0.3 mm×0.32 μm), poly-dimethyl-

xiloxane chemically bounded stationary phase. The carrier gas was pure He, with a flow rate of 1 cm^3 min^{-1}.

Injector temperature was 270 °C, interface temperature was 270 °C.

MS conditions: EE: 70 eV, (EI+) and full SCAN mode were applied. Data acquisition and instrument control was performed by a PDP 11/53 microcomputer.

2. Results

Lentinus edodes mycelium was produced successfully in submerged liquid culture and a considerable reduction could be achieved in the time required for biomass production as compared to the traditional technologies. The morphology of the mycelium was a pellet form in the batch culture. The biomass productivity (dry weight of mycelium) was 1.0–1.2% in the shaken flasks (after 14 days incubation time) and 0.8–1.0% in the fermenter (after 10 days incubation time).

Glucose concentration reduced upto 72 h incubation time in the fermenter (at the beginning the glucose concentration was 2.2% and after 72 h it was 1.5%) and then this value did not change to a large extent.

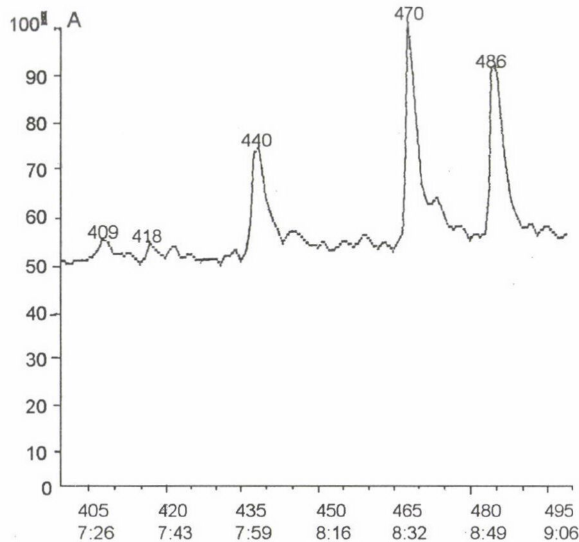

Fig. 1. Total ion chromatogram of Shiitake mycelium, the peak number 470 corresponds to eritadenine

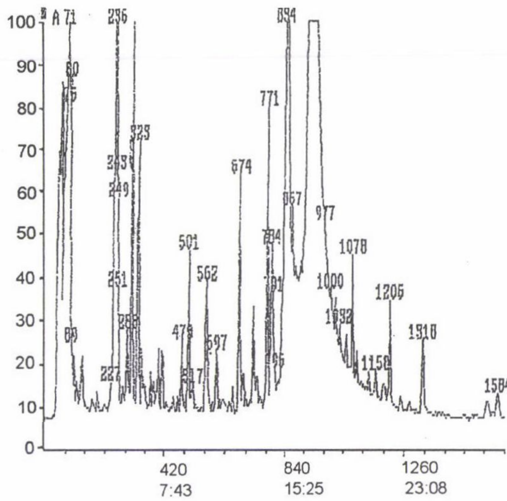

Fig. 2. Total ion chromatogram of aroma components in Shiitake mushroom, peak number 674 means the lenthionine in the fresh mushroom

Results concerning the bioactive compound eritadenine and the cyclic volatile compounds were the followings: on the basis of the GC-MS measurements we proved that eritadenine was produced in the mycelium too (not only in the fruiting body), the amount of the bioactive compound was 73.7 mg 100 g^{-1} in the Shiitake mycelium. Figure 1 shows the total ion chromatogram of the Shiitake mycelium, the peak number 470 corresponds to eritadenine.

Based on NBS library data lenthionine, trithiolane and other aroma compounds were successfully identified in the mycelium and in the mushroom as well. Shiitake mushroom is rich in various aroma components as it can be seen on the Fig. 2. The peak number 674 means the lenthionine in the fresh mushroom.

The volatile compounds of the Shiitake biomass produced in submerged culture are partly shown in Fig. 3. Peak 663 means the lenthionine in the mycelium.

3. Conclusions

The presence of the bioactive compound eritadenine was proved in the Shiitake mycelium as well, and it opens an opportunity to apply the Shiitake biomass as a healthy food ingredient to spices, sauces, etc. For this purpose we would like to work out the technology for preservation of Shiitake mycelium and to prepare a final product

for the market. Furthermore we want to set the fermentation parameters in order to increase the biomass productivity and to get an economic process too.

SAITO and YASUMOTO (1975) measured the eritadenine concentration in several species of edible mushrooms, and their results indicated that only Shiitake contained eritadenine at relatively high levels, 60–70 mg 100 g^{-1} in the cap and 40 mg 100 g^{-1} in the stem.

Summarizing the results the edible mushroom Shiitake was produced under controlled circumstances in liquid submerged culture, the biomass (mycelium) production can be performed the whole year around this way. The studies of SONG and CHO (1987) has also proved that the growth rate of *Lentinus edodes* can be increased five-fold by using airlift fermenter. Additionally this biomass contains the bioactive compound eritadenine too, its beneficial, hypocholesterolemic effect was proved by many researchers.

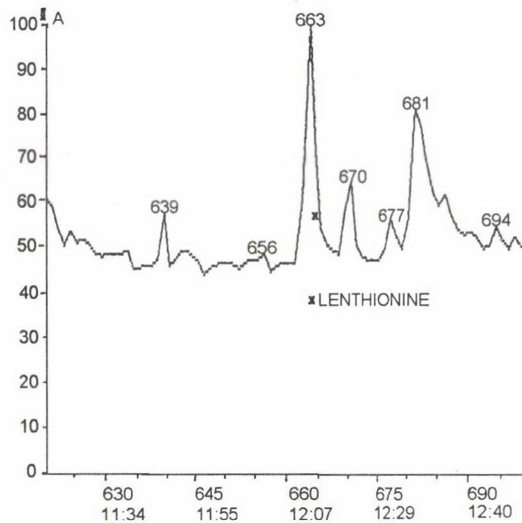

Fig. 3. Total ion chromatogram of aroma components in Shiitake biomass, peak number 663 corresponds to lenthionine

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FUNCTIONAL PROPERTIES OF THE ENZYMATICALLY MODIFIED SOYA PROTEIN ISOLATE

E. SÜLE[a,b], GY. HAJÓS[a] and S. TÖMÖSKÖZI[b]

[a] Department of Biochemistry II, Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary
[b] Department of Biochemistry and Food Technology, Technical University of Budapest, H-1111 Budapest, Műegyetem rkp. 3. Hungary

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Proteolytic modification reactions are mostly suitable for inducing conformational change, that may result in modification of the functional properties of proteins. In this work the effects of the peptic, α-chymotryptic, papaic modification and the effective covalent Met-enrichment by enzyme-catalysed process (EPM) on the functional properties of soya protein isolate were investigated.

The foam activity showed better results in case of the enzymatically modified soya protein isolate products compared to the untreated protein isolate while the stability remained the same. The emulsifying properties of the modified products did not improve.

Keywords: soya protein isolate, enzymatic modification, EPM, functional properties

Plant proteins, the major source of proteins for the majority of world's population, are usually deficient in one or more of the essential amino acids. For example, the limiting essential amino acid of legume seeds (e.g. soybean) is the methionine.

To improve the functionalities of proteins, chemical modification has been extensively studied (JOHNSON & BREKKE, 1983; KIM & KINSELLA, 1987). Chemically modified proteins in foods will have to clear regulatory safety guidelines. In contrast, enzyme-modified proteins are not likely to be of safety concern, and thus enzymatic modification may be a viable alternative to chemical modification.

During enzymatic modification, L-amino acids are partially incorporated into the peptide chains of a protein hydrolysate (FUJIMAKI et al., 1977). Covalent amino acid enrichment of peptides can improve their biological value and modify their functional properties. Enzymatic peptide modification of proteins (EPM) (HAJÓS et al., 1988, 1996) may alter their net charge, hydrophobicity, their structure, surface activity and thus might induce improvement in their functional properties (LUDWIG et al., 1995; SÜLE et al., 1995).

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Aims of this study were the followings:

– Preparation of soya protein isolate,

– Proteolytic modification of soya protein isolate,

– Covalent attachment of methionine to peptides of hydrolysates of soya protein isolate during EPM reaction,

– Investigation of subunit degradation and changes in the molecular mass of peptides during proteolytic treatment,

– Improvement of functional properties of the enzymatically modified soya protein isolates.

1. Materials and methods

1.1. Materials

1.1.1. Substrates:

– *Bovine casein* (Reanal, Budapest, Hungary)

– *Soybean:* type: "Borostyán" source: Bolyi Agric. Ltd Hungary

– *Soya protein isolate* (SPI) was obtained by alkaline extraction (WISSER & THOMAS, 1987).

pH:	8.0
extraction time:	2×60 min
centrifugation:	5000 r.p.m., 30 min
dialysis:	24 h, at 4 °C
freeze-drying:	by lyophilization (Instrument: Christ Alpha 1–4)

1.1.2. Enzymes:

α-chymotrypsin (5 Umg^{-1})	(Sigma) EC 3.4.21.1
trypsin (6 Umg^{-1})	(Serva) EC 3.4.21.4
pepsin (20 mAnson)	(Calbiochem) EC 3.4.23.1
papain (1 $MCUmg^{-1}$)	(Calbiochem) EC 3.4.22.2
carboxypeptidase A (50 Umg^{-1})	(Serva) EC 3.4.17.1
carboxypeptidase B (50 Umg^{-1})	(Serva) EC 3.4.17.2

1.1.3. Modified proteins:

1.1.3.1. Hydrolysates – Soya protein isolate was hydrolysed with α-chymotrypsin and trypsin at pH 7.5, with pepsin at pH 2.0, and with papain at pH 6.0, respectively. The incubation was carried out at 37 °C for 2 h. The reaction mixture was stirred during hydrolysis and then freeze-dried.

1.1.3.2. EPM products – The concentration of hydrolysates was 20% w/v in the EPM reaction. A methionine ethyl ester : substrate ratio of 1:5 was used for the incorporation in the enzyme catalysed reaction with amino acid enrichment. After incubation, the products with methionine incorporation were simultaneously dialysed for 1.5 days through a cellophane membrane against distilled water then freeze-dried.

1.2. Methods

1.2.1. Carboxypeptidase treatment. A suspension of the EPM product (5 mg) in 100 μl 0.1 mol NH_4HCO_3 buffer was incubated with a mixture of carboxypeptidase A (1 μl) and carboxypeptidase B (1 μl). Samples (10 μl) were investigated after incubating for 0, 10, 30, and 60 min, respectively, and the samples were immediately mixed with an equal volume of conc. HCl to stop the enzyme reaction. Free amino acids were separated by thin layer ion-exchange chromatography.

1.2.2. Electrophoretic and chromatographic methods

1.2.2.1. SDS-PAGE – The molecular mass distribution of protein fractions was investigated by SDS-PAGE according to DELINCÉE & HAJÓS, (1984). The protein zones were stained with Coomassie Brilliant Blue.

1.2.2.2. Thin-layer ion exchange chromatography – Free amino acids were separated by thin-layer ion exchange chromatography (DÉVÉNYI, 1976). A Biotec Fischer videodensitometer was used for quantitative determination.

1.2.3. Methods for determination of functional properties

1.2.3.1. Foam properties (foaming activity index (FPI), foaming stability index (FSI)) were measured using the method of TÖMÖSKÖZI and PUNGOR (1993).

1.2.3.2. Emulsifying properties emulsifying activity index (EAI) and emulsifying stability index (ESI)) were determined by the method of PEARCE & KINSELLA (1978) and of KATO and co-workers (1985) respectively.

2. Results

The electrophoretic patterns of protein fractions of the soya protein isolate, and the tryptic, peptic, α-chymotryptic and papaic hydrolysates separated by SDS-PAGE are shown in Fig. 1. The distribution in the molecular weight of proteins during tryptic hydrolysis does not change because of the presence of trypsin inhibitors in the soya protein isolate. Therefore the tryptic hydrolysate was not further investigated.

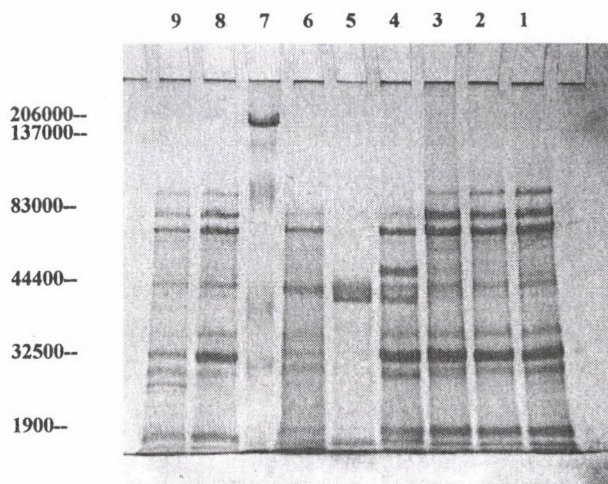

Fig. 1. Molecular mass distribution of different soya protein samples separated by SDS-PAGE. 1: Defatted soya flour; 2: soya protein isolate; 3: tryptic hydrolysate of soya protein isolate; 4: α-chymotryptic hydrolysate of soya protein isolate; 5: peptic hydrolysate of soya protein isolate; 6: papaic hydrolysate of soya protein isolate; 7: standard; 8: soya protein isolate (SPI); 9: residue of isolation

The effect of carboxypeptidase on samples are shown in Fig. 2. In sample taken at 0 min, where HCl was added together with exopeptidase to prevent digestion only a small amount of methionine was detectable. This finding proved, that both the excess methionine ethyl-ester and the free methionine were removed from the reaction mixture during dialysis. In samples obtained after 10–60 min, increasing amounts of free amino acids were detected as a results of exopeptidase reaction. It is seen in Fig 2. that methionine was covalently incorporated into the proteolysates of soya protein isolates during an enzymatic peptide modification process in the presence of pepsin.

Functional properties of the following products were measured: casein as a control (casein has very good functional properties), soya protein isolate, α-chymotryptic, papaic, peptic hydrolysates of SPI and EPM (Met-enriched product of peptic hydrolysate prepared by pepsin catalysis). Foam properties and emulsifying properties of the samples are presented in Figs. 3 and 4. Both emulsifying properties decreased during enzymatic treatment of soya protein isolate. The emulsifying stability of soya protein isolate was very low compared to casein. The peptic hydrolysate and EPM product has not emulsifying stability at all. Foaming activity increased in case of peptic hydrolysate and of EPM product. The foaming activity of these samples is essentially better, than that of the casein. The α-chymotryptic and papaic hydrolysis did not significantly alter the foaming activity. The enzymatic treatment, except for α-chymotryptic hydrolysis, reduced foaming stability.

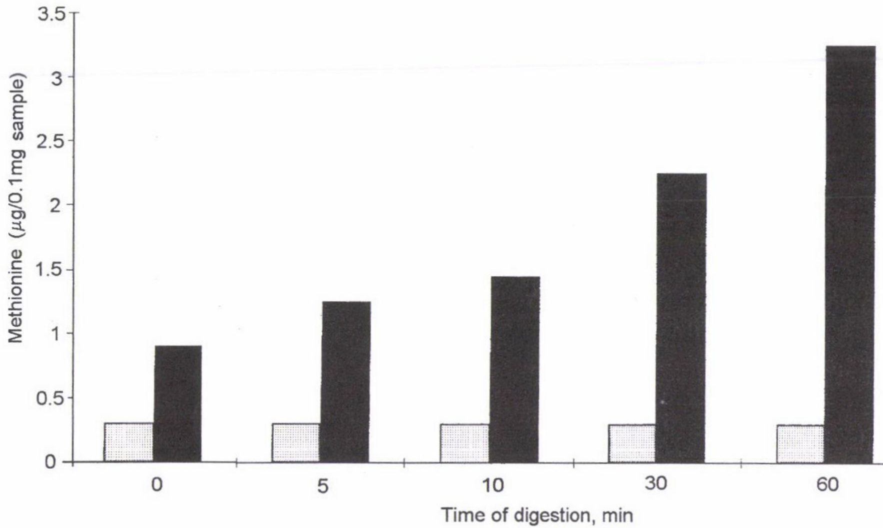

Fig. 2. Determination of methionine incorporation into the C terminals of the enzymatically modified soya protein isolate by carboxypeptidase treatment. The samples are: peptic hydrolysate of SPI (Soya protein isolate) and EPM (Met-enriched product of peptid hydrolysate prepared by pepsin catalysis). ▒: Peptic hydr. of SPI; ■: EPM

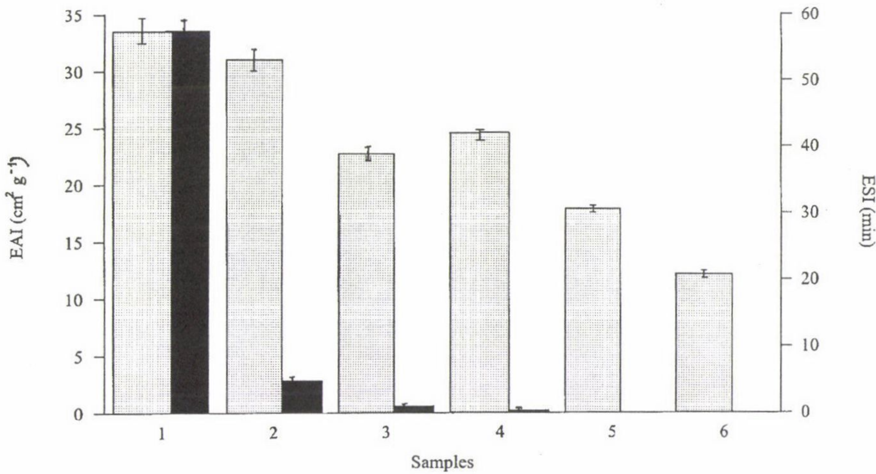

Fig. 3. Emulsifying activity by turbidimetric method (PEARCE & KINSELLA, (1978) and stability by conductometric method (KATO et al., 1985) of soya protein isolate (SPI) and enzymatically modified products. 1: casein; 2: soya protein isolate (SPI); 3: α-chymotryptic hydrolysate of SPI; 4: papaic hydrolysate of SPI; 5: peptic hydrolysate of SPI; 6: peptic catalyzed EPM product with Met-enrichment from peptic hydrolysate of SPI. ▒: Emulsifying activity index (EAI, $cm^2\ g^{-1}$); ■: Emulsifying stability index (ESI, min)

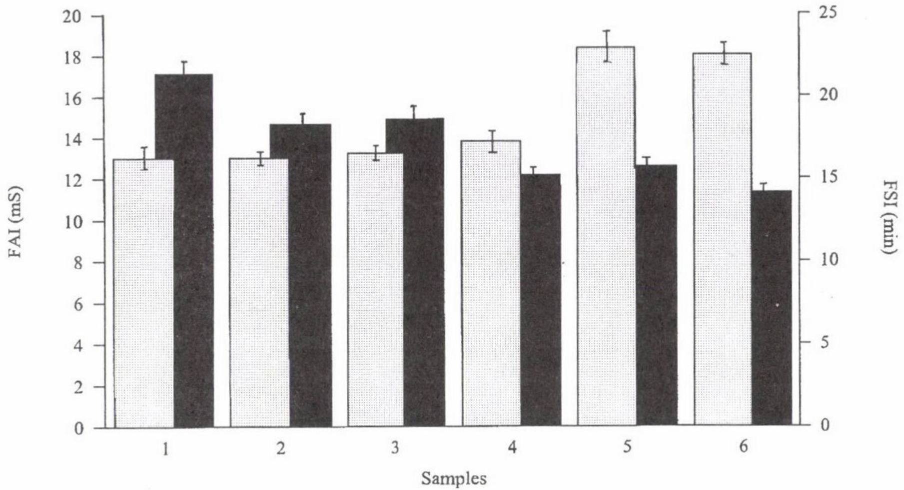

Fig. 4. Foam properties of soya protein isolate (SPI) and enzymatically modified products by conductometric (TÖMÖSKÖZI & PUNGOR, 1993) method. 1: casein; 2: soya protein isolate (SPI); 3: α-chymotryptic hydrolysate of SPI; 4: papaic hydrolysate of SPI; 5: peptic hydrolysate of SPI; 6: peptic catalyzed EPM product with Met-enrichment from peptic hydrolysate of SPI. ▒: Foam activity index (FAI, mS); ■: Foam stability index (FSI, min)

3. Conclusion

These results suggest that the enzymatic hydrolysis and the enzymatic peptide modification with covalent Met-enrichment might be a suitable way for improving the functional properties, mainly the foam activity of soya protein isolate together with improvement of their biological and nutritional values.

*

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ABSTRACTS
of papers presented at the
"VAS KÁROLY" SCIENTIFIC SESSION

organized by
THE UNIVERSITY OF HORTICULTURE AND FOOD INDUSTRY

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Budapest, Hungary

SECTION OF FOOD SCIENCE

RAPID AND NON-DESTRUCTIVE TEST METHODS FOR FOODS

A. FEKETE

Physics-Control Department, University of Horticulture and Food Industry, H-1118 Budapest, Villányi út 35. Hungary

Physical, chemical and microbioligical test methods are used for the assessment or the monitoring of food quality characteristics. Lately rapid and non-destructive test methods are more and more accepted, therefore the importance of the test methods for the measurement of the physical properties of foods are encouraged.

The objective of the work reported herein was to determine the field of application for the test methods as follows: mechanical (by compression or shear), vibrational (by impact, resonance, and acoustics, such as sonic and ultrasonic methods), electric impedance (by specific conductivity and dielectric properties) and by optical methods, such as by shape and size and colour.

Compression test method was developed for the non-destructive measurement of the compression stress with a definite known and limited deformation. The coefficient of elasticity was introduced for the characterization of the foods. A compression test method developed and used for the determination of firmness and ripeness of different apple, apricot, pear, cherry, tomato and cucumber varieties.

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The impact test method was used to determine the ratio of the maximum impact force to the time of the maximum force, to characterize the firmness and ripeness of apples, pears, peaches, etc.

The resonance test method was used to determine the stiffness coefficient of the material tested, as follows:

$$s = -F/x$$

where F: force, N

x: displacement, m

This stiffness coefficient turned out to be a good characteristic of firmness, ripeness and shelf life of the flesh of apples, tomatoes and others.

The sonic test method can be used to measure the transmission of the sound through the material, therefore it is suitable for the assessment of firmness of internal texture of apples, peaches, tomatoes, etc.

The velocity, the attenuation and the reflection of ultrasound can be measured to determine ripeness of apple, avocado and melon and the internal deteriorations, such as hollow hearts especially in potatoes.

The electric conductivity can be used to characterize the moisture content of grain, the number of somatic cells in milk and therefore the infection of the udder, and the nitrogen content in liquid manure. The electric impedance is a good measure of the moisture content of grains and the irradiation of potatoes and milk powder.

Machine vision and image processing was found to be suitable for the identification of cereal, fruit and vegetable varieties. Furthermore the image processing system and the color analysis software have a large field of application, including ripeness assessment of apples and tomatoes and the identification of pepper (paprika) varieties.

The conclusion is that several characteristics of the agricultural materials can be assessed or determined by rapid and non-destructive test methods. These characteristics are as follows: firmness, ripeness, bruise, storage period, internal deteriorations (such as hollow heart), moisture content, variety (according to shape, size and colour), nitrogen content and irradiation.

MACHINE VISION APPLICATIONS TO QUALITY CHARACTERIZATION OF FOOD PRODUCTS

J. FELFÖLDI, F. FIRTHA and E. GYŐRI

Physics-Control Department, University of Horticulture and Food Industry, H-1118 Budapest, Villányi út 35. Hungary

The quality of the products is a complex characteristic determined by a lot of parameters: either physical or other properties. There is a definite need for characterization of the quality on the basis of simple, fast, non-destructive physical parameters. Having an appropriate measuring method this type of parameters can be the visual properties (size, shape, colour) as well. Our objective was to work out methods for recording and processing digitized colour images of the produces and for quantitative characterization of colour and shape.

The machine vision system consists of a 3-chip 1/2" colour video-camera, a 3*8 bit true-time and true-colour image digitizing PC-card and a PC (486 DX4-100). A true-colour bitmap-file of 320*200 pixels was recorded and stored for each sample tested, that is 3 colour components (Red, Green and Blue) of each image-pixel are stored. The images are processed by the software developed for the system. The main task of the image processing are:

– **segmentation** of the object and the background;

– determination of the outline of the object, storage of the polar coordinates (R, φ) of the outline pixels (**outline-function**);

– calculation of the Fast Fourier Transform of the outline-function to characterize the main symmetry properties of the object;

– calculation of the average R, G, B colour components of the pixels belonging to the produce tested.

Method was developed for 2-dimensional, graphical representation of the average colour of the produces. A quantitative, one-dimensional **colour coefficient** was introduced on the base of the graphical representation for objective characterization of the changes in the colour of the produces and for comparison with other, quantitative parameters.

The shape can be characterized on the base of the outline-function. Discriminant analysis of the FFT-components of the outline-function was done to classify the samples or to detect shape-defects.

The system developed was used to characterize the colour of fruits and vegetables (cherry, tomato) in different ripening states, to describe the colour-change during the storage (mushroom), to distinguish different varieties by shape (apple,

onion) and to recognize shape-defects (biscuits). On the basis of the results the machine vision system and the image processing methods are suitable for measurement and objective, quantitative assessment of shape and colour properties of produces and food products.

QUESTIONS OF METHODOLOGY AT MEASURING APPLE COLOURS

J. ZANA and P. LÁSZLÓ

Physics-Control Department, University of Horticulture and Food Industry, H-1118 Budapest, Villányi út 35. Hungary

Testing materials of biological origin is difficult, because the searched properties are varying. In the research there is a problem whether we should test the materials by their original characteristics or we are allowed to pick them for better results in variances.

When testing fruits we find factors causing variance. These effects are:

– the cultivar, sort, or variety;
– the habitat;
– the generation;
– the type of soil, the agricultural technique;
– the weather, the rainfall;
– the tested side is covered by base colour or cover colour;
– the picking time (which varies from year to year);
– the maturity level;
– the transport and storage;
– the position on the tree: on the top, at the axis, at the end of the branch, in shadow or not, at the windy side, at the rainy side;
– the state of the plant;
– cross effect of these (e.g. testing early matured cultivar after bad weather circumstances and testing the other cultivars in the same year among better circumstances).

We have to decide what we want to test. Excluding the samples originating from circumstances that we do not want to test, the data population will be false and will describe only a part of the real population – but we will get better variances.

Results from three different colorimetries were compared. The first samples were tested by a Hungarian three-stimulous colorimeter, the MOMCOLOR 100. We used a

home-built connection to store the data in Commodore-type computers. This sample is to describe the data storing type:

11.5
1.04
23.47
12.32
C

The group of numbers represents the X1, X2, Y and Z colour stimuli, the character represents the type of the next test e.g. it was tested by its flesh colour.

Our next colorimeter is the Ultrascan by Hunterlab, Inc. It works connected to a computer. Its data format is the next:

SAMPLE ID
f1
7/12/1993
STDZ MODE
RSIN/UVL OFF/UVF OUT/SAV
XYZ
17.58
16.62
8.37
CIELab
47.78
6.99
27.22

This form was changed when the measuring software was changed. We tested apples, vegetables and other produces such as tobacco leaves.

When the data were evaluated on the colour triangle we found samples of great saturation at the same cultivar but only in one year (1994). The samples were different in colour, e.g. in 1993 the Jonagold apples were the reddest in hue of the cover colour. The variance of the base colour was much more less. Considering the outliers in the distributions the CIE 1976 CIELAB a^* results were in the same range but they were distributable by their b^* values. Computing the C^* chrome values one generation (1994) was different from the others. To find differences in the lightness the CIELAB proved better than the CIE Y and the COLOROID lightness V was even better for showing the differences in generation.

Testing chrysanthemum leaves we found small variance in hue but great variance in saturation. The distribution of hue values was close to the normal distribution.

When tobacco leaves were tested it was a surprise that there was no difference at all in CIE 1931 Y lightness but at the y colour-co-ordinate the difference was

noticeable. Computing their COLOROID hue *A* we found an increase in variance according to the treatment by metallic component of the fertiliser. The hue was shifted towards the green colours at the top of the plants. Difference cannot be found when tested the hue at the middle of the leaves against to their top.

ACOUSTIC BASED FIRMNESS ASSESSMENT OF FRUITS, VEGETABLES AND FOOD PRODUCTS

J. FELFÖLDI

Physics-Control Department, University of Horticulture and Food Industry, H–1118 Budapest, Villányi út 35. Hungary

The firmness of the raw materials of food industry – especially that of the fruits and vegetables – is an important characteristic for the processing or storage. Generally compression methods – mostly destructive ones – are used to determine the firmness. However the industry and the research work need non-destructive methods making possible the further use or investigation of the individual produces tested.

The acoustic behavior of the produces (mechanical resonance, speed of wave propagation, etc.) depends on the elastic properties. The analysis of the mechanical resonance of the produces offers the possibility of the non-destructive, global assessment of the firmness. Our aim was to develop a measuring system and method suitable for recording and analysis of the acoustic response of the produce, hit with small energy and to characterize the firmness on the base of the acoustic behavior.

The computer based measuring system consists of

– a sensitive microphone with preamplifier and filter unit;

– A/D card to digitize the microphone-signal (PC-LAB 818);

– PC-compatible computer for the measurement control and data assessment.

The software developed for the system is for control of the measurement (sampling and storing the acoustic response signal of the sample with set rate) and for determination of the dominant frequency (f_0). The mass of the sample is also measured and the firmness is characterized as $f_0^{2*}m$ *acoustic firmness coefficient.*

The firmness of different fruits and vegetables was tested with the system. For apricot and tomato varieties correlation was found between the acoustic firmness coefficient and other firmness parameters (*such as the coefficient of elasticity*). For tomatoes the coefficient was found suitable for analysis of the firmness change during the storage and for discrimination between groups of different growing conditions. For apple and apricot significant difference was found between the acoustic firmness coefficients of samples of different ripening states.

Summarizing the results, the firmness-assessment based on the analysis of the acoustic response of the produce was found to be suitable for quick and objective determination of the firmness of fruits and vegetables. Because of the repeatability and non-destructivity the acoustic firmness coefficient is especially suitable for measurement of the firmness change during the ripening or storage and for analysis of the small firmness-differences due to the different methods of storing or processing.

STUDY OF PROTEIN INTERACTIONS

ZS. CSERHALMI and B. CZUKOR

Central Food Research Institute, H–1022 Budapest, Herman Ottó út 15. Hungary

The variety of food protein additives increases step by step. Besides of inself used protein additives of plant and animal origin the use of mixed protein additives came to the front. For their use it is very important to know the protein interactions.

The protein solubility, water- and fat binding ability, emulsifying activity, emulsion stability, gelation, protein surface hydrophobicity and thermal denaturation of five individual protein products of plant and animal origin (soy protein isolate, soy protein concentrate, pea protein isolate, sodium caseinate, meat protein extract from fresh slaughtered pork *L. dorsi*) and their mixes in the rate of 1:1, 1:2, 2:1 were studied. These properties were measured by well known, standard methods.

The solubility of the individual proteins was between 25 and 100%, the water binding ability between 454 and 600%, fat binding ability between 160 and 306%, emulsifying activity between 63 and 98%, emulsion stability between 65 and 94%, surface hydrophobicity between 134 and 424.

The DSC thermograms of the individual proteins showed endothermic peaks at 59 °C and 80 °C in the case of the soy protein products, at 88 °C in the case of pea protein isolate and at 63 °C in the case of meat protein extract.

Among the individual proteins the soy protein isolate and concentrate formed heat-induced protein gels. The hardness was 11 N in case of isolate and 6 N in case of concentrate.

The emulsifying properties, gelation and fat binding ability of mixed protein systems significantly differed from those of the individual proteins. The emulsifying activity of mixed protein systems in some cases was worse than that of the individual proteins. However, the emulsion stability of mixed protein systems was better than that of the individual proteins depending on the mix rates. The fat binding ability of mixed protein systems including meat extract was worse than that of meat extract, but was better than that of soy protein, milk protein and pea protein products. The fat binding

ability of the mixed protein systems including soy, milk and pea protein was worse than that of the individual proteins. The mixed protein systems did not formed heat-induced gels.

The obtained results raise further questions that will be studied in the future.

ANTIGENIC CHARACTER AND PROTEIN STRUCTURE

GY. HAJÓS [a] E. SZERDAHELYI[a], É. GELENCSÉR[a] and M. POLGÁR[b]

[a]Central Food Research Institute, H–1022 Budapest, Herman Ottó út 15. Hungary
[b]Madarász Children's Hospital, H–1131, Madarász V. út 21–24. Hungary

The knowledge of the influence of structure on protein biological functions and properties is essential for their rational use as foods and for the improvement of the safety of food proteins.

Proteins of several foods have been identified as common allergens such as: milk, legumes, eggs, cereals, seafood etc. However, many patients suffering from a given food protein allergy, are often sensitive also to other food proteins. The identification of food allergens is mainly based on clinical observations before and after administration of an allergen-free diet to patients suffering from a given allergy.

The antigenic specificity of proteins in restricted areas of the molecule, known as antigenic determinants or epitopes, which are recognized by the combining sites or paratopes of certain immunoglobulin molecules. The precision of steric and chemical fit between epitope and paratope necessary for achieving this type of immunological recognition is highly variable.

The most common way of classifying epitopes consists in distinguishing continuous (sequential) and discontinuous (conformational) epitopes. The label continuous epitope is given to any short linear peptide fragment of the antigen that is able to bind to antibodies raised against the intact protein. Usually these antibodies cross-react only weakly with the peptide and the continuous epitope identified in this manner is unlikely to mimic exactly the conformation and structure of the corresponding epitope in the intact protein.

It is also concluded that no common universal structural characteristic of the allergenic molecules has been found so far. However, it may be possible to eliminate the allergenic components or to reduce their level by several new food-processing techniques, first of all by enzymatic modification processes.

Enzymatic modification, heat treatment (as an enzyme inactivating step) and separation processes result in a significant reduction of the allergenic character of the proteins. However, there are still problems in obtaining peptides of lower allergenicity.

The first step is the selection of enzymes which attack the antigenic site of the protein. The heat treatment of food proteins leads to the loss of conformational epitopes during denaturation, but proteolysis and enzymatic modification may lead to the loss of sequential epitopes.

An enzymatic peptide modification (EPM) process has been recently elaborated for tailoring peptides and proteins of special nutritional character.

The main aim of research of enzymatic modification process is to formulate high quality proteins mainly from plants to fit healthy lifestyles. For human health and safety aspects the use of proteinases in protein modification should offer better products than those which could be obtained by corresponding chemical modification methods. However, as enzymatic modification leads to conformational changes in proteins, their physicochemical and biological properties will be altered.

The fact that the allergenic character of proteins could be decreased by this EPM method is due probably to the following factors:

– Controlled enzymatic hydrolysis can alter the surface character of proteins, may destroy epitopes and thus may reduce the allergenicity of proteins or peptides.

Therefore, the length of peptide chains in a proteolytic hydrolysate is very important for producing physiologically optimal hydrolysates as peptide-based hypoallergenic infant formulas.

– Modification of the structures of sequential and conformational determinants by transpeptidation leads to a favorable change in the antigenicity of protein chains.

– Covalent incorporation of designed amino acids modifies the structure of the determinant groups of the proteins and prevents the formation of new determinants.

Thus the allergenicity of proteins may be satisfactorily reduced by a designed enzymatic modification of immunogenicity and antigenicity.

SUPERCRITICAL EXTRACTION AND STUDY OF POLYLPHENOLS

K. VÁSÁRHELYI-PERÉDI[a], I. ÉLIÁS[a], V. ILLÉS[b] and O. SZALAI[b]

[a]Central Food Research Institute, H–1022 Budapest, Herman Ottó 15. Hungary
[b]Veszprém University, Faculty for Chemical Procedures, H–8200 Veszprém, Egyetem u. 10. Hungary

Polyphenols are natural components of numerous plants, seeds, fruits and roots, and can be regarded as general micro-components. Their role, significance is not fully clarified yet. They are not toxic, but their structure may change when the plants are processed by the food industry. Through those changes they may cause enzymatic or autolitic browning, may alter the taste or flavour, and so their presence used to be

considered undesirable. Quite recently views on them changed, some researchers consider them to have antioxidant, bactericide and/or anticarcinogen effect due to their capacity of linking radicals. With our work we join the research aiming at clarifying, proving the effects of these compounds.

Our aim has been to extract, separate and characterize the plant polyphenols, the determination of their biological activities, the study of the technological applicability of these complexes for the production of functional foods for the advantage of consumers and of the food industry. The initial results of this complex theme has been presented actually. Our work has begun with the study of the most important Hungarian oil plant, the sunflower polyphenols. Our aim has been to assess in which phase of the vegetative development of the sunflower are polyphenols in the greatest amounts produced, in which part of the plant they can be found and how their composition undergoes changes. We have collected samples once in every other week from the variety of sunflower which is publicly produced, starting from the time when the plant developed buds up to the harvesting period. Seed commencements and seeds were separated, other parts of the plant (stalk+leaf+plate) homogenized. The samples of plant and the seeds in the different phases of development were extracted by two methods: the traditional extraction with solvent and supercritical fluid slovent mix (CO_2/ethanol or C_3H_8/ethanol). Supercritical extractions were carried out with the apparatus with 400 bar effective pressure of 114 cm^3 working volume in dynamic system, belonging to the Veszrpém University. The total polyphenol content of the obtained extracts was determined by spectrophotometer, the polyphenol composition with HPLC measuring methods.

The following conclusions can be drawn from our results:

– In the course of the plant's development the total polyphenol content dropped by 77%, from 4.7% to 1.1%. The rapid drop occurred during days 80 and 120 in the plant's development.

– The total polyphenol content dropped also in the seed, but to a lesser extent, from 3.0% to 2.2%, that is by 27%. The drop occurred between days 80 and 110, after that age the value remained unchanged.

– The result suggests, that the plants contain more kinds of polyphenols than the seeds and during the development period the number and level of these compounds decrease.

– In the polyphenol composition the chlorogenic acid dominated both in the plant and the seed during the entire development period. Besides chlorogenic acid isomers, as well as caffeic acid, ferulic acid, sinapic acid and coumaric acid could be identified.

– The quantity of the dominant chlorogenic acid is increased in the plant and the seed during the development phase and finally it contributes 50% in the plant and 60% in the seed to the polyphenol content.

– Extraction by supercritical fluid mix solvent (under the employed conditions) leads to a selective, but not full solution of polyphenols. To increase the output the extraction method needs to be changed.

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QUALIFICATION OF WHEAT FLOUR WITH FARINOGRAPH IN HUNGARY AND ABROAD

L. SZALAI

Department for Grain and Industrial Plant Technology, University of Horticulture and Food Industry, H–1118 Budapest, Somlói út 14–16. Hungary

The farinograph gives the most profound information about the baking quality of flour almost for seventy years. The farinograms are made on the same way all over the world. The courses of analysis are written in ICC, ISO and AACC standards in the same way, those are based on theoretical and practical experiences. This agrees substantially with the process that proceeds from Jenő Hankóczy from that time when he made the farinograph. The aspects of evaluation are not the same in our country and in the European Community or in the United States of America. We are going to annul this inconsistency in a short time. The evaluation used in Hungary is delivered in this lecture and is written in Hungarian standard (MSZ 6369/6). Nowadays there are more and more evaluations first of all in papers of scientific institutes according to the AACC and ICC instruction, which cause trouble in interpretation with the use of the same or similar term in many times. In this lecture we will survey the influence of modification in the field of method and application as well.

– We will deal with the influence of decreasing the mass of dough from the aspect of kneading parameters and of geometrical formation of instrument.

– We will examine the consequence of changing the revolution per minute of kneading elements.

– We will analyse the consequence of change that ensues in material of kneading bowl.

We will discuss the problem of adjustment and verification of damping of instrument.

– We will examine the instrument comparability to the legal metrological conditions applied in trade.

– We are going to compare the enforceability of positional and evaluational aspect according to the present day modern measurement technique.

– We make a recommendation for more exact definition of the parameters applied in national and international methods.

– We make comparison between the Hungarian and foreign methods in evaluation of the same analytical samples and we will demonstrate the correlation between the evaluation methods.

PRODUCTION AND PROPERTY EXAMINATION OF PEA BASIS DOUGHS MEETING SPECIAL NUTRITION REQUIREMENTS

E. T. KOVÁCS[a], D. J. VAN ZUILICHEM[b], C. VIDAL-VALVERDE[c] and L. GERŐ

[a]University College of Food Industry, Department Food Chemistry and Analysis and Department Microbiology and Biotechnology, H–6724 Szeged, Mars tér 7. Hungary
[b]Agricultural University, Wageningen, Department Food Process Engineering. The Netherlands
[c]CSIS Institut de Fermentaciones Industriales, Madrid, Spain

The climate and soil in Hungary are fairly favourable for growing peas. The yellow peas grown under Hungarian conditions are especially of good quality. The protein content of pea is 23–25%, the dietary fibre content of the seed is 19%, starch content is 50% and those of the other components are 7%. Peas do not contain any gluten protein, so good quality dough structure can be obtained from it by heat treatment as well as by additives. The pea basis mixture is suitable to produce dough rich in proteins which is key importance for patients with coeliac disease. The emulsifiers as additives interact with the proteins, lipids and carbohydrates and result in a protein-lipid-carbohydrate complex. The obtained complex is important from the point of view of the product. The antinutritive substances in the pea also should be taken into account, but their quantity can be diminished by the processing technology.

The authors are examining the properties of the pea basis doughs in model-systems. Different types of emulsifiers are applied and the quality improving effects are monitored. After extrusion cooking of pea the dough with emulsifiers were produced. The influence of heat treatment and the examination of interactions are carried out with electrophoresis. The change of antinutritive substance is traced too which is important from point of view of the product.

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The experiments are carried out in the framework of MKM 474/1996 project.

DETERMINATION OF OCHRATOXIN A IN COFFEE BEANS, ROASTED AND INSTANT COFFEE

A. PERNECZKY, K. MATYASOVSZKY and J. SOHÁR

Institute of Food Hygiene and Nutrition, H–1097 Budapest, Gyáli út 3/a. Hungary

In the recent years there is a growing concern worldwide about the effect of mycotoxins. Beside alfatoxins special attention has been paid to the ochratoxins especially ochratoxin A (OTA) both in the field of health care and analytical chemistry.

Ochratoxin A is a toxic mold metabolite produced by *Aspergillus ochraceus* and *Penicillium verrucosum*. OTA consists of a dihydroisocumarin moiety linked through its 7-carboxyl group to L-phenylalanine by an amid bond.

OTA may cause kidney disease and it was found to be carcinogenic, nephrotoxic and genotoxic. JECFA established the provisional tolerable weekly intake (PTWI) in 100 ng/kg bw. For the provisional tolerable daily intake (PTDI) the Nordic Expert Committee recommended 5 ng/kg bw.

OTA has been detected in a variety of foods and feeds mostly from countries with temperate climate. OTA was also found in human blood sera samples and mother milk. For humans the main sources of intake seem to be cereals, coffee products, beer and pork. The daily intake of OTA was calculated to be about 100–150 ng.

The OTA content of green coffee may decrease during roasting where the maximal loss of OTA is estimated to be between 2–29 %. The carry-over of ochratoxin A from green beans through roasting into the coffee brew was found about 50%.

In Hungary the regulatory limit for OTA in green coffee is 15 μg/kg, for roasted coffee and other cereals 10 μg/kg.

For determination of OTA in green coffee and coffee products we used thin layer chromatography (TLC), high pressure liquid chromatography (HPLC) and indirect competitive enzyme-linked immunosorbent assay (ELISA).

Green and roasted coffee samples were extracted with chloroform in the presence of phosphoric acid then transferred to $NaHCO_3$ phase followed by C18 catridge cleanup. The dry residue was dissolved in acetonitril 5% acetic acid, which served as the mobile phase on a Sperisorb RP 18 analytical column. OTA was detected with a fluorescence detector at the detection limit of 0.5 μg/kg. The recovery was 93%.

Determining OTA in roasted and instant coffee for clean-up an immunoaffinity column was used instead of C18 catridge before HPLC analysis. The recovery was 94%.

The ELISA method was found to be appropriate for determining OTA in raw coffee and also in coffee products with the detection limit of 0.5 μg/kg.

In the past two years the ochratoxin A content of 1400 green coffee samples was within the range of 0.5 to 80 μg/kg. In 2.9% of the samples the OTA level was over the regulatory limit. Since our extensive investigation in commercial coffee imported to Hungary the OTA level started to decrease, and starting from March 1995 was always below the regulatory limit. OTA was detected in roasted coffee within the range of 0.5 μg/kg. The OTA content of instant coffee samples was less than 10 μg/kg.

RECOGNITION OF RAW COFFEE BEANS BY GC-MS MEASUREMENT OF THE TRADE SAMPLE EXTRACTS

K. KORÁNY and M. AMTMANN

Department of Food Chemistry and Human Nutrition, University of Horticulture and Food Industry, H–1118 Budapest, Somlói út 14–16. Hungary

Methods controlling the imported raw coffee cargoes by their visual and sensoric check and microbiological investigation are not sufficient. They give no information on the chemical substances ensuring the identification of provenance and variety. The significance of the problem can easily be judged considering that Hungary imports 35000 tons of coffee bean with a cost of 10 billion (10^{10}) forints yearly. From economical point of view finding a suitable qualifying – classifying procedure would be of primary importance, consequently the development of new analitical methods measuring the characteristic composition is a must.

In our experiments raw coffee samples of controlled provenance and variety have been investigated: Honduras (*Arabica*), Santos (*Arabica*), Salvador (*Arabica*), Kenya (*Arabica*), Uganda Standard (*Robusta*), Uganda G 6 (*Robusta*), Indonesian (*Robusta*), Vietnamese (*Robusta*).

Elaborating the new recognition method the following tasks have been solved:

– extraction method producing the representative extract of the coffee beans being tested has been worked out;

– in the samples 22 compounds have been identified by GC-MS;

– data evaluation method (relative chromatogram creation) indicating sensitively the differences among the coffees has been elaborated;

– the suitability of the relative chromatogram creation method has been proved by the identification of 8 original coffee samples.

Due to our results it could be established that the structure of the relative chromatograms are independent of the errors of both the sample preparation and the chromatography and the coffee samples could perfectly be distinguished and identified in every case.

EXTRACTION OF CARDAMOM OIL WITH SUPER- AND SUBCRITICAL CO_2 AND PROPANE

H. G. DAOOD[a], V. ILLÉS[b], É. KARSAI[c] and P.A. BIACS[a]

[a]Central Food Research Institute, H–1022 Budapest, Herman Ottó út 15. Hungary
[b]University of Veszprém, H–8200 Veszprém, Egyetem u. 10. Hungary
[c]University of Horticulture and Food Industry, H–1118 Budapest, Villányi út 29–33. Hungary

Cardamom (*Elettaria cardamomum* W.M.) is widely used as spice and medical herb. Its flavour is incorporated in processed foods by adding ground seeds, hydrodistilled oil and solvent-extracted oleoresin. The pleasant aroma profile is attributed to the essential oils that have therapeutic benefit as well (LEWIS, 1984).

Extraction of cardamom by hydrodistillation and solvent extraction brings about substantial change in the chemical composition of the volatile constituents of the recovered oils. To get cardamom oil devoid of these limitations CO_2 extraction seemed to be ideal (GOPALAKRISHNAN & NARAYANAN, 1988). In the present work the effect of super- and subcritical conditions of CO_2 and propane extraction of cardamom oil were studied. The yield and solvent extractability were examined as a function of extraction conditions such as temperature, pressure and solvent/solid ratio. Of the quality components of the recovered oil, pigment (chlorophylls and carotenoids), volatile constituents, fatty acids and tocopherol, were determined by modern techniques including high-performance liquid chromatography (HPLC) and gas chromatography – mass spectrophotometry (GC-MS).

The results indicated that cardamom oil was easy to extract by liquid CO_2 even at subcritical conditions with very cost-effective ratio of solvent/solid. Addition of ethanol to CO_2 did not improve solvent extractability. Propane was found more capable to recover seed oil than CO_2 at subcritical conditions and with lower ratio of solvent/solid. Propane-extracted oil contained higher level of vital pigments than CO_2-extracted samples. Supercritical fluid extraction (SFE) conditions were found to affect fatty acid and volatile content of the oils.

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CHARACTERISATION OF WINE AROMA COMPOUNDS USING SOLID PHASE MICROEXTRACTION (SPME)

GY. VAS[a] and K. VÉKEY[b]

[a]Station of the Research Institute for Viticulture and Enology of the Ministry of Agriculture, Eger, H–3301 Eger, Kőlyuktető, Pf. 83. Hungary
[b]Central Research Institute for Chemistry, Hungarian Academy of Sciences, H–1025 Budapest, Pusztaszeri út 59–67. Hungary

Wine is a fermented alcoholic drink that is made from grapes. Wine – from the chemists' point of view – is a very complex mixture, containing more than 2000 different identified compounds. We can characterise these compounds by the origin (originated from the grape, formed during the fermentation etc.), by the chemical composition etc. The wine aroma compounds are influenced by various factors (soil, climate, grape variety etc.). Every wine making process puts its own "fingerprint" into the wine. This example show that wine aroma determination is very important and useful for the enologists.

Wine aroma characterisation require sample preparation, before chromatographic analysis. The most popular sample preparation method is solvent-solvent extraction. In spite of the popularity of the solvent-solvent extraction it has some disadvantages. These are:

– time consuming (about 3–4 h/sample),
– expensive (depending on the solvents, 3000–5000 Ft/sample),
– most volatile components may be lost during concentration,
– solvents change the equilibrium of wine,
– solvent impurities appear in the aroma chromatograms,
– poor reproducibility.

Every year our chromatography lab has to do a large amount of wine aroma characterisation. It seems logical to find a sample preparation method without these disadvantages.

Solid Phase Microextraction (SPME) is a new sample preparation method which is efficient and economic. It was developed by Pawliszyn and co-workers in 1990. Using this sample preparation method, the time and cost of analyses is reduced by a factor of 10. Using SPME as a head-space sampling the method is very sensitive for wine esters and terpene alcohols.

We successfully use the SPME technique for certain fields of wine aroma characterisation.

FUNCTIONAL FOODS: CHALLENGES AND PITFALLS FOR THEIR PRACTICE

E. DWORSCHÁK

National Institute of Food Hygiene and Nutrition, H–1097 Budapest, Gyáli út 3/a. Hungary

Functional foods help us to function better and more effectively in many aspects of our lives, including helping us directly in the prevention and treatment of illness and disease.

Japanese health prescriptions underline the natural origin and the scientific judgement on the active components of the functional foods.

Classification by components, the following main groups are known as base for functional foods:

- Dietary fibres;
- Oligosaccharides and sugar alcohols;
- Amino acids, peptides, proteins;
- Vitamins;
- Bacteria producing lactic acid;
- Essential minerals;
- Polyunsaturated fatty acids;
- Phytochemicals and other plant antioxidants.

On the base of home nutritional surveys and the recent scientific results the following challenges and pitfalls should be kept in mind in the course of development of functional foods.

Calcium and zinc deficiences need the development of new products with elevated mineral concentrations.

Iron supplementation presumes more danger than benificial effect.

For antioxidant vitamins the reference values of requirement should not be exceeded because of the ambivalent effects.

It is suggested to increase the rate of n-3/n-6 fatty acids avoiding the overdosage of the required 0.4 g/day of n-3 fatty acids.

RISKS OF GENETIC ENGINEERING AND BIOTECHNOLOGY

P.A. BIACS

Central Food Research Institute, KÉKI, H–1022 Budapest, Herman Ottó u. 15. Hungary

Consumers of a modern society often disagree to change genetic material and to be involved into inheritance character, therefore reject the new, but unconventional technique of genetic engineering. Only few scientists are dealing with genetic engineering, so less are informed in its risks and hazards which gives an opportunity to mislead others. People's opinion seems to be shocked to learn about transplantation of human genoms or to be informed on appearance of unknown genetic material. From many risk factors an emphasis is given to changing the natural environment by giving dominance/majority to genetically modified organisms and causing unbalanced situation within the nature. Besides genetically modified plants and animals the main target of research is still the kingdom of bacteria, yeasts and moulds. Traditional fermentation products are well known in the food industry and in many households, so genetic modification of those microorganisms caused less resistance in the public. Food items from traditional animal or plant sources and modified by genetic engineering are also considered to be safe. Two main criteria of food safety regarding GMO's are: 1. to avoid intake of harmful substances, 2. to disclose random incorporation of unknown genetic materials.

One of the most important task is to determine the presence of pharmacologically or toxicologically active groups. Similarly to pesticide and drug residues there is a need to establish a limit of genetic material which is generally regarded as safe (GRAS). Substances derived from genetic materials are considered to be food additives.

Industrial branches using biotechnology in economically significant level set criteria for using microbial cultures, plant tissue cultures, transgenic animals and enzymes produced from those organisms:

– raw materials should be cheap and available without any barriers of trade;
– reasonable yield from renewable sources;
– economic output after processing;
– considerable interest for such products worldwide;
– profit generated from sale;
– satisfying all security, legal and environmental restrictions.

In case of fulfilling those criteria and showing strict similarity to those products already set GRAS-criteria there is no need for a long-lasting registration procedure.

ROLE OF SPECIAL MUSHROOMS IN OBTAINING PARAMEDICINAL FOOD PRODUCTS

CS. LAMPER[a] and I. BEREK[b]

[a]Department of Food Chemistry and Food Analysis
[b]Department of Food Biology and Biotechnology, College of Food Industry, H–6724 Szeged, Mars tér 7. Hungary

In the past years mushroom growing in Hungary has become one of the most rapidly developing branches of agriculture, but this statement concerns, first of all, the champignon. The sale of Hungarian mushroom products abroad could be realized by healthy competition in the market and by broadening our export possibilities. The majority of cultivated mushroom is for fresh consumption all over the world, and only an insignificant amount is processed.

As modern eating habits are introduced, dishes made of different kinds of mushrooms have come into the focus of attention. From alimentary point of view it is important that mushrooms have low calory value, but their saturation value is high due to their undigestible fibres. Beside the well known champignon and oyster mushroom the shiitake mushroom also gains more and more popularity.

The health protecting effects of *Lentinus edodes* (shiitake) have been examined for decades by Japanese researchers, although the examinations have been extended to other mushroom types, as the traditions of the far-east folk healing have also been taken into consideration. Some of the positive effects: antiviral and anticarcigonen effects, it keeps cholesterol level at an optimal value, as well as, it strengthens the immune system. In addition, its positive effects have been observed in several other fields, but these results need further confirmation.

It can be stated that the consumption value of shiitake mushroom is augmented by its therapeutic value. In this way it is obvious that beside medicinal herbs, certain mushrooms (shiitake) must be paid special attention. In the present stage of research the isolation of some active components (eritadenine, lentinan) has been performed on the basis of mechanism of action. The isolation of other active components is required in further researches, as well as for paramedicinal food products in food industry. Keeping in mind the above mentioned facts, our aim is to utilize fermentation possibilities and to develop certain food industrial products in this line. If we are able to verify the significance of shiitake in different fields of the therapy, food industrial firms will face a great challenge of producing curative articles, which will surely enjoy great popularity.

ORGANOLEPTIC INVESTIGATION OF TASTE INTERACTIONS

A. S. SZABÓ[a] and R. JÓNÁS[b]

[a]University of Horticulture and Food Industry, H–1118 Budapest, Somlói út 14–16. Hungary
[b]University of Agricultural Sciences, H–2103 Gödöllő, Páter K. u. 1. Hungary

It is a well known fact that during qualification of foodstuffs sensory evaluation of quality is of primary importance.

Previously investigations were carried out to study the taste-interactions in model-solutions and real foodstuffs (e.g. fruit-juice, wine). Existence of interaction between sweet and sour tastes has been proven on the basis of modification of threshold-values, in comparison of solutions containing only one component.

On the basis of the results for the saccharose-citric acid system it was established that the presence of citric acid significantly decreases the perceptibility of sweet taste.

The measurements carried out with aspartame proved that the increase of concentration increases the recognition of the taste. But the presence of citric acid decreased the perceptibility of aspartame only slightly.

PRODUCTION AND EVALUATION OF AMYLOLYTIC ENZYMES OF THE THERMOPHILIC FUNGUS THERMOMYCES LANUGINOSUS

G. RÓNASZÉKI, Á. HOSCHKE and J. REZESSY-SZABÓ

Department of Brewery and Distillery, University of Horticulture and Food Industry,
H–1118 Budapest, Ménesi út 45. Hungary

There is a huge demand for polysaccharide degrading enzymes all over the world. Amylolytic enzymes which include endo-amylase (α-amylase), exo-amylase (β-amylase, glucoamylase) and debranching amylase (pullulanase) for processing starch is preferred over acid hydrolysis because of the high specificity of enzymes, homogeneous end products, mild reaction conditions, lower energy requirements and the absence of undesirable side reactions. However, the efficient hydrolysis of natural starch not only requires the above mentioned enzymes but also requires these enzymes to be stable and active at temperatures around 90–95 °C and over a pH region of 4.0–6.5. Although α-amylase from *Bacillus* species is stable and active between 90–95 °C and used widely in food and other industrial applications, the narrow pH optimum makes it less suitable for industrial processing of starch from the economic

view point. Furthermore, the above bacterium does not produce appreciable amounts of glucoamylase which has to be used from an additional source. Currently, the glucoamylase from *Aspergillus* is used in industries but this enzyme is stable and active only up to 55–60 °C and under pH 5.5. Therefore, a thermophilic fungus such as *Thermomyces lanuginosus* which would produce thermostable amylase and glucoamylase appeared to be an ideal source for developing economically attractive methods for industrial processing of starch.

Nineteen *Thermomyces languginosus* strains were screened. According to the results of the rapid screening all strains have shown amylolytic activity. Applying shaken flask cultivation technique 8 strains appeared to be promising in respect of amylolytic activity. Five strains seemed to have relatively good glucoamylase activity and 5 strains were selected for further investigation regarding α-amylase activity.

The main goal of this study is to find *Thermomyces lanuginosus* strains producing thermostable amylolytic enzymes with distinguished respect of glucoamylase enzyme. Our strains seemed to be very promising since we have experienced that the temperature optima of α-amylase and glucoamylase at the investigated strains are 70 °C. It is at least 5–10 °C higher than that of the industrial glucoamylase enzymes. These results suggest, that it might be worth determining the temperature optima of all the other strains as well. The pH optimum in case of glucoamylase is 4.6 and in case of α-amylase is 5.0. We consider it very important, that our strains should be investigated for their thermostability however from the crude ferment broth we had already made some measurements at different temperatures. Observing the effect of Ca^{++} ions we have established that the α-amylase activity doubled. With chromatographic methods we determined the protein components and identified active fractions from the crude ferment broth. By the optimization of media composition, culture conditions and inoculation method we are expecting to enhance the productivity of the strains. With the investigation of the carbohydrate profiles during fermentation we want to establish relationship between the glucoamylase synthesis and the glucose concentration. We hope this information can help to bring us closer to reach the maximum enzyme production. Eliminating the supposed glucose repression we want to stabilise the maximum enzyme production level. Afterwards we are going to define the active protein fractions in the culture filtrate. We are planning to purify the glucoamylase and α-amylase enzymes for the determination of kinetic parameters, enzyme stability, the pH and the temperature optima.

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STRUCTURE, BIOSYNTHESIS, DEGRADATION AND FUNCTION OF PLANT FRUCTANS. RESULTS AND QUESTIONS

L. BOROSS[a], W. PRAZNIK[b], E. BÁNYAI-STEFANOVITS[a] and I. KEREPESI[c]

[a]Department of Chemistry and Biochemistry, University of Horticulture and Food Industry, H–1118 Budapest, Villányi út 45. Hungary
[b]Center for Analytical Chemistry for Agrobiotechnology, Tulln. Austria
[c]Department of Chemistry and Biochemistry, Janus Pannonius University, Pécs. Hungary

In the frame of Austrian-Hungarian Research Cooperation we wanted to extend the knowledge about the plant fructans. Similarly to starch, fructans are reserve polysaccharides, some plants contain only starch, some others fructans and some other plants contain both polysaccharides. The functions and advantages of fructans in different plants have not been clarified yet and are being intensively investigated at present by some research groups in the world.

Our aim was to elaborate analytical methods for quantitative determination of plant fructans. Our procedure is suitable for estimation of the amounts of some individual oligofructans and our multienzymic analytical methods simplify the determination of the quantities of various carbohydrates in the presence of each other in various plant extracts.

For separations and purifications of the fructan-hydrolysing endo- and exoinulinase enzymes, we developed micropreparative electrophoretic and ionexchange chromatographic methods. We obtained immobilized preparations of excellent catalytic activity by covalent binding of those enzymes. Application of immobilized inulinases in laboratory bioreactors and the hydrolysis of inulin into fructose molecules with these bioreactors were studied.

Experiments were done to determine changes in the carbohydrate composition and the biosynthesis of fructans in wheat seedlings during their development. It was found that various environmental stress effects influence the fructan content and composition of wheat.

FACTORS EFFECTING THE DIACETYL PRODUCTION OF BREWER'S YEAST

J. REZESSY-SZABÓ and Á. HOSCHKE

Department of Brewery and Distillery, University of Horticulture and Food Industry, H–1118 Budapest, Ménesi út 45. Hungary

Beer is an alcoholic beverage with low alcohol content and possesses characteristic flavour. This special flavour composition is defined by the raw materials and the metabolism of the brewing yeast.

Beer fermentation can be divided into two stages: one is the fermentation process which is followed by the lagering or maturation. During the fermentation alcohol and aroma components are formed and in course of the maturation beer gains its own final aroma.

The strong flavour potential of diacetyl which belongs to the vicinal diketones is the origin of numerous off-flavours in beer. In lagers the flavour threshold of diacetyl is 0.1–0.2 mg l^{-1} in order and when diacetyl is present in excess it imparts a characteristic sickly-sweet/butterscotch flavour to beer.

The vicinal diketones are side products of the iso-leucine and valine pathway. α-acetolactate is a common precursor of valine and diacetyl. One part of α-acetolactate is metabolised in valine pathway and its excess is secreted into the wort where it is spontaneously decarboxylated and converted to diacetyl in non-enzymatic way. Yeast cells take up diacetyl and as they have considerable capacity for diacetyl reduction so diacetyl is transformed into acetoin then 2,3-butanediol. The spontaneous decarboxylation of α-acetolactate is going on very slowly at the temperature of brewing. So this process is the rate limiting step. Duration of maturation depends on the yeast strain and technology applied.

Diacetyl production of brewer's yeasts is defined by their genetic construction and environmental factors of the fermentation. Selecting mutants resistant to the herbicide sulfomethuron methyl in this population one can find mutants which produce lower amounts of diacetyl. The explanation of this phenomenon is that the target of sulfomenthuron methyl herbicide is the gene encoding α-acetolactate synthase.

Other possibility for the reduction of diacetyl production is to ensure adequate valine concentration in course of the fermentation. Because valine blocks the first enzyme of the synthetic pathway due to the feedback repression, so diacetyl production of brewer's yeast can be controlled by the above mentioned way.

Yeast strains harbouring gene encoding α-acetolactate decarboxylase have already been constructed by recombinant DNA technique, so they are capable to covert

α-acetolactate direct to acetoin hereby the diacetyl problem emerging during brewering process can be eliminated.

COMPARISON OF FERMENTATION VOLATILES FROM WINES PRODUCED BY YEAST STRAINS FROM TOKAJ WINE REGION

É. MIKLÓSY and V. PÖLÖS

Research Institute for Viticulture and Enology of the Ministry of Agriculture,
H–6000 Kecskemét, Kisfái 182. Hungary

The fermentation aroma of wines is highly influenced by the applied yeast strains.

Volatile substances of wines (alcohols and fatty acid esters) fermented with 6 *Saccharomyces cerevisiae* yeast strains were studied. The yeast strains were isolated from spontan yeast flora of musts from Tokaj wine region.

Volatiles were analysed after liquid-liquid extraction by GC–MSD. The concentrations of 7 alcohols (2-methyl-1-1-propanol, butanol, i-amyl-alcohol, hexanol, octanol, decanol, 2-phenyl-ethanol) and 12 esters (ethyl acetate, ethyl propionate, ethyl hexanoate, ethyl lactate, ethyl octanoate, ethyl decanoate, propyl acetate, i-butyl acetate, butyl acetate, i-amyl acetate, hexyl acetate, 2-phenethyl acetate) were used for characterization of the yeast strains.

The yeast strains were grouped on the basis of their ability producing esters and that of the ratio of ester and alcohol production.

EXAMINATION OF ETHANOL TOLERANCE OF LACTIC ACID BACTERIA CAUSING SPOILAGE IN WINES. RELATIONS WITH THE MEMBRANE LIPID COMPOSITION

Z. P. KALMAR[a] and A. LONVAUD-FUNEL[b]

[a]Research Institute for Viticulture and Enology of the Ministry of Agriculture,
H–6000 Kecskemét, Kisfái 182. Hungary
[b]Faculté d'Œnologie, Université Victor Segalen Bordeaux II, 351, cours de la Libération,
F–33405 Talence, Cedex. France

The alcohol tolerance of four lactic acid bacteria: *Lactobacillus plantarum* IOEB9113, IOEB9106, *Lactobacillus hilgardii* IOEB720 and *Lactobacillus fructivorans* IOEB546 isolated from spoiled wines, from Pineau de Charentes and Porto

which wine products had been preserved by fortification with ethanol during their elaboration, was studied in this work. The alcohol tolerance of strains being trained to tolerate different ethanol concentrations in modified MRS medium was determined by the effect of different alcohol concentrations on the growth kinetic parameters of the strains (changes in optical density, growth rate and OD max.). The membrane lipid content (polar and neutral lipids, phospholipid content, total fatty acid composition) of the biomass of these strains cultivated in this alcoholic medium and being stayed in stationary phase was also determined.

The examined strains can easily be adapted to 10–15 vol% alcohol content as an effect of the 5 vol% alcoholic grade applied during precultivation. The alcohol concentration of 5–10–15 vol% decreases growth at *L. hilgardii* and *L. plantarum* but the strains are capable to reach 1.0 values of optical density. However the growth rate of the *L. fructivorans* strain increased in the function of alcohol concentration.

The membrane lipid extracts of the examined strains have not contained hopanoic components above the limit value of 1 μg/g dry weight. The phospholipid, PG and saturated fatty acid content of the extracts decreased in the function of alcohol concentration. At the same time, the content of unsaturated fatty acids ($C18:1cis^{11}$), some glycolipids and the average length of carbon chain of total fatty acids increased. The numerous changes are described by principal components analysis and some relations between unsaturated fatty acids and some glycolipid forms were statistically demonstrated. During starvation, some lipids could act as growth or survival factor. For a better alcohol tolerance, unsaturated fatty acid ($C18:1cis^{11}$ or cis^{9}) availability and their incorporation in specific glycolipid fractions may be very important.

THE EFFECT OF ENVIRONMENTAL CONDITIONS ON BACTERIOCIN ACTIVITY

J. KROMMER and Z. INCZE

Hungarian Meat Research Institute, H–1097 Budapest, Gubacsi út 6/b. Hungary

In the recent years the number of publications dealing with bacteriocins produced by *Lactobacilli* has increased. Bacteriocins are protein-like compounds which can control the growth of several pathogenic and spoilage bacteria. Some years ago a pathogenic microorganism, *Listeria monocytogenes*, was given attention to because of its being more resistant to the environmental conditions than the other pathogenic bacteria known. The aim of our study was to investigate whether it is possible to inhibit the *Listeria monocytogenes* using bacteriocin producing *Lactobacillus* strains.

Two Lactobacillus (*Lactobacillus helveticus* and *Lactobacillus sake*) and a Leuconostoc species (*Leuconostoc gelidum*) were selected.

After preliminary investigations the bacteriocin produced by the *Lactobacillus sake* strain was found to be the most promising one for the further research. This strain was grown in MRS media with different pH set either by buffer (to 5.5, 6.0, 6.5, 7.0, 7.5) or by acid or alkali (to 5.0, 6.0, 7.0) and incubated at room temperature. After 0, 4, 8, 16, 20, 24 and 48 h of incubation the pH of the media, the CFU/ml (colony forming units) and the bacteriocin activity (by agar spot test) were determined.

In another test the *Lactobacillus sake* strain was grown in different media (MRS, APT=All Purpose Tween, TSB=Tryptose Soy Broth and MRS with reduced carbohydrate content, as well as sterilized milk). After 0, 4, 8, 16, 20, 24 and 48 h of incubation at room temperature the CFU/ml and bacteriocin activity against *Listeria monocytogenes* as indicator microorganism were determined.

In order to achieve "low cost production" several inexpensive substrates were also tested. The strain was grown in milk whey alone or in combination with yeast extract (4 g/l) or glucose (20 g/l) or yeast extract (4 g/l) and glucose (20 g/l). After 0, 4, 8, 16, 20 and 24 h of incubation at room temperature the CFU/ml as well as bacteriocin activity were determined.

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MODELLING BACTERIAL GROWTH KINETICS

L. MÉSZÁROS

Department of Refrigeration and Livestock Products Technology, University of Horticulture and Food Industry, H–1118 Budapest, Ménesi út 45. Hungary

The microbial state is one of the most important influencing factors determining safety and stability of the foods, in particular that of with high water content. The micro-organisms of different origin could growht up to an unacceptable level during processing and storage of the foods. One of the main aim of the different preservation techniques used (i.e. chilling, reducing the pH, lowering the water activity, using preservatives, packaging in vacuum- or modified atmosphere etc.) is to prevent the growth of micro-organisms. Changing the composition of the food, or alternation in the used methods for production, packing or storage could drastically affect the stability

and safety of the product. It is often hard even for specialists to quantitatively predict or reconstruct how this changes affect the microbial load of the product. The disadvantage of the traditional shelf-life test is, that the results are valid only for the investigated product and for the conditions used. Further, to judge the effects of alternating conditions, temperature for instance, during storage and distribution by using the traditional approach is enormously laborious and expensive.

The efficiency of the traditional "challenge test" could be improved by using the results of a modelling approach; called predictive microbiology. This promising methodology, growing into an individual discipline in the last decade, could be efficiently used to describe the growth responses of micro-organisms to environmental conditions. The paper introduces the concept of predictive microbiology. Experimental results for growth characteristics (maximum growth rate, lag-phase duration and maximum population density) of *Listeria monocytogenes* in synthetic liquid- and solid nutrient media and in Bologna-type sausage are used for explanation of the potential benefits and limitations of this approach.

HEAVY METAL ION TOXICITY ON VARIOUS SOIL AND RHIZOSPHERE MICROORGANISMS

M. KECSKÉS[a], B. BIRÓ[b], H. E. A. F. BAYOUMI[a], A. KISS[a], Z. KISS[a], N. KUCSMA[a], GY. VÁRADY[a] and Z. NAÁR[c]

[a]Ph. D. Subprogram of Agricultural-, Environmental Microbiology Soil Biotechnology, Department of Food Sciences (GATE-KÉKI) and Environmental Microbiological Research Group of the Hungarian Academy of Sciences, H–1022 Budapest, Herman Ottó út 15. Hungary
[b]Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences, H–1022 Budapest, Herman Ottó út 15. Hungary
[c]Eszterházy Károly Teacher's Training College, Department of Botany, Eger. Hungary

The heavy metals constitute a group of about 40 elements. Although many of them are essential for the growth, they are also reported to have toxic effects, mainly as a result of their ability to denaturate protein molecules. The phenomenon of microbial resistance therefore has a fundamental importance. According to the literature data there is a controversy about the tolerance limits of the different isolates and microbial groups, that is highly influenced by the origine of strains, the in vitro techniques and also by the materials used. The aim of this study was to develope a standardised method for investigating the microbial metal tolerance limits using various groups and isolates of beneficial microorganisms.

Soil and rhizosphere microorganisms tested belong to the taxa Rhizobium, Azospirillum, Pseudomonas and Trichoderma, that are known to fix atmospheric

nitrogen to produce siderophore or antibiotics against the phytopatogenic fungi and have therefore a plant growth promoting effect (BURR & CAESAR, 1984; GHISALBEERTI & SIVASITHAMPARAM, 1991; HILTNER, 1904). Concentration levels of some heavy metals (Cd, Cu, Ni and Zn) or metal-combinations (with Fe or Co) were tested in vitro using a modified liquid method. One particular method, when applied throughout a series of examinations should be suitable for screening the sensitivity of different strains. Taking this into consideration, the broth culture method was preferred in this study, and because of the precipitations between the studied chemicals and medium components, the inorganic salts were omitted (BIRÓ et. al., 1995). Rate of colonised soil particles originating from a metal spicked field-experiment was calculated in case of moulds and Trichoderma species.

Authors established, that

– Pseudomonads, having a quick colonising ability in the rhizosphere were found to be the less sensitive group to the heavy metals and also to other chemicals (pesticides) in an earlier study (BALÁZSY et al., 1984).

– Resistance to the different metals on the other hand were highly influenced by the strain origin, the in vitro techniques and the rates used. There was no correlation found between the sensitivity of different taxa and the order of metals investigated.

– Essential metals such as ferric ion (Fe^{2+}) may compensate the harmful effects of toxic metals (Cu, Ni, Zn, Cd) using the unique standardised method. Concentration of toxic and essential metals was highly depending on the media constituents.

– Rate of colonised soil particles by Trichoderma fungi were found to be increasing in correlation with used metals (Ni, Zn 90–810 kg ha^{-1}), that were below the European permitted limits. Species composition of the abundant Trichoderma genus has also changed considerably as a function of different heavy metals.

Although the microbial resistance were found to be highly dependent on the environmental conditions, using the modified standardised methods, it was possible to isolate tolerant representatives as potential candidates for inocula components.

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SECTION OF FOOD ENGINEER

DEVELOPMENT OF ORIGIN CONTROL SYSTEM OF THE WINE REGIONS IN NORTH TRANSDANUBIA

I. BALOGH, A. LŐRINCZ, F. BÉNYEI and G. ZANATHY

Department of Viticulture, University of Horticulture and Food Industry,
H–1118 Budapest, Villányi út 35. Hungary

Department of Viticulture participated in subprogram of Collective Wine Marketing program called "Formation of origin control system of Hungarian wines for the entire wine economy", as the regional responsible body for North-Transdanubian Area Subject sponsored first by NGKM (Ministry of International Trade Connections), later by ITD Hungary from the Foundation of Trade Development. Professional supervising of the process work was performed by SZBKI of Kecskemét (Research Institute of Viticulture and Oenology). Project started at the same time in all wine regions belonging to the Area (wine regions of Ászár-Neszmély, Etyek, Mór, Pannonhalma-Sokoróalja, Somló, Sopron). Our main duties were: in the first year to solve the registration works, establishing origin control system (vineyard cataster, cellar cataster) and in the second year to do the tasks of regulation and development connected with origin control system.

- We process the history of wine region and emphasize the traditions of viticulture and wine production during our development work. We describe wine region's ecological circumstances and explored the important characteristics of origin to be controlled.
- Data of 4886 ha vineyard on area of 41 communities had been recorded; only 70 percent of these are suitable for production and marketing.
- Cellar cataster entry was made from 82 oenologycal units of community. The total storage capacity of these is 918867 hl.
- We drew up the suggested list of potencially origin controlled products with the help of surveys spread widely in wine regions. Suggestions for 65 different products were recived.
- We clarified specifications of apellation of origin controlled wines (OCW).
- The suggested list was limited to the place and variety of origin.
- We fixed the boundaries of growing lands of the chosen products. We took into account only the vineyards found on lands classified into the first and second

class of growing land cataster. Those vineyards that had a considerable grapevine shortage (21%<) and objectionable biological productivity were neglected.

- We described the features of viticulture, processing of grapes and wine of potencially origin controlled products, specifying the professional guidelines, the constitutional and institutional limits of the product regulation and the control (pints of control).
- We revealed the background of the production and integration of the potencially origin controlled wines. According to our estimation, in the 6 wine regions altogether 107000 hl of origin controlled wine (OCW) are to be expected. Four of the wines are made from variety Olasz rizling, three from Rajnai rizling, two from Chardonnay, two from Tramini, one from Ezerjó, Furmint, Korai piros veltelini, Leányka, Zöld veltelini, Kékfrankos and Zweigelt.

BIOGENIC AMINES IN HUNGARIAN WINES

M. KÁLLAY and M. BÓDY-SZALKAI

Department of Enology, University for Horticulture and Food Industry,
H–1118 Budapest, Villányi út 35. Hungary

The determination of nutritional physiological components, clearing up their origin and relationship with the technological processes is one of the most important fields of wine research. Biogenic amines belong to these components too. Their presence is important not only for the reasons of sanitary regulations but also from the point of view of quality assurance.

Biogenic amines are usually formed from their respective precursor amino acids by enzymatic way. In spite of the fact that the concentration of biogenic amines in wine is lower than in some protein rich foods (fish, meat, dairy product) the alcohol may enhance the effect of amines present in wine by directly or indirectly inhibiting the amine oxidase. Histamine and tyramine may cause migraine, hangover and some allergological symptoms in sensitive people.

Different clean up methods and detection systems were elaborated and compared in order to determine biogenic amine content of wines. The effect of certain fining agents (such as bentonite and activated carbons) and malolactic fermentation on the biogenic amine content (mainly histamine, tyramine and serotonine) were investigated.

Our essential results are similar to the published data. The biogenic amine (mainly histamine) content in red wines is usually higher than in white wines. The

reason of this can be the difference in technological processes. The biogenic amine content of the Hungarian wines is not higher than the international limits. The treatment of the wine results in significant variation in the quantity of biogenic amines.

THE ROLE OF THE ICP-AES IN THE WINEQUALITY CONTROL

M. BÓDY-SZALKAI and P. FODOR

University of Horticulture and Food Industry, H–1118 Budapest, Villányi út 35. Hungary

Metal analysis of wines has a great importance from technological and safety aspect, too. Single element techniques (atomic absorption spectroscopy, flame-photometry etc.) can be find in the standards. ICP-AES ((Inductively Coupled Plasma–Atomic Emission Spectrometry) is one of the widely used multielement technique. It was investigated for the analysis of wine samples.

Because of the difficult matrix of the wine samples different sample preparation methods were compared: acid digestion in PFTE (polytetrafluor-ethylene) vessel under pressure and using microwave energy, evaporation of the alcohol content using IR (infrared) lamp and microwave oven, dilution of the samples. The result of ICP-AES measurements were evaluated using F and T statistical methods. The recoveries for different sample preparation methods were measured after standard addition of all measured elements. The critical comparison of methods revealed differences between the sample preparation methods from the point of wiev of precision, reproducibility, accuracy and fastness.

The sodium, potassium, calcium and iron content was compared in case of three different analytical method. The comparation was based on the linearity of the calibration curves, the correlation coefficient of the calibration curves, the standard addition method and the matrix effect.

A possibly use of ICP-AES is shown in the wine quality assurance. Effect of different bentonite preparations and the same of fining conditions were examined as acting on metallic composition of wines by fining tests. The change of metal content was followed by using ICP-AES technique. Significant alterations of metal content, in consequence of fining treatments were observed. It was the type of wine, the temperature of treatment and the class of the agent that influenced the degree of alteration of the metal composition. Fining treatments induced changes in amount of metals and by it, influenced the stability of wines.

CHANGE OF THE YEAST FLORA OF TOKAJ ASZU WINES DURING FERMENTATION

I. MAGYAR, G. BÚJDOSÓ and E. MIKLÓS

University of Horticulture and Food Industry, H–1118 Budapest, Villányi út 35. Hungary

Microbiology of Tokaj Aszu making has been poorly studied. The aim of the present study was to get a better knowledge on the taxonomic composition and the evolution of the local yeast flora, focusing on the fermentative yeast species that take part in Aszu vinification.

The yeast composition of Aszu wines was investigated at various steps of the processing in two wineries in Mád, and Sárospatak. Within the limits of the method used, we paid special attention to the quantitative changes of the individual genera and species.

The examinations in Mád revealed that on the surface of the noble rotted grapes *Candida stellata* and other *Candida* species predominated, followed by the sugar tolerant *Zygosacch. rouxii*. We were unable to isolate Saccharomyces species by direct plating. During fermentation, *Sacch. cerevisiae* strains got dominant over 9% (V/V) ethanol content. The prevailing *Sacch. cerevisiae* strains of Aszu wine were presumably different from those of the base wine.

From the results it seems that the main domestic *Saccharomyces* yeasts involved in Aszu fermentation are different in the two wineries studied in this work. Comparing the different isolates belonging to the same physiological race, minor phenotypic differences were reproducibly detected, allowing a presumption that several strains of the same race may be present in a wine. Identity or diversity of the isolates will be verified by molecular biological methods.

HIGH-GRAVITY BREWING WITH UNMALTED GRAINS

B. HEGYES-VECSERI and G. KABAI

University of Horticulture and Food Industry, H–1118 Budapest, Villányi út 35. Hungary

Recently there is a growing interest in the technology making the increase of capacity and energy-saving possible with relatively little input. In Hungary most beers are made with unmalted grains, which decreases the cost.

The aim of our project was to optimalize the technology of high-gravity brewing combined with unmalted grains, as it is being the current practice in Hugarian breweries.

We carried out our experiments at the Faculty of Weihenstephan at Technische Universität München and the Department of Brewery and Distillery of University of Horticulture and Food Industry, Budapest.

As the first step we produced wort out of malt and unmalted grains. After defining the most important components of the wort we fermented it in high gravity and normal condition. We checked the change of the main parameters during fermentation and storage. After storage we set all types of beer at the same concentration values and completed the analytical and sensory evaluation.

Summarizing the results we have come to the followings:

– diluting high-gravity wort before fermentation to normal concentration values results in the same soluble nitrogen content as wort produced by traditional method;

– it seems favorable to produce beer with unmalted grains with high-gravity brewing technology, because as a result of higher percentage of soluble nitrogen content, the quantity of diacetyl resulting during fermentation is near or below the tasting limit;

– high-gravity wort needs longer fermentation period than normal beer;

– when fertilizing high-gravity wort the soluble oxigen content must be set at a higher value, thus the gravity of esters and alcohols with more carbonnuclei equals that of in normal beer after redilution;

– during the sensory evaluation of beer ready to consume we came to the conclusion that beer types with rice adjunct have the best quality, and the poorer quality types are that with barley. There was a minimal difference between the types of beer with the same adjunct fermented in high-gravity and diluted condition.

As a summary we can declare that high-gravity brewing with unmalted grains is an economical technology, which results in the increase of capacity without investment. The quality of beer ready to consume is equal to that made in traditional way.

CHANGE OF THE QUALITY OF GRAPE JUICES DURING STORAGE IN DIFFERENT PACKAGING MATERIALS

Á. NAGY

University of Horticulture and Food Industry, H–1118 Budapest, Villányi út 35. Hungary

The subject of the storage experiment was a white grape juice of 100% fruit content, filled into various packaging materials: green and hyaline bottles, yellow and hyaline PET bottles as well as TETRA BRIK and PKL COMBIBLOC aseptic packs.

The experimental products were stored for 12 months under different circumstances:

– at 5 °C in the dark

– at ambient room temperature under natural lighting or in the dark

– at 33 °C under natural lighting or in the dark.

In every month samples were taken for chemical and sensory analysis. This paper focuses on the colour changes detected by spectrophotometry (absorbance readings at 420 nm) and also by sensory evaluation.

The main conclusion was that the storage temperature had a major impact on the colour of the products, but the effect of packaging material was also significant. From the aspect of the juice quality, bottles were found superior to PET containers during storage. Comparing the two types of aseptic packs, TETRA BRIK provided significantly better colour stability.

REMOVAL OF HEAVY METALS FROM BEVERAGES WITH DIVERGAN HM RESIN

J. PANYIK and I. SZOIMMER

University of Horticulture and Food Industry, H–1118 Budapest, Villányi út 35. Hungary

The relatively high metal content in beverages (soft drinks, wine, spirit) is usually coming from the technological process.

The heavy metal content may be harmful from food safety point of view.
Heavy metals can be removed by K-ferrocyanid fining, but this product is dangerous from many point of views:

– HCN may form due to overdose of K-ferrocyanide;

– Berliner-blue precipitation deposit is dangerous on the environment. The PVPP based resins have been authorised since 1990 in the EU countries, for removal of phenolic compounds and stabilisation of colour in the beverage industries.

A recently developed variation of PVPP (PI/PV) could serve as an important means of heavy metal fixation. In the present study, the heavy metal content of sour sherry sine, alcoholic sour cherry juice, Tokaj-type wines and concentrated apple juice was reduced by Divergan HM resin.

The applied technological temperature was between 10–20 °C. The resin was dispersed by continuous stirring. On the basis of the results we concluded that a complete removal of Fe, Cu, Zn and Al ions could be achieved by Divergan HM.

High temperature and continuous stirring significantly accelerated the fixing rate of heavy metals. High ethanol content also had a beneficial effect on the process.

The advantages of Divergan HM resin are:

– it can be used in case of finished products, without disturbing the clarity already achieved;

– no risk of overdose;

– in contrast to K-ferrocyanide, not only the free ions, but also the chemically bound forms can be removed;

– no waste material dangerous for the environment is formed.

METHODS OF DETERMINING THE CARBON DIOXIDE CONTENT IN CHAMPAGNES

B. BÖRÖCZKY

University of Horticulture and Food Industry, H–1118 Budapest, Villányi út 35. Hungary

During the more than 300 years of making champagne there were many modifications applied. Manufacturing technologies were improved to increase and maintain the quality, the taste and the purity of the produced champagne. One of the most important qualifying parameters is the quality and quantity content of carbon dioxide.

My experimental purpose was comparing and modifying the different methods of determining the carbon dioxide content. The studies were made complete by organoleptic tests and statistical analysis.

The primary aim was to develop a simple, quick and repeatable producing method that can be used in factory laboratories. A new way was suggested for comparing the bound carbon dioxide content of champagnes produced by different

methods (champagne: bulk process sparkling wine, quality champagne: disgorging by filtration or cleaning by freezing process), deducing the manufacturing technology from determining the carbon dioxide content.

Champagnes produced by HUNGAROVIN Rt. were used for the experiments. I examined first three different kind of bulk process sparkling wines, then three champagnes (Gála, Hungaria Extra Dry, François President) produced by different manufacturing methods.

I chose four from quantity determination methods found in technical literature: using pressure gauge for bottles; a method based on loss of weight; method of distillation (O.I.V. 1978), titrimetric method (O.I.V. 1990).

It proves the up-to-dateness and importance of the topic that there were no regular and objective examinations made neither in countries producing champagne, nor in Hungary.

On the evidence of our examinations, the method based on loss of weight shows no difference between manufacturing technologies, demanding sample and time.

O.I.V.'s method of distillation requires much (and corrosive) solution. The required device is fragile and it's not easy to keep clean. The method demands sample and time, ambiguously repeatable.

O.I.V.'s titrimetric method can be processed easily and quickly. It is repeatable, has got low dispersion (0.00–0.15), thus average values can be compared. We suggest to use this method in connection with other drinks that contain carbon dioxide: reduced wines slightly refreshed before bottling; pearly wines; sparkling wines; also soft drinks and beers.

Measuring by devices never can replace sense impressions. These impressions must confirm the results of analytical methods.

At the end I made a survey in Budapest on preferences of customers. Results say the most popular champagnes are made by Törley Champagne Factory. The most liked products of this factory were 'Charmant doux' and 'Muscateller doux'. This fact shows that customers make decisions on sugar content rather than carbon dioxide content.

STUDY ON WINE PHENOLICS HAVING BENEFICIAL PHYSIOLOGICAL EFFECT

M. KÁLLAY and Z. TÖRÖK

Department of Enology, University of Horticulture and Food Industry,
H-1118 Budapest, Villányi út 35. Hungary

It is widely known that certain phenolic compounds of wine have protective effect relating cardio-vascular diseases. In recent years more and more paper have been published on one of these phenolics, resveratrol.

Resveratrol is a phytoalexin produced by plant as a response to stress or fungal infection (e.g. *Botrytis cinerea*). Concerning the presence of resveratrol in grape, two hypothesis can be found in the literature: (1) Grape varieties having genetically high resveratrol content are resistant to fungal diseases. (2) It is the fungal infection that induces resveratrol synthesis in the grape.

The objective of the present study was to investigate the effect of noble rot (caused by *Botrytis cinerea*) on the resveratrol concentration in Tokaj wines. Authentic wine samples as well as commercial products were analysed for resveratrol. Preparation of wine samples was performed by liophylization and liquid-liquid extraction. Resveratrol concentration was determined by HPLC. The main results and conclusions were as follows.

– High concentration of resveratrol (20 mg/kg) was found in noble rotted (botrytized) berries;

– Occurrence of resveratrol in wines was contingent and the concentration highly varied. No significant correlation with vintage, vineyard location or "puttony" number was revealed;

– Significant quantity of resveratrol is bonded in glycosides.

In conclusion, resveratrol (produced by the grape in response to Botrytis infection) can be found in special quality Tokaj wines, in certain cases at a considerable concentration (5–6 mg/l).

EXPERIENCES OF PREPARATIONS FOR ACCREDITATION OF FOOD TESTING LABORATORY

K. HORVÁTH-ALMÁSSY, O. BARA-HERCZEGH, V. MÉNESI-BARTA and GY. MAGYARI

University of Horticulture and Food Industry, College of Food Industry,
Department of Food Chemistry and Food Analytics,
H–6724 Szeged, Mars tér 7. Hungary

In 1995, a Food Testing Laboratory began to work in co-operation of three departments at the University of Horticulture and Food Industry, College of Food Industry, Szeged. It consists of three testing groups: chemical-analytical, microbiological, and physical groups.

The task of Laboratory is testing food quality in the South Hungarian Region. The tests are determined by the demands of potential customers (small- and middle food processing firms), and the capacity of Laboratory.

In 1996, we decided together with the head of College the preparation of Laboratory for Accreditation, according to the standard EN 45002. The quality assurance system of Laboratory has been done according to the standard EN 45001, and the system of quality manual has been done according to the directives of AR 12.

The qualification of Testing Laboratory includes three main fields:

1. Certification of quality assurance system;
2. Certification of incorruptibility;
3. Certification of proficiency.

The functional system and circumstances of laboratory ready for accreditation, must be documented in the quality manual.

The preparation is going on as scheduled with the help of consultants.

The most important experiences and results are as follows:

– The collective of laboratory accepted the approach of quality;
– The members of laboratory have participated in building of the system, and order;
– During some years the discipline began to relax, but now it is strict again;
– The documentation according to the directives of AR 12 is working well;
– The directives of AR 12 give a help into the system of quality manual;
– The trust of customers has been improved;
– Experiences of Good Laboratory Practice have been converted to the education;
– The working relationships of groups improved by co-operation.

INTERNATIONAL GUIDELINES FOR THE QUALITY ASSURANCE OF FOOD PRODUCTS

I. FÁBRI[a], V. TABAJDI-PINTÉR[b], M. HARKAY-VINKLER[c], CS. MOHÁCSI-FARKAS[c], J. BECZNER[d], E. SZABÓ[d] and E. ZUKÁL[e]

[a]FAO/WHO Hungarian Codex Committee on Food Hygiene, H–1097 Budapest, Mester u. 81.
[b]National Food Investigation Institute, H–1097 Budapest, Mester u. 81.
[c]University of Horticulture and Food Industry, H–1118 Budapest, Somlói u. 14-16.
[d]Central Food Research Institute, H–1022 Budapest, Herman O. u. 15.
[e]National Meat Research Institute, H–1097 Budapest, Gubacsi u. 6/b. Hungary

The overall quality of food products involves the safety, the wholesomeness, including the nutritional as well as the sensorical values and their preservation throughout the shelf-life, and the suitability for consumption. The nutritional and sensorical values are determined by chemical, physical, biological and organoleptic properties. For the elimination of hazards endangering the safety and suitability for consumption of foods the HACCP system has been worked out.

The "Guidelines of the Application of HACCP system" to ensure the safety (as well as other quality parameters) of food was edited by FAO/WHO Codex Alimentarius Commission in 1993 and its adoption in the food industry is mandatory in the European Union and in the USA. The essential basic implementation of HACCP is the application of the "General Principles of Food Hygiene" worked out also by the FAO/WHO Codex Alimentarius Commission. The expectations from the food products concerning their quality (safety, wholesomeness, suitability for consumption), moreover the connections among the different procedures for ensuring food quality will be discussed.

QUALITY ASSURANCE OR QUALITY MANAGEMENT

E. PALLAGI

College of Food Industry, H–6724 Szeged, Mars tér 7. Hungary

During the last decade the number of companies which have developed a quality assurance system in accordance with the requirements of ISO 9000 standards has been growing rapidly. In 1996 the number of certified food companies was over 30, and there are a lot of firms which are working on the development of quality system.

The question is, wether the quality assurance alone will be enough to strengthen or to keep on their competitiveness? On the basis of the results of the certified food

companies we can conclude, that they do not have significantly better position in the market than before. The hope concerning the raising competitiveness and better financial results were not proved in most cases. The new requirements are not only the conformance to requirements but to surpass the expectation of the customers. The new competitive view of quality means a competition on two levels. One is at the level of perceived quality, the other is the efficiency of processes. So the companies must adapt a new quality philosophy and practice, which enables them to provide greater value for the customers money than the competitors without cost raising. The adaptation of the total quality management could be the solution, because it with the implementation of the principle of continuous improvement, provides real competitive advantages.

ROLE OF COMPUTERS IN SENSORY ANALYSIS

M. ERDÉLYI and I. SÁRKÖZI

University of Horticulture and Food Industry, H–1118 Budapest, Villányi út 29-33. Hungary

For the support of different aspects of sensory analysis a number of computer software were developed during the last decade. However, just a few are used internationally, because it is recommended for as many partners as possible to use the same system (partners like food producers, institutions, traders, etc.).

Some well-known sensory softwares are for example: “Compusense”, “PSA”, “Sensorex”, “Taste” and there are separate statistical tools available like “Senpak”, “Senstools”, etc.

The focus is on the reproduction of sensory results as it is common with analytical data. International sensory reproduction, that is production of more and more objective results from a basicly subjective assessment, needs to apply standardized steps during the whole process: ISO based selection and training of assessors; ISO based or internationally used system for the design and preparation of sessions; ISO test methods as well as ISO or internationally accepted methods for the statistical evaluation and data processing.

At the present lecture authors use “ISOSENSE”, a newly developed integrated modular sensory software, for the demonstration of the title. Modular structure means here that the system can be build up from separate modules (test types), all of them are available and run even alone. Functions like test description, design, randomisation, coding, sample positioning, screen and printed questioner design, manual or scanner based automatic data entry, data base handling, test conform statistical analysis, printed report, etc., are integrated in all modules.

Modular structure backs small companies to build their sensory system step by step. From the beginning they have the same ISO based module what the institutions are using for a certain task. This uniform application supports reproduction of quality sensory results.

Just two areas are mentioned here for the demonstration of advantages using computer softwares for sensory analysis: a, selection and training of assessors; b, preparation design of session.

– ISO standards for identification of tastes as well as for recognition of thresholds suggest flexible sample numbers (9–15, 8–11, respectively) and "random multiple sample positioning" in the latter case. During training period the panel leader can give personally fitted "difficulties" to each candidate. However, at the selection stage computer randomization can help balancing "difficulties" for each assessor.

– Creation of correct balanced sample positioning, Block Design, is one of the most important element of test serie preparation. Randomised complete designs, balanced incomplete block designs are almost not possible without the use of software routines – even if formulas are published in the fifties...

Beside final statistical evaluation the use of high level mathematical formulas in the preparation process can be daily practice at small, "one computer" companies, at the "multies" and at the institutions as well. This uniformity is an important element in the good reproduction of results...

MARKETING PROBLEMS OF A MINOR REGION CALLED "HOMOKHÁT"

M. KIS

University of Horticulture and Food Industry, College of Food Industry,
H–6724 Szeged, Mars tér 7. Hungary

The Regional Development Association of the Local Governments in the "Homokhát" region was set up for the general development of this minor region in 1994.

The Association was formed on the initiative of the 12 local governments of the community constituting a coherent economic, social and geographical unit.

The agreement was concluded for the general purpose exploiting the advantages of the cooperation at regional level through which the special planning and development should become feasible to ensure a coordinated, consistent progress for all settlements.

Both the internal i.e. the local resources, individuals and the different relief funds, subsidies as well as the external investors can be mobilized or applied for only, if well founded concrete projects are created concerning the cultural, educational, health, environmental and infrastructural issues.

PROBLEMS OF THE ADAPTATION OF ENVIRONMENTAL MANAGEMENT SYSTEM IN FOOD PROCESSING

E. SZABÓ

Central Food Research Institute, H–1022 Budapest, Herman Ottó út 15. Hungary

In the interest of the maintenance of development, environment protection should get an increasing role in our everyday activity.

It was a German industrial company who set in 1972 first its activity to be in harmony with the environment as its operational target.

The International Chamber of Commerce (ICC) formulated in 1991 in 16 basic principles of environment economy the characteristics of environmental behaviour to be followed (company priority, integrated economy, continuous improvement, etc.). In 1992, a standard was elaborated under the number BS 7750 for the system of environmental management to be introduced (KIR). The review and the issue of this as ISO 14001 standard can be awaited in the near future. In 1993, a directive was issued in the European Union with the number 1836/93 for the elaboration and audition of the system of environmental management of the enterprises.

It is quite unambiguous on base of the above, that management on the level of the higher leadership, the treatment of problems connected with environment contamination to be on the same rank in the target system of the undertaking are belonging to the tasks of the present.

The environment – conscious consideration and practice in the life of a company get more and more expressed in a more and more sharper way in the obligatory prescriptions, as well. This kind of business mind gets more and more involved as factor of competitiveness.

The ambition to create the equality of conditions in the economical competition from the part of countries being more developed than ourselves acts in the direction of unified requirements and the unity and indivisibility of the earthly environment cannot be left out attention, either.

The documents mentioned above are being detailed in the lecture: an analysis presenting all similarities and all differences. The elaboration of KIR being in

correspondence with ISO 14001 standard can be recommended for the food processors. This requires the solution of tasks of similar type and working method as the establishment of the quality assurance system according to ISO 9000 and for this reason, a greater readiness of acceptance can be awaited from the companies having already experiences in the system development. It is highly essential, that the quality assurance system and the environmental management system should be established not separately, independently from each other, but in an integrated way. The establishment of KIR – in spite of its actuality – can be only a target setting of longer distance being in knowledge of conceptional and economical conditions. For this reason, the lecture enumerates finally some domains of matters (application of rationalization technics for various problems, participation in the education, joining some professional social organizations dealing with environment-conscious business management, trans-illumination for environmental protection, etc.), where the advance helps the preparedness of the enterprise for the realization of the KIR. Further, it is quite indispensable to make complex research work for the study of domains connected with environment protection (especially the packaging wastes) the results of which can orient the enterprises towards the direction of certain solutions. Moreover, the effect analysis of the economical means of regulation of environmental protection (penalties, taxes, various fees, etc.) and the refinement of these measures would be needed, as well.

It can be stated, that only a close cooperation and interaction of science and practice can develop a mass of knowledges with the use of which the business management can be realized on a high level with great efficiency from the aspects of environmental goals. In this perception, it is our task for the future to operate in full compliance with these trends.

APPLICABILITY OF MEMBRANE SEPARATION IN FOOD TECHNOLOGY

GY. VATAI[a], E. BÉKÁSSY-MOLNÁR[a] and T. MIODRAG[b]

[a]University of Horticulture and Food Industry, H–1118 Budapest, Villányi út 35. Hungary
[b]University of Novi Sad, Novi Sad. Yugoslavia

Membrane separation is one of the most dinamically developing separation technologies in the last decade. Application of membrane separation is very adventageous because it is a “cold” separation method and economically more effective than classical separations (evaporation, distillation). The most widely used membrane separation techniques in food technology are ultra- and microfiltration.

In the next five years the application of new membrane separations is expected, namely membrane absorption/desorption, -distillation and -extraction as well as membrane bioreactors. In the above mentioned separations the membrane serves as a contact surface between the two phases. For this purposes the hollow fiber membrane modules are the most efficient, because the specific contact area is up to 10.000 m^3/m^2.

In the frame of this presentation application of membrane distillation, membrane absorption/desorption, membrane extraction as well as membrane bioreactor investigations are presented.

FLOW-THROUGH TYPE HEAT TREATMENT AND FOOD QUALITY CHANGE

I. KÖRMENDY

Department of Canning Technology, University of Horticulture and Food Technology, H–1118 Budapest, Ménesi út 45. Hungary

Heat treatment in a flow-through type system differs considerably from the heat treatment of food in packaged condition. In the first case the food elements reside for different time intervals inside a processing unit, while in the second case the residence time of the elements is identical, though their time dependent temperature variation differs.

Quality attribute (concentration of microbes, enzyme activity, concentration of vitamins or deleterious components, sensory attributes) variation in flow-through type processing can be treated on results from the chemical reactor technics, such as macro- and micromixing principles. The methods of macromixing (complete segregation) are undoubtedly exact.

Possibilities for using macromixing principles for the heat treatment of food are: food consisting of real macro-particles; destruction of microbes, if destruction kinetics depends on the heat resistance distribution of the individual cells (at constant temperature); first order quality attribute variation.

Two important results have been presented in the lecture, these are based on the macro-mixing principle and relate to an apparatus of serially connected units (e.g. heating, constant temperature and cooling units). Conditions for the exactness of results: Constant temperature and food volume inside each unit (naturally units' values differ); unidirectional flow at the inlet and outlet ports of the units; steady state conditions and constant initial attribute intensity (at the inlet port of the first unit); independence of residence time distributions of the units; quality attribute follows the linear mixing law; quality attribute variation at constant temperature depends only on

the product of rate constant and time. The last criterion is also necessary when calculating the equivalent heat treatment time.

The first result serves for the calculation of the mean quality attribute intensity at the outlet of the apparatus, i. e. the discharge port of the last unit. Here the known, weighted mean creating definite integral, valid for one unit, has been generalized for serially connected units: The residence time distribution of the units are to be transformed to time multiplied by rate constant (kt) distributions, then follows the calculation of the overall distribution of kt, then the creation of the weighted mean.

The second result is a simplification method belonging to the first result: Weighted mean of the exit attribute intensity is calculated by the use of the overall residence time distribution and the proper average of the units' rate constants is applied in the weighted mean calculating integral. There are applications, where the average rate constant can be determined by weighting the units' rate constants according to their mean residence time (i. e. expected value). Validation has been done by using Laplace transforms, and only first order attribute intensity variation has been involved. The first result is based on the separate measurement of the residence time distributions of all participating units, while in the second case only the overall residence time distribution is involved.

EXAMINATION OF THE HEAT TRANSFER FOR COARSE FOODS

E. BOZI

Chemical and Food Engineering Department, Technical University,
Budapest, H–1111 Budapest, Műegyetem rakpart 3. Hungary

Sterilising is one of the most important and basic treatment in the food industry. For suiting the up-to date quality requirements and expectations we have to know exactly the heat transfer that takes place and the physical properties of the treated food.

Measuring the heat transfer process for many kind of foods requires much time and is in many cases impracticable and unrealisable. Creating a computing program we can quickly and easily compute the temperature distribution inside the heat treated material, from which we can calculate the sterilising grade. The calculation is based upon solving the heat transfer differential equation. If the temperature of the heat transmission medium is constant during the heat-treatment, it's easy to use the analitical solution of the differential equation what we can find in the literature. If the temperature of the heat-transmission medium is not constant, a numerical method is more suitable to use for solving the differential equation for counting the temperature distribution inside the material. This calculation method gives a chance to estimate the

sterilising time exactly, which is necessary for reaching the requested sterilising grade with having the quality requirement in sight, this means minimizing the damage in taste, colour and vitamin content. With this numerical computation method we can count the heat treatment cycling curve in the case of a cooling process as well.

The heat treatment of coarse materials is a difficult problem. There are different experiments for sterilising this kind of foods. They can't be sterilised in a plate-heat-exchanger and in Ohmical way the end-cooling is not worked out. But using a special forming scraped-surfaced-heat-exchanger, the same device serves for the heat-treatment and the cooling. During this process the temperature of the flowing water is not constant.

We have created a computation model for cylinder and spherical shape, programming in MATLAB, which uses the Crank-Nikolson method for the numerical solution of the heat-transfer differential equation in case of the differential boundary conditions. First we checked our model comparing with the analitical way of solution when the outside temperature is not constant in case of the boundary conditions of the first and the third. We made certain that our computational model gives the same result as the analitical solution.

Second we carried out measurements with different kind of foods. The results gave us evidences that the measured and the computed data show a similarity of the highest degree.

We continue the modelling in the next step with creating a computational model for the numerical solution of the differential equation of diffusion.

With the aid of the numerical solutions of the heat-transfer differential equation and the differential equation of diffusion we can compute a parallel modell of the heat and mass transfer during a heat transfer process if the outside temperature is not constant.

EXAMINATION OF HEAT TRANSMISSION IN SCRAPED-SURFACE HEAT EXCHANGER

SZ. MUTSY

Chemical and Food Engineering Department, Technical University, Budapest,
H–1111 Budapest, Műegyetem rkp. 3. Hungary

The most up to date aseptic manufacturing process meets best the requirements. In the course of this process food industrial products follow a heat-treatment in continuous operation, they are stored sterile and they are packed at sterile conditions just before delivery.

An important equipment during heat-treatment phase is the scraped-surface heat exchanger, which is perfectly suitable for high viscosity non-Newtonian food products. In this equipment, considering fluid mechanics and thermology, complex processes take place which are not well defined yet. Our research group has investigated the question of these processes and the correction of actual theories for several years. I am involved in the examination and optimization of the processes of heat transmission and fluid mechanics.

A part of my work is to observe the heat transmission coefficient under different influences, where the dependent variables are the material flow and the speed of scraper elements while the steam pressure (running side), the flow of cooling water, the inlet temperature of primary material and the geometry of the heat-exchanger equipment are constant.

On the other hand I examine the pressure loss evolving around the heat exchanger during operation of the equipment. These examinations take place at the same conditions as the former ones.

The results are processed in form of dimensionless numbers (Nu, Re_{rot}, Re_{ax}, Re, Pr, Eu).

In the scraped-surface heat exchanger the material streams through a ring section, meanwhile the inner wall turns compared to the outside wall. Under this constraint the material is deflected from the original axial movement. We can describe this actual movement as the composition of an axial stream characterized by the axial Reynold number (Re_{ax}) and a rotational stream characterized by the rotational Reynold number (Re_{rot}).

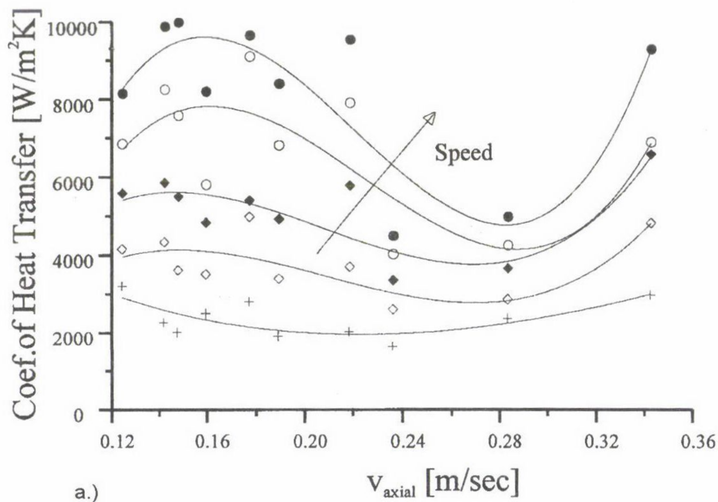
In the extreme case, when the axial velocity of the material is zero or close to it, the so-called Couette stream is formed. If we increased the speed of the rotational element (in our case the axle with the scraping elements) over a critical value, we would find that toroid, the so-called Taylor's eddies appear. The critical value depends on the material and the geometrical shaping.

The Taylor's eddies were examined and the critical values determined for the first time by TAYLOR (1935), who also introduced a dimensionless number, the so-called Taylor number, measured its critical value for several material and geometrical shapes (HO et al., 1964). Others have attempted to describe the transition between the two streams, that means they established through measurements a critical Re_{rot} number, which depends on the geometrical dimensions of the heat exchanger (TROMMELEN & BEEK, 1971). These two numbers are not equivalent, and it is a matter of debate whether the first or the second one gives a better approach to this process. For this reason there are several theories describing the process of heat transmission, therefore the relationship between heat transmission and material stream is also described by several, often very different theories. For instance CUEVAS and co-workers (1982)

measured heat transmission at high material stream and found that on the one hand at small speed values the outlet temperature increases in parallel to the increase of the speed, meanwhile at higher speed values this correlation diminishes or can even turn into inverse correlation. On the other hand at small material flow the outlet temperature is higher than at high flows. The $Nu=ARe_{rot}^{B}Re_{ax}^{C}Pr^{D}(m/m)^{E}$ equation was determined by dimension analysis. MAINGONNAT and CORRIEN (1983) determined by measurements the A, B and C values $Nu=2{,}24Re_{rot}^{0.56}Re_{ax}^{0.017}Pr^{0.33}$ and concluded that the axial velocity had negligible influence on the heat transmission coefficient, that means can be determined by the rotational Reynold number $Nu=2.28Re_{rot}^{0.56}Pr^{0.33}$.

BOTH-FEHÉR (1995) has developed a simplified theory where instead of two, only one Reynolds number is used which is derived from the vectorial addition of the rotational and axial components. So the criterial equation becomes simplier: $Nu=ARe^{B}Pr^{D}$.

According to my measurements the axial stream has a smaller influence on the heat transmission but in a quite different way than that of the effect of the rotational stream. Nevertheless our results show clearly that the Re_{ax} is not negligible therefore the more expedient way is measuring their influence on heat transmission apart and take both into account.



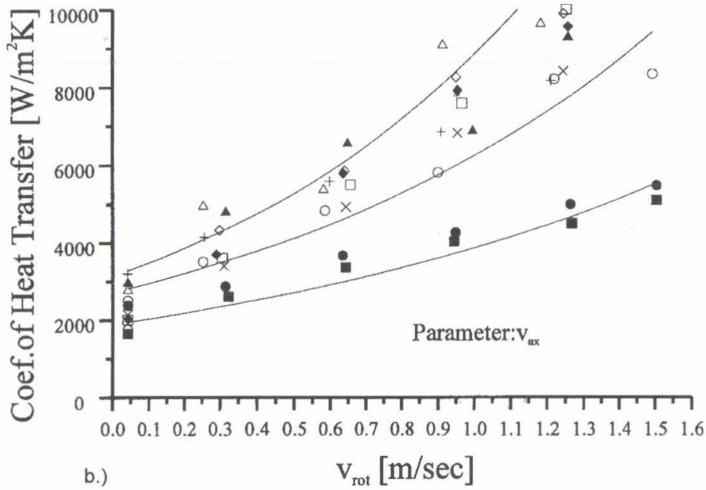


Fig. 1. The coefficient of heat transfer in the function of a./ rotation speed, parameter is the axial speed, b./ axial speed, parameter is the rotation speed.

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TECHNOLOGICAL VALUE OF INULIN CONTAINING CROPS

J. BARTA, GY. PÁTKAI and B. GION

Department of Canning Technology, University of Horticulture and Food Industry, H–1118 Budapest, Ménesi út 45. Hungary

Presence and concentration of inulin in different plant sorts and the technology of inulin production from inulin containing crops was studied on the basis of the literature.

Laboratory analyses have been carried out on 6 varieties of Jerusalem artichoke: Bárdi 3, Büki 20, Gyöngyvér, Rika, Sugárka and Waldspindel, originated from the same growing area: NÖMI Agrobotanical Center Tápiószele.

The objective of our work was the investigation of those chemical and physical properties of the above mentioned varieties, which are significant concerning their processing, and investigation of the changes in the tubers harvested in autumn or in spring.

On the basis of the analysis of variance the variety "Waldspindel" has the highest solid content. Difference between its solid content and that of the variety "Bárdi 3" with the lowest solid content is about 5% (w/w). Total solid content of the tubers is decreasing from autumn to spring significantly as a function of the harvesting time, and the losses run to 3±1% (w/w) during storage of 22 weeks in the soil throughout the winter.

Level of *total sugars* means the sugar content after hydrolysis measured as reducing sugar. There are significant differences in the sugar content of the investigated varieties. The varieties "Waldspindel", Büki 20 and Rika have the highest sugar content. The sugar concentration of the varieties "Sugárka", Bárdi 3 and Gyöngyvér is significantly lower than that of the other ones.

The sugar content of the tubers is generally decreasing during the winter, but this fact is not true for all investigated varieties.

The reducing sugar content of the variety Waldspindel is 0.55%, that value is rising above the others.

The reducing sugar content of the variety Büki 20 is only 0.24%, significantly lower than that of the other varieties. Decreasing sequence of the varieties concerning their reducing sugar content is as follows: Waldspindel, Sugárka, Bárdi 3, Rika, Gyöngyvér and Büki 20.

The linear regression analysis showed no significant relation between harvesting time and reducing sugar content.

On the basis of our results the variety "Gyöngyvér" has a very low *glucose concentration*. Varieties "Büki 20" and "Waldspindel" have a higher glucose content than the other four varieties.

Increasing glucose content could be observed during 22 weeks of storage throughout the winter in the soil. The increase amounts to 0.035% (w/w) weekly.

Ash content, macro-, microelements: The analysis of variance carried out in point of view of the different varieties ensures, that the high content of soluble solids is not accompanied by a high ash content. Although the variety "Waldspindel" has also the highest ash-content, but the variety Rika has a significantly lower ash content than the others, besides the high level of soluble solids.

The inulin content was calculated as the difference of total sugar content and reducing sugar concentration, and the fructose content as the difference between total sugar content (measured after hydrolysis) and the glucose content.

Based on the varieties with the most advantageous chemical composition an inulin retaining technology was developed, and as finished product demineralized inulin-concentrate of good quality was produced.

THE INFLUENCE OF THE RAW MATERIAL ON THE QUALITY OF APPLE CONCENTRATE

E. HORVÁTH and M. STÉGER-MÁTHÉ

Department of Canning Technology, University of Horticulture and Food Industry, H–1118 Budapest, Villányi út 35. Hungary

The Hungarian apple culture is in the period of changing the variety. That probably can affect the technology and the quality of concentrate.

In our experiment (working together with the Department for Fruitgrowing of the University of Horticulture and Food Industry) we have tested the suitability of 20 new varieties and aspirants for variety for concentrate processing.

In this work we determined the raw material's composition (ref. corr., titratable acid, malic acid, tartaric acid, citric acid, lactic acid, glucose, fructose, D-sorbitol, saccharose, minerals) which influence the concentrate quality, and we made also the qualification of the concentrates from apple varieties according to the international standards.

Our tests showed the differences in the components of apple varieties, especially the acid content and quantity of certain minerals effecting the keeping quality of the concentrate and thus the market value. So for example the keeping quality of

concentrate made of Granny Smith, Mutsu, Idared and Prima varieties is better than that made of Jonathan, Jonnee, Jonika and Jonagold.

Our results underline the importance of co-operation between food technologists and fruit growers and breeders to preserve the quality of apple concentrate.

ESTIMATION OF MASS TRANSFER COEFFICIENT IN HOT AIR DRYING

B. GION, I. KÖRMENDY and J. BARTA

Department of Canning Technology, University of Horticulture and Food Industry, H–1118 Budapest, Ménesi út 45. Hungary

The determination of heat and mass transfer in porous capillary bodies is of great practical importance in food technological processes. Typical problems include the drying of fruits and vegetables.

Knowledge of temperature and moisture distribution in these products is important for quality evaluation, equipment and process design, and the evaluation of storage. Development of a mathematical model of coupled heat and mass transfer for the prediction of moisture and temperature profile during drying in a slice shaped solid fruit or vegetable is described.

Altering, non-constant physical and thermal properties were incorporated in this model. The model was applied for air drying of carrot. A finite difference method was used in the solution of coupled heat and mass transfer equations at different times during drying. Experimental studies were carried out on drying of carrot slices (10×10× 4 mm) in a hot air tunnel drier at 70, 80, 90, 100, 110 °C and air flow of 0.7 m s^{-1}. The diffusion coefficient was obtained by the mathematical method. Applying the result of the model a good agreement was found between experimental and calculated data.

NUTRITIONAL IMPORTANCE OF THE SPROUT VEGETABLES AND THEIR APPLICABILITY IN THE FROZEN FOOD INDUSTRY

P. BONTOVICS

Development and Quality Institute of Frozen Food Industry, H–1094 Budapest, Márton u. 3/b. Hungary

Nowadays the reform nutrition and the healthy lifestyle come to the front and several customers are interested in the sprouts of different vegetables. Their nutritional

value is the high vitamin and mineral content. The increasing interest for foods including sprouts is also clear.

The sprouts of different vegetables are very sensitive to the different treatments during processing. Therefore we have to take into consideration the changes in the food after blanching and freezing. These are the changes in vitamin B_2 content and the peroxidase activity during blanching, as well as the formation of colour and dry matter content during freezing. Changes in sensory properties can occur during processing.

The analyses started with eight different seeds (barley, bean, lentil, millet, oats, rye, soya, wheat), from which two were ejected (barley and oats) after measuring the sprout weight. Green sprouts (sprouted in light) and white sprouts (sprouted in dark) were analysed.

The determinations were extended to the frozen and frozen stored sprouts and their dry matter content, colour and to the enzyme activity.

There was no difference in the dry matter content between the raw and frozen sprouts.

In view of results for the colour, vitamin content and enzyme activity the soya, bean and lentil sprouts were suitable for freezing. The primary object is in this case the decoration or completing meals with sprouts.

The profile analysis of these three sprouts was carried out. It was found on the basis of sensory analysis, that the soya sprout is the most suitable for using in frozen products.

EVALUATION OF CURRANT VARIETIES FROM THE VIEW OF THE FOOD PROCESSING INDUSTRY

M. STÉGER-MÁTHÉ, E. HORVÁTH and J. BARTA

Department of Canning Technology, University of Horticulture and Food Industry, H–1118 Budapest, Ménesi út 45. Hungary

With the development of the food processing industry the fruit consuming habit has changed all over the world. There is a little less fresh fruit in the modern diet, but the portion of the different preserved fruit products is larger. During the last few years currant reached a distinguished position among the berry producing shrubs. The demand has especially increased for blackcurrant as it has a high vitamin content.

To ensure the quality of the food processing industry products the role of the variety is important, and it is determining in the cases of certain products. Therefore it is especially important for the farms producing mainly for the food processing industry to consider the demands of the processing plant before planting. The condition for this

is the evaluation of the growable varieties from the view of the food processing industry and choosing the varieties to be planted according to the final use.

In the experiments with 13 currant varieties our primary aim is to examine the components of nutrition physiology importance (such as minerals, acids, carbohydrate, vitamin C) and their change during processing. Our other aim is to determine the components having processing technology importance (such as pectin, water soluble dry materials, pH) and to examine their effect on the processability. In the second phase of our experiments we wish to investigate the quality and storability of the concentrates and drinks made of currant varieties.

The results presented are from this year investigations. We wish to continue our work 2 more years, therefore steady consequences regarding the varieties can only be drawn after the 3 year experiment series.

EFFECT OF IRRADIATION ON THE MACRO- AND MICROSTRUCTURE OF FRUITS

E. KOVÁCS

Department of Microbiology, Central Food Research Institute, H–1022 Budapest, Herman Ottó út 15. Hungary

This program was initiated to promote the quality of fruits and to increase the export possibilities. In the last 30 years a number of fruits (strawberries, red current, cherry, sour cherry, peaches, apricots, apples, pears) cultivated in Hungary were studied. Our results are summarized on this special occasion.

Irradiation (2–3 kGy) reduced spoilage of fruits, but this very positive effect is connected with softening of fruits. Just after irradiation all fruits became significantly softer, but during storage time softening of irradiated fruits only slightly increased. At the end of storage generally there were no significant differences between irradiated and unirradiated fruits. Softening was influenced by season, ripeness and cultivars, etc. Calcium treatment before irradiation (1 kGy) moderated the softening of apples and pears (KOVÁCS et al., 1988). Packaging also reduced softening during storage of the control and irradiated (2.5 kGy) cherry (Germersdorfi óriás) (KOVÁCS et al., 1995).

The tissue structure of fruits in respect of softening was investigated by scanning and transmission electron microscope (SEM, TEM) and chemical analysis. Middle lamellae of cell wall in apple (cv Golden Delicious) changed as a function of increasing radiation dose. Transmission electron micrographes (TEM) showed, that a radiation dose of 0.5 kGy induced minimal swelling of middle lamellae, 1 kGy increased swelling strongly. A dose of 2.5 kGy caused less swelling in middle lamellae as 1 kGy,

and an unknown electron dense material could also be observed in it. At a dose of 5 kGy middle lamellae completely disappeared (KOVÁCS, 1995).

By SEM technique the tissue structure of skin and cortex of apple (Mutsu, Gloster) and pear (Hardenpont) was studied. Differences were observed among apple cultivars in the shrinkage of cells of tissues without any treatment but stored (Golden Delicious, Empire), irradiation (1–2.5 kGy) increased differences between the investigated cultivars (KOVÁCS et al., 1997). Calcium inhibited shrinkage, and the combination of calcium with irradiation (1 kGy) moderated shrinkage of cells in apple (Mutsu, Gloster) and pear cortex (Hardenpont) (KOVÁCS et al., 1988). The role of calcium in the combined treatments is not yet clear. Calcium probably influences several properties of the cells at the same time. Among these changes the effect of calcium on the membranes and mitochondria seems to be also important. The effectiveness of this combined treatment depended on the season and harvest stage of apple. In spite of open questions, the calcium in combination with irradiation helped to preserve the quality of apples, not only the structure, but taste, organic acid content as well (KOVÁCS & DJEDJRO, 1994).

The content of total pectin (Golden Delicious, Empire) in cell walls was reduced during storage and after irradiation (KOVÁCS et al., 1997).

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INVESTIGATION OF IRRADIATED SPICES IN THE FRAME OF THE COPERNICUS PROGRAM

J. KISPÉTER[a] and S. BARABÁSSY[b]

[a]University of Horticulture and Food Industry, College of Food Industry, H–6724 Szeged, Mars tér 7. Hungary
[b]University of Horticulture and Food Industry, H–1118 Budapest, Villányi út 35. Hungary

The ionizing treatment is used on large scale for the microbial decontamination of spices. The irradiated state of the different foodstuffs is detected using the changes in the physical, chemical, and biological properties.

The European Committee of Normalisation accepted 5 standardised methods based on large research co-operation and inter-laboratory trial investigations.

In the CIPA-CT94-0134 COPERNICUS Project six countries (France, Portugal, Belgium, Bulgaria, Poland and Hungary) take part in the period of 1995–1997. This is a co-ordinated research and inter-comparison work to detect irradiated spices and dried fruits using the accepted standards and to elaborate new methods.

In this paper we present the results of our group (Hungary) achieved in 1995.

As investigated samples black and white pepper and Hungarian paprika were used. The samples were treated by gamma (Co 60) and electron beam irradiation.
For the investigation four physical methods were used:

- Viscosity (rheological) method;
- Thermoluminescence method;
- Electron spin resonance method;
- Near infrared spectroscopy method.

The obtained results showed significantly the fact of the irradiation for all detection methods and it was possible to detect whether the samples were pre-irradiated.

These methods could be suitable in the future to be used in authorised reference laboratories for the control of the imported and exported foodstuffs.

OBJECTIVE QUALIFICATION OF PACKAGING MATERIALS FOR CONSUMER PACKAGES USED TO DRIED FOODS; BASIC METHODS AND RESULTS

J. MONSPART-SÉNYI and I. VARSÁNYI

Department of Canning Technology, University of Horticulture and Food Industry,
H–1118 Budapest, Ménesi út 45. Hungary

One of the main duties of packaging is to keep the quality and the quantity of packed foods from processing to consuming. In the case of dried foods with high volatile oil and aroma content the barrier property (water vapour and aroma), the oil and the grease resistancy are important aspects of packaging material selection.

One of the goals of our researches was to test some flexible packaging materials produced in Hungary and to determine their barrier behaviour.

The investigations formed three groups: mechanical test, package forming test and permeability test. The mechanical tests cover strengthen test, elongation test, tensile test, bursting test. In the frame of package forming test the effects of various technologies were determined on the quality of seals (mechanical strength and permeability). The water vapour and the gas permeability of the packaging materials were also tested.

After the test results achieved the packaging materials were grouped for short-term, middle-term and long-term shelf-life packaging of dried foods. The representative materials belonging to each group are as follows: paper based packaging materials coated by emulsified polymers or micro-waxes; paper and polymer combinations; paper – alu foil – polymer lamination. The results proved that the investigated Hungarian flexible packaging materials are suitable for dried food packaging with high aroma content in pouch form of 5–1000 g filling mass. The tested materials are well printable with consumer information and also advertisment.

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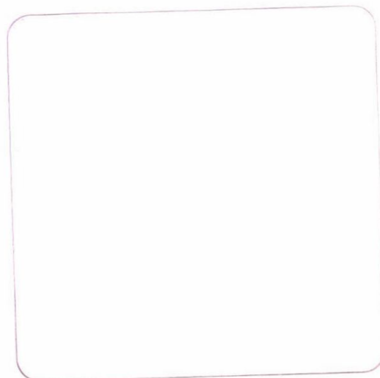

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ACTA ALIMENTARIA

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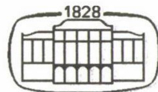

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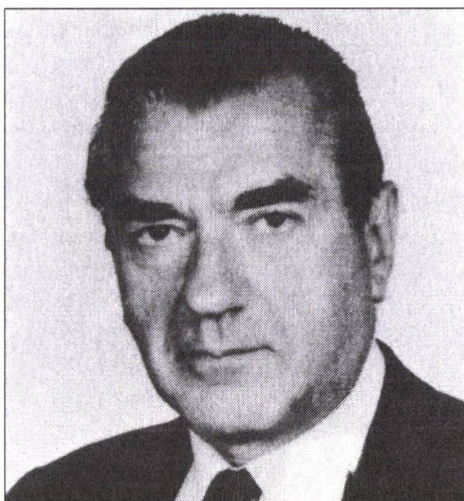
IN MEMORIAM I. VARSÁNYI
1931-1997



Dr. Iván Varsányi, University Professor and Doctor of Technical Sciences, passed away with tragic unexpectedness on 13 August 1997. His name was well known all over the world for the readers of Acta Alimentaria. From 1976 Dr. Iván Varsányi was Co-ordinating Editor of Acta Alimentaria. The readers had the opportunity to become acquainted with his conscientious and thorough work for 20 years.

Iván Varsányi was born on October 23, 1931, Budapest. He graduated as organic chemist from the Chemical Engineering Faculty of the Technical University of Budapest in 1955. In the academic year of 1966/67 he was a postgraduate fellow at the Department of Chemical Engineering of the University of Iowa (USA). He became University Doctor in 1970 in the field of food technology at the University of Horticulture, Budapest. He obtained the degree of Candidate in Chemical Sciences in 1973, the degree of Doctor of Technical Sciences in 1993, at the Hungarian Academy of Sciences. In 1985 he became Honorary University Associate Professor, then Honorary University Professor in 1992, and Dr. habil. in 1994 at the University of Horticulture and Food Industry. His activities were acknowledged by numerous commemorative medals and awards in his native country and abroad.

After the years in the industry he worked at the Institute for Logistics and Packaging. He joined the Central Food Research Institute in 1962 and served there for almost 30 years at first as scientific officer, later as Scientific Secretary, then Head of the Department of Research Management from 1980. His scientific activities were

primarily focused on the packaging materials of foods, the interaction between the food and packaging, the changes in quality and quantity of the fresh and processed food, and their prediction. He was engaged in science organisation and scientific planning, scientific documentation, library development, the application of appropriate information systems for the characterisation of the produce of agrarian-economy which are compatible with international systems.

His university career started as invited lecturer in 1974, visiting Associate Professor (1986), and later Professor (1988), respectively. He was appointed in 1991 as the Head of Department of Canning Technology at the University of Horticulture and Food Industry until 1996. He was invited speaker also to international institutions and organisations.

He took part in graduate and postgraduate training both in Hungary and abroad from 1969. From 1977 he delivered special seminar course entitled "Selected chapters from the packaging technique of preserving industry" at the University of Horticulture and Food Industry.

He was successful participant in international projects. His results were published in nearly 150 publications, one third of which is in foreign languages. He was co-author of two books, and had two books published, the third manuscript was not finished because of his death. He took an active part in the scientific public life and work of committees and board of leaders of scientific societies. He worked as an expert for the UNIDO (1972), FAO (1982), and UNCTAD/GATT (1987), the specialized Organisations of the United Nations abroad, and he also was a lecturer of UNIDO and FAO Seminars. He was a member of IUFoST, ILSI, INFOODS, International Langual Steering Committee, International Association of Packing Educators, EASE and the representative of Hungary in the Management Committee of Co-operation in Science and Technology as well as co-representative at EUROFOODS. He performed editorial board activities for several papers, next to being the Co-ordinating Editor of *Acta Alimentaria* from 1976 he also was Editorial Board Member for the *Journal of Food Science and Technology* from 1994.

Dr Iván Varsányi had a rich career accompanied by great success. The enumeration of achievements is an overview of a hard working life, which passed with a strong will-to-do in mind. His activities were acknowledged both in Hungary and abroad.

He was in a lot of countries on study tours as an expert of lecturer. He became to know great many people, and even more people knew him and his activities. He shared his rich experience with his colleagues and pupils. His work will be continued. His co-workers pupils and friends both in Hungary and abroad will remember him for a long time and preserve his memory.

I.F. Kiss

Acta Alimentaria, Vol. 26 (4), pp. 345–358 (1997)

PREPARATION OF CHROMIUM ENRICHED YEASTS

J. HEGÓCZKI[a], Á. SUHAJDA[b], B. JANZSÓ[b] and G. VERECZKEY[a]

[a] Department of Bioengineering, Central Food Research Institute,
H–1111 Budapest, Budafoki út 59. Hungary
[b] Department of Agricultural Chemical Technology, Technical University, Budapest,
H–1521 Budapest, Szt. Gellért tér 4. Hungary

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Chromium (Cr) is an essential micronutrient for humans. Its main action is thought to be regulation of blood sugar, since chromium deficiency is associated with diabetic-like symptoms and Cr-supplement is associated with enhanced glucose tolerance and insulin sensitivity. The beneficial dietary form of Cr is an organometallic compound called glucose tolerance factor (GTF). Although it is known that brewer's yeast is a good natural source of GTF-Cr, the concentration is usually quite low (2 to 4 $\mu g\ g^{-1}$ yeast solids). Since the organically bound form of chromium found in brewer's yeast is known to be both assimilable and efficient as a dietary source of chromium to alleviate deficiencies and impaired glucose tolerance, it would be desirable to provide supplemental chromium in a more concentrated useful form that could be used in small amounts as a dietary chromium supplement.

Under adequate circumstances yeasts are capable of accumulating in their cells and incorporating into organic compounds large amounts of trace elements.

In present investigations chromium has been introduced into *Saccharomyces cerevisiae*, *Candida utilis* and *Schizosaccharomyces pombe* cells. These yeast strains are able to assimilate Cr(III)-chloride and accumulate chromium in their cells. In case of *Saccharomyces cerevisiae* Cr incorporation into the cells in exponential phase led to approximately 10–15% protein increase compared to normal baker's yeast.

These new natural trace element resources are more suitable for and favourable to both human and animal organisms.

Keywords: trace elements, chromium, enrichment, yeast

Chromium (Cr) has been known as an essential nutrient for animals and humans for at least 30 years. In case of plants chromium is not an essential trace element, although some researchers consider Cr has stimulative effects.

Its documented beneficial role in human health maintenance spans from blood sugar to cholesterol regulation and its deficiency has been linked with increased incidence of age related non-insulin diabetes, hypoglycaemia, food allergies, high blood levels of placque-forming cholesterol, and coronary heart disease. Furthermore,

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adequate intake of dietary Cr can be an important aspect of successful muscle toning, athletic fitness, and weight loss programs (BURTON, 1995).

Inorganic Cr salts are not as readily absorbed or biologically active as organic forms of chromium. The beneficial dietary form of Cr is an organometallic compound called glucose tolerance factor (GTF). Good natural sources of GTF-Cr are brewer's yeast, organ meats, pepper, and oatmeal. Although the exact chemical structure of GTF has been elusive, SCHWARZ and MERTZ (1959) determined it to consist of one atom of Cr^{3+} bound to several molecules of niacin, and probably the amino acids found in glutathione (glutamic acid, glycine, and cysteine). Without Cr^{3+} at its core, GTF is inactive.

GTF is required for normal metabolism of carbohydrates, proteins, and lipids (MERTZ, 1982). The Cr^{3+} atom of GTF is thought to facilitate interactions between insulin and insulin receptors on target tissues such as muscle and fat (MOORADIAN & MORLEY, 1987). In this way, GTF potentiates anabolic activities of insulin. Insulin's main function is to regulate blood glucose levels.

GTF also aids in the conversion of thyroxine (T_4) to triiodothyronine (T_3), the thyroid hormone that increases metabolic rate causing increased oxygen consumption, heat production, metabolism of fats, proteins and carbohydrates in the liver, kidney, heart and muscle, cardiac output, neutral irritability, and RNA and protein synthesis.

In the USA the recommended daily allowance (RDA) of dietary Cr for adult humans is 50 to 200 μg (in Hungary it is 120 μg; BÍRÓ & LIDNER, 1988). Under these guidelines, up to 90% of American adults are Cr deficient because they typically consume only 40–60% of the minimum requirement (average 25 μg per adult day; ANDERSON, 1987). Although in Hungary the data are not known exactly, the situation can be similar. Part of the problem is that most foods naturally rich in Cr are considered unpalatable (e.g. brewer's yeast and organ meats), and that processing removes up to 80% of the Cr found in other foods (e.g. grains). However, Cr deficiency can also be caused or confounded by factors such as high intakes of dietary sugar, refined carbohydrates, carboloading (commonly done by athletes), strenuous exercise, pregnancy (maternal transfer to the developing fetus), disease, and numerous other forms of emotional, physical, metabolic, and environmental stress (ANDERSON et al., 1990; 1991). Therefore factors that chronically elevate blood glucose levels are a significant cause of Cr deficiency.

It was observed that natural organic chromium complexes isolated from brewer's yeast or certain other natural products differed from simple inorganic chromium compounds in respect to the rate of intestinal absorption, tissue distribution, and in other effects. When chromium deficiency occurs, dietary supplement with high levels of inorganic chromium compounds is needed to restore chromium sufficiency, as

contrasted to the much lower levels of organically bound chromium that are required to counteract nutritional deficiency.

Since the organically bound form of chromium found in yeast is known to be both assimilable and efficient as a dietary source of chromium to alleviate deficiencies and impaired glucose tolerance, it would be desirable to provide supplemental chromium in a more concentrated useful form that could be used in small amounts as a dietary chromium supplement.

Under certain conditions yeasts are able to take up trace elements and form organic bonds at concentrations several times higher than the normal level. These novel trace element resources are more suitable for, and favourable to, both human and animal organisms (JANZSÓ et al., 1990).

The recommended daily allowance (RDA) of dietary Cr for adult humans is 120 μg in Hungary. We supposed that the remarkable part (15–30%) of chromium in this form is really absorbed. Considering these facts RDA can be ensured by the consumption of approximately 0.5 g from 1000 $\mu g\ g^{-1}$ chromium yeast.

1. Materials and methods

In our investigations we enriched chromium in yeast cells. The accumulating organisms were *Saccharomyces cerevisiae* (baker's yeast), *Candida utilis* (yeast for feedstuff) and *Schizosaccharomyces pombe.*

The chromium salt used to treat the yeasts was a water-soluble, non-toxic chromium source: chromic chloride hexahydrate ($CrCl_3 \cdot 6H_2O$).

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 media were identical.

The medium was poured into 750 cm^3 Erlenmeyer flasks (150 cm^3 in each) and sterilized. After inoculation from agar surface yeast was grown in shaking flasks (350 rpm, 30 °C, 20–24 h). The composition of growth medium is shown in Table 1.

In our lab scale experiments we used two accumulation methods: enrichment in growing cells and enrichment in non-growing cells.

In the first method Cr was added to the culture in the exponential phase of yeast growth. Cultivation was carried out in 10 dm^3 laboratory scale fermenters with 5 dm^3 actual broth volume at 30 °C, 700 rpm agitation, batch technique, 1 $v\ v^{-1}\ min^{-1}$ aeration. The amount of inoculum was 10% of the broth volume. The composition of medium is shown in Table 2.

Table 1

Composition of nutrient medium for shaking flasks

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

However, this method usually has a disadvantage. In most cases the addition of microelements during cultivation more or less inhibits the yeast growth.

Table 2

Composition of nutrient medium for lab fermenter

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, 15 min. in autoclave

In the second method we used separated baker's yeast milk (*Saccharomyces cerevisiae*). The uptake period was essentially non-growth period where the yeast cells in the fermenter were in an aqueous suspension in the presence of the dissolved chromium salt. The circumstances (aeration, agitation, initial pH, volume) were the same as those in the growth phase accumulation. This method has numerous advantages in comparison to "classic" growing cell accumulation:

- the required amount of trace element can be calculated exactly;
- the inhibitory effect of trace element can be eliminated almost completely;
- the fermentation technique becomes significantly simpler;
  - reduced fermentation time (4–8 h);

– it can be carried out under semi-sterile conditions;
– the fermentation can be monitored by the measurement of dissolved oxygen level;
• the economy of production is improved.

In 1982 SKOGERSON issued a US Patent with the name of *"Production of chromium yeasts"*. In his patent he described another method for preparation of chromium yeast. First he produced about 1000 $\mu g\ g^{-1}$ chromium yeast called "*seed yeast*" with a resting cell method. Then he cultivated this "*seed yeast*" to get about 50–200 $\mu g\ g^{-1}$ chromium yeast, which could be utilized directly without inert material dilution. In our experiments we worked out such a resting cell method – in addition to "classic" growing cell method – in which we used separated yeast milk from a yeast factory for accumulation instead of production of biomass. (Advantages are listed in the previous paragraph.) With our method we are able to accumulate not only chromium but numerous other microelements easily as against the growing cell method.

In the course of cultivation yeast growth was followed by cell density measurement. Samples were measured spectrophotometrically at 550 nm in comparison with non-inoculated medium, applying 10-fold dilution. For dilution 0.01 n HCl was used.

Glucose contents of the samples were determined by Schoorl's reduction method. Separation of biomass from the nutrient media was carried out by centrifugation for 30 min at 3000 rpm. After being washed with tap water three times and repeated centrifugation the yeast biomass was supplemented with acetone in excess. Upon the effect of this the biomass precipitated in the form of fine flocculants and could be filtered with a water jet pump. After filtration yeast was dried at room temperature and stored in small bottles until analysis.

Cr contents of the samples were 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 left 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 analyser. Samples were prepared (except for determination of cysteine) by the following steps: 10 cm^3 3 mol dm^{-3} p-toluene sulphonic acid (containing 0.2% triptamine) was added to a sample (corresponding to 30 mg protein), then hydrolysed at 110 °C for 22 h. After partial neutralization with 2 mol dm^{-3} NaOH sample was filled up to end volume with distilled water (TÖMÖSKÖZI et al., 1993).

For determination of cysteine, 10 cm^3 6 mol dm^{-3} HCl (containing 2.5% DMSO) was added to a sample (corresponding to 60 mg protein), then after bubbling through with nitrogen gas (3 min), samples were hydrolysed at 10 °C for 22 h. When hydrolysis

was completed, samples were partially neutralized with 10 cm^3 5 mol dm^{-3} NaOH solution and finally supplemented to a volume of 25 cm^3 with Na-citrate (pH=2.2). There is another method for determination of cysteine: determination in form of cysteine acid after oxidation with performic acid to avoid undesired cysteine oxidation or reduction transformation during protein hydrolysis. Although this method is as accurate as our determination (both methods' reproducibility and standard error are 1–3 per cent) we chose the first cysteine determination method because of easier realization. In this case forming cysteine acid and hydrolysis occur in one step, while in the second method the cysteine oxidation and hydrolysis take place in two steps one after the other. The determination with DMSO takes one day, while the determination with performic acid takes two days (SIMON-SARKADI & SZERZŐ, 1985).

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 570 and 440 nm

For the measurement of Cr 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 at –24 °C until used. Before the ICP measurement the thawed samples were centrifuged (30,000 rpm, 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 $CrCl_3 \cdot 6H_2O$ in different concentrations. We wanted to know, whether the above described Cr introduction affects yeast growth and whether the yeast is capable of accumulating chromium. Our results are presented in Fig. 1 and Table 3.

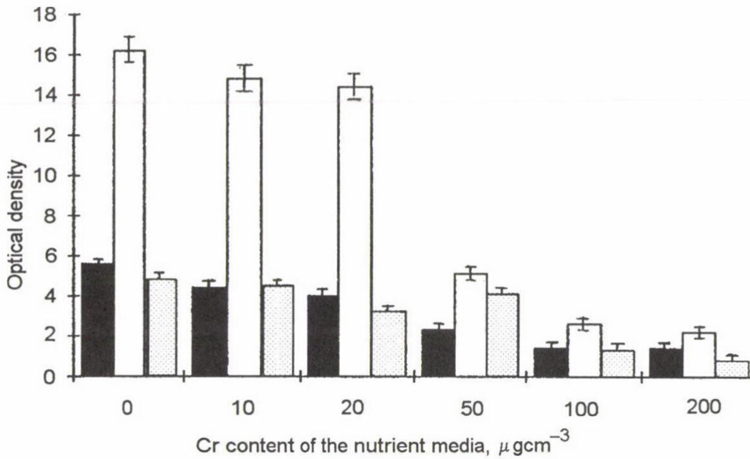

Fig. 1. Optical density of yeasts cultivated in shaking flasks with $CrCl_3 \cdot 6H_2O$ supply (sample number: n=3; probability level: P=0.05). ■: *Sacch. cerevisiae*; □: *Candida utilis*; ▒: *Schizosacch. pombe*

Table 3

Significance table of optical density values as a function of Cr content (shaking flasks)

Yeast	Cr content of the nutrient media (μg cm^{-3})					
	0	10	20	50	100	200
Sacch. cerevisiae	a	b	b	c	d	d
Candida utilis	a	b	b	c	d	d
Schizosacch. pombe	a	a	b	c	d	d

In one row the same letters mean that there is no significant difference at P=0.05 probability level

The data clearly show that chromium(III) hinders yeast growth in some degree already at low concentrations (10–20 μg cm^{-3}). The growth inhibition of Cr is increased remarkably at 50 μg cm^{-3} and above. At 200 μg cm^{-3} the inhibition of Cr is so high that the biomass production is almost totally repressed.

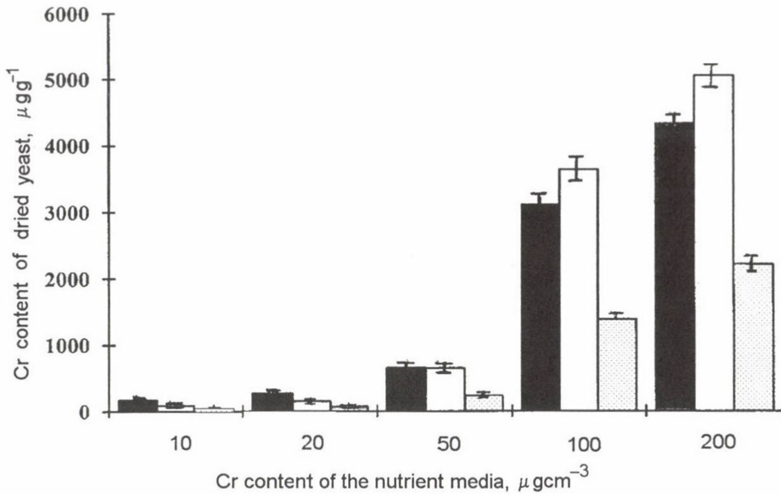

Fig. 2. Cr uptake of yeasts cultivated in shaking flasks with $CrCl_3 \cdot 6H_2O$ supply (n=3; P=0.05). ■: *Sacch. cerevisiae*; □: *Candida utilis*; ▒: *Schizosacch. pombe*

Table 4

Significance table of Cr accumulation values (shaking flasks)

Yeast	Cr content of the nutrient media ($\mu g\ cm^{-3}$)				
	10	20	50	100	200
Sacch. cerevisiae	a	b	c	d	e
Candida utilis	a	b	c	d	e
Schizosacch. pombe	a	b	c	d	e

In one row the same letters mean that there is no significant difference at P=0.05 probability level

From Fig. 2 and Table 4 it seems obvious that Cr incorporation is proportional to the amount introduced. Considerable Cr uptake (200–700 $\mu g\ g^{-1}$) occurred only at 50 $\mu g\ cm^{-3}$ Cr addition. There are remarkable Cr accumulations at 100 and 200 $\mu g\ cm^{-3}$ Cr addition. However, due to the inhibiting effect of Cr in the course of the following lab scale experiments we supplied the nutrient medium with 50 and 100 $\mu g\ cm^{-3}$ concentration of chromium.

In these assays we used growth phase accumulation method. The yeast strains were cultivated in laboratory scale fermenter, where Cr was added in 50 and

100 μg cm^{-3} concentrations at the beginning of the exponential phase of yeast growth. Our results have been summarized in Fig. 3 and Table 5.

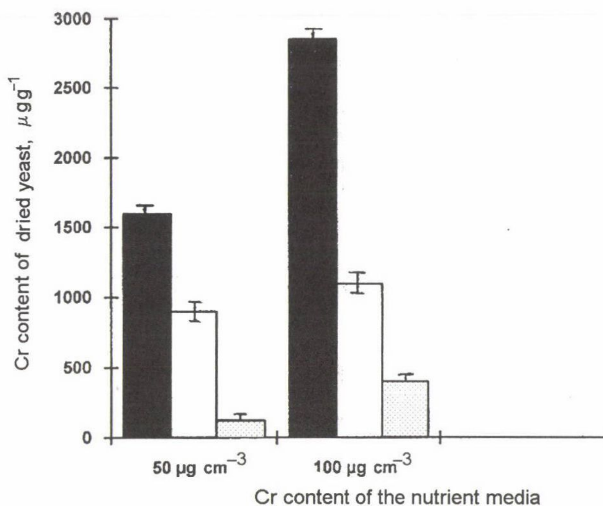

Fig. 3. Cr uptake of yeasts cultivated in lab fermenter with $CrCl_3{\cdot}6H_2O$ supply (n=3; P=0.05). ■: *Sacch. cerevisiae*; □: *Candida utilis*; ▒: *Schizosacch. pombe*

Table 5

Significance table of Cr accumulation values (lab fermenter)

Yeast	Cr content of the nutrient media (μg cm^{-3})	
	50	100
Saccharomyces cerevisiae	a,1	b,1
Candida utilis	a,2	b,2
Schizosaccharomyces pombe	a,3	b,3

In one row the same letters mean that there is no significant difference at P=0.05 probability level
In one column the same numbers mean that there is no significant difference at P=0.05 probability level

Apparently Cr uptake is also proportional to the amount introduced. The highest Cr incorporation was in *Saccharomyces cerevisiae* followed by *Candida utilis* and *Schizosaccharomyces pombe*.

The next experiments in lab scale fermenters were carried out with *Saccharomyces cerevisiae* by detailed examinations of 50 μg cm^{-3} Cr addition. Changing the conditions of cultivation the effects of another inorganic Cr compound supply /$Cr_2(SO_4)_3$/, pH and aeration are presented. In Fig. 4 data for Cr uptake are collected, in Fig. 5 those with pH adjusted, to 5.0 are presented.

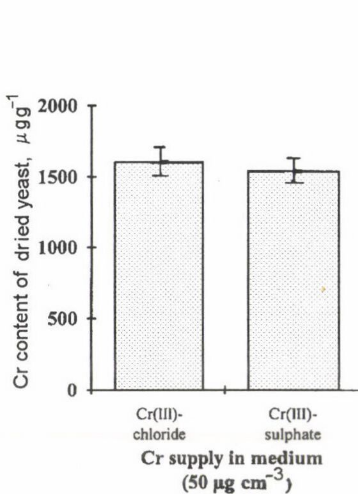

Fig. 4. Effect of inorganic Cr-compound supply (n=3; P=0.05)

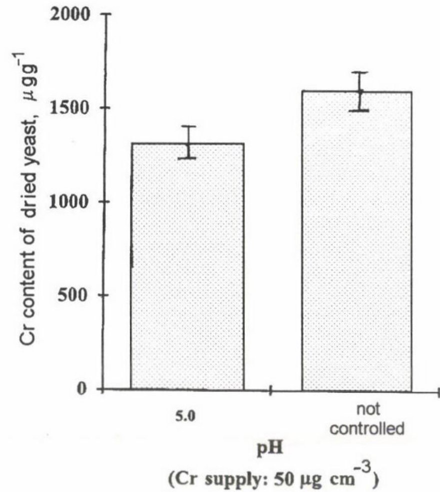

Fig. 5. Effect of pH (n=3; P=0.05)

No significant differences can be observed (at P=0.05 probability level) in Cr accumulation and yeast growth between the experiments with Cr(III)-chloride and Cr(III)-sulphate.

With the increase (and simultaneous control) of the pH value, Cr uptake decreased in comparison with productions without pH adjustment.

The results in Fig. 6 were obtained by cultivation with reduced aeration. Under conditions of reduced aeration (0.5 v v^{-1} min^{-1}) Cr absorption in baker's yeast was significantly lower (more than 50%, P=0.05) than observed with 1 v v^{-1} min^{-1} aeration.

In the next investigations we applied a new accumulation method: the non-growth phase (resting cell) method. This procedure is more simple and accurate than the previous "classic" growing cell method. Another advantage is that the strong inhibitory effect of chromium can be eliminated almost completely.

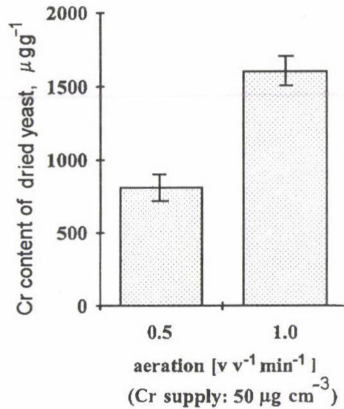

Fig. 6. Effect of aeration (n=3; P=0.05)

In the non-growth phase accumulation Cr(III)-chloride was added to the resting baker's yeast cells. Based on dry matter content of separated yeast milk (200 g dm^{-3}) we calculated the theoretical Cr requirement. In practice we added double amount into the medium. Our results have been summarized in Table 6. The difference between the two sets was in accumulation time.

Table 6

Cr-enrichment in separated yeast milk (non-growth phase accumulation, n= 3)

	Accumulation A		Accumulation B
Amount of yeast milk		2 dm^3 (400 g dry matter)	
Trace element		Chromium in form of $CrCl_3 \cdot 6H_2O$	
Desired Cr content		1000 μg g^{-1}	
Theoretical Cr addition		400 mg	
Experimental Cr addition		800 mg	
Accumulation time	4 h		24 h
Weight of dried Cr-yeast	522 g		588 g
Cr content of dried yeast	233 μg g^{-1} (SD=18)		535 μg g^{-1} (SD=23)
Residual Cr conc. in medium	22 μg cm^{-3}		51 μg cm^{-3}

We could achieve the desired Cr content in neither enrichment. The Cr uptake was twice as high in accumulation B, where the accumulation time was longer than in accumulation A.

For comparison of growth phase and non-growth accumulation methods we studied Cr incorporation and amino acid content of Cr-yeasts.

Cr containing cells were disrupted in ultrasonic wave equipment. After separation of the solid moieties and the filtrate Cr contents of the fractions were measured by ICP. The results are given in Table 7.

Table 7

Study of Cr incorporation (n=3)

Accumulation	Cr uptake	Incorporated Cr
In growth phase	1600 $\mu g\ g^{-1}$ (SD=34)	1412 $\mu g\ g^{-1}$ (SD=53) 88%
In non-growth phase	535 $\mu g\ g^{-1}$ (SD=23)	304 $\mu g\ g^{-1}$ (SD=35) 57%

Results obtained verify that great part of accumulated chromium is incorporated probably into organic compounds.

Amino acid analysis has also been performed for chromium containing yeasts obtained by enrichment in cultivation and in resting cells. These data are shown in Table 8 compared to amino acid composition of the control yeast. Cr uptake caused simultaneous increase in amino acid concentration.

Chemical analysis of chromium enriched baker's yeast has revealed further interesting information. In growth phase accumulation Cr increased protein content of the yeast with 13 per cent. This increase can be of importance in industrial production of yeast. The protein content did not change practically in non-growth phase accumulation.

A conspicuous result is the cysteine content decrease in both chromium yeast samples in comparison to the normal yeast (cysteine content: 14% and 17% respectively). A possible reason of this decrease is that the high chromium content may catalyse the breakdown of cysteine.

3. Conclusion

Saccharomyces cerevisiae (baker's yeast), *Candida utilis* (yeast for feedstuff) and *Schizosaccharomyces pombe* are able to assimilate Cr(III)-chloride and accumulate chromium in their cells. The addition of chromium resulted in the following best Cr uptake values summarized in Table 9.

Experiments with *Saccharomyces cerevisiae* in lab scale fermenters were carried out by detailed examinations. Two accumulation methods were applied; the growing cell and the resting cell method.

Table 8

Amino acid content of Cr enriched and control baker's yeast

Amino acid	Control yeast	Cr enriched yeast: enrichment in growth phase (1600 μg g^{-1} Cr)		Cr enriched yeast: enrich. in non-growth phase (535 μg g^{-1} Cr)	
	mg	mg	%	mg	%
Asp	40.9	53.2	130	45.5	111
Thr	26.4	25.1	95	20.5	78
Ser	18.1	24.0	133	19.9	110
Glu	60.5	59.9	99	62.4	103
Pro	18.4	16.9	92	29.3	159
Gly	17.1	19.7	115	16.7	98
Ala	19.5	24.3	125	21.0	108
Cys	3.6	0.5	50	0.6	60
Val	18.4	26.9	146	18.5	101
Met	5.0	8.0	160	3.0	60
Ile	14.9	24.0	161	15.3	103
Leu	28.4	35.7	126	25.5	90
Tyr	16.2	19.7	122	14.8	91
Phe	19.3	25.8	134	19.5	100
His	11.1	16.3	147	14.8	133
Lys	46.9	41.8	89	35.0	75
Arg	31.4	25.9	82	22.2	70
Total	396.1	447.7	113	384.5	97

Table 9

Chromium enrichment in yeasts

	Enrichment in growth phase: Cr content in medium: 50 mg cm^{-3}	Enrichment in growth phase: Cr content in medium: 100 mg cm^{-3}	Enrich. in non-growth phase: Cr content in medium 160 mg cm^{-3} (for 400 g yeast)
Saccharomyces cerevisiae	1600 μg g^{-1}	2850 μg g^{-1}	535 μg g^{-1}
Candida utilis	900 μg g^{-1}	1035 μg g^{-1}	–
Schizosaccharomyces pombe	125 μg g^{-1}	400 μg g^{-1}	–

In the first procedure that we have used for about ten years the chromium-chloride addition occurred in the exponential growth phase of the baker's yeast. Changing the conditions of cultivation the effect of pH and aeration is presented. From

these results it seems obvious that Cr uptake decreased with the increasing (and simultaneous control) pH value or with the decrease of aeration.

Cr incorporation into the cells in exponential phase led to approximately 10–15% increase in protein content compared to normal baker's yeast.

In the new resting cell method the chromium-chloride was added in the non-growth (resting) phase of the baker's yeast. With this method we managed to eliminate the strong inhibitory effect of chromium we experienced in preliminary shaking flask and lab scale growing cell experiments. Another benefit of this method is the simplification of the fermentation technique. Although the Cr accumulation was about 25–50% of the Cr-yeast produced by growing cell method, the weight of bio-mass was 20 times higher than in case of growing cell method (Table 9).

On the basis of cell disruption by ultrasonic digestion it seems that greater part of chromium (60–90%) accumulated in the yeast produced by any methods is very likely present in organic bond.

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EFFECT OF CHILLING AND IRRADIATION ON THE ULTRASTRUCTURE OF THE MEMBRANES AND MITOCHONDRIA OF FRUITS AND VEGETABLES

Review

E. KOVÁCS

Department of Microbiology, Central Food Research Institute, H–1022 Budapest, Herman Ottó út 15. Hungary

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Ultrastructural effects of irradiation were investigated on the specific constituents of plant cells, primarily in fruits.

The effect of aging and environmental factors, especially cold, chilling and irradiation on the membranes and mitochondria are summarized. During fruit ripening, progressive loss of membrane function occurs. The changes were correlated with the lipid composition of the membranes. There is also evidence of changes in membrane viscosity/fluid. Temperature dependent changes can be observed in membranes. Low temperature (10 °C, chilling) have a drastic effect on cellular structure. Mitochondria are swollen, and membranes are disorganized.

Irradiation induced membrane permeability (electrolyte leakage), and free radicals were formed as a result of ionizing radiation. The changes noticed in the matrix and cristae of mitochondria occur mainly at radiation doses higher (5 kGy) than practical doses (upto 2.5 kGy).

Calcium helped to prevent the increase in microviscosity in membranes, protected cells against free radicals and preserved the ultrastructure of cell compartments.

Keywords: fruits, vegetables, mitochondria, membranes, lipids, ultrastructure, irradiation, free radicals, chilling, calcium

1. Introduction

Postharvest physiologists are interested in studying the biochemical processes during shelf life of fruits and vegetable.

There are a number of references about the chemical and biochemical changes of foods, however recently the ultrastructural studies have become increasingly important.

The aim of this study is to compare the present knowledge on the ultrastructure of environmental factors (chilled, low oxygen storage, etc.) and irradiated fruits and vegetables.

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This paper is focused on the effect of irradiation on mitochondria and membranes. Mitochondria are chosen because their role in the biological oxidation is very important. The research on functional and structural changes of membrane lipids due to chilling and irradiation is particularly important.

2. The effect of aging and environmental factors on the structure and functionality of membranes and mitochondria

Physicochemical changes in membrane structure and properties:

- enzymes mediating lipid degradation during senescence,
- the mechanism of induction of senescence by ethylene,
- ATP-ase activity during senescence,
- compartmentalization of membrane lipid degradation,
- involvement of free radicals in senescence processes,
- interdependence between cell wall and plasma membrane (PALIYATH & DROILLARD, 1992).

Generally, the early phase of aging is characterized by an increased saturation of fatty acids resulting in a net increase in microviscosity of membrane lipids. Membrane lipid peroxidation also induced senescence. The protein in the membrane is altered as a result of enzymic degradation, which leads to cross-linking. Electrolyte leakage increases during ripening of fruits. Ion leakage has been used as an indicator of damage to the plasma membrane (MARANGONI et al., 1996). Decreases in temperature resulted in a transition of lipids from the liquid crystalline to a gel phase bilayer configuration. The creation of discrete gel phase regions can be detected by freeze-fracture electron microscopy and appear as smooth, particle-free domains in fracture face views of the membrane. The lipid phase separation was associated with functional changes in the membrane, including loss of selective permeability (QUINN, 1989). The greatest changes in membrane properties can be seen as the fruits reached its climacteric and this corresponded with changes in the sterol:phospholipid ratio in the membranes. However, during the transition from climacteric to post-climacteric, the increased saturation of fatty acids also contributes to increased membrane viscosity. Decreases were also found in fatty acid unsaturation level, but primarily in the post-climacteric stage of ripening (LURIE & BEN-ARIE, 1983). Ethylene promotes senescence, and indirectly influences membrane deterioration (THOMPSON, 1986).

Changes in physical and chemical properties of the plasma membrane from hypodermal mesocarp tissue of netted muskmelon fruit progress from immaturity to maturity, and with storage at 4 or 24 °C. An increase in the saturation index of the plasma membrane phospholipids, increased permeability of tissue were observed in

mesocarp tissue of muskmelon. It is suggested that biochemical changes affecting the lipid matrix of the plasma membrane influence fruit membrane permeability (LESTER & STEIN, 1993).

Slow changes in the metabolism of banana fruits occur before the respiratory climacteric begins. Some of these changes, such as increases in respiration rate, soluble solids, and the linolenic acid content of phospholipids, are qualitatively similar to changes that occur more rapidly during the climacteric. The phospholipids of ripening bananas undergo similar changes either the fruits are held in ethylene free air or treated with ethylene or propylene. The total content of phospholipids does not change during ripening, but the proportion of linoleic acid increases and decreases respectively. These changes in membrane lipid composition may be incidental to the mechanism of ripening, but the lipids may have a regulatory function. Membrane lipids do not regulate climacteric respiration directly, since in fruit treated with propylene respiration increased before lipid polyunsaturation increased. Some other of the metabolic changes that occur during the climacteric could perhaps be affected by the membrane lipids, whether by control of substrate concentration or modulation of enzyme activity (WADE, 1995).

Sterol content in senescing membranes can either increase (DUXBURY et al., 1991; LURIE & BEN-ARIE, 1983), or remain constant (MAKHLOUF et al., 1990), while phospholipids are rapidly lost during senescence (PALIYATH & DROILLARD, 1992). Changes in phospholipids and sterol contents bring about an increased sterol:phospholipid ratio that has been considered responsible for the increase in membrane microviscosity observed by many authors in apples (LURIE & BEN-ARIE, 1983; LURIE et al. 1987), tomatoes (LEGGE et al., 1986), banana (WADE, 1995), broccoli (DESCHENE et al., 1991) and muskmelon (LESTER & STEIN, 1993).

A common feature accompanying senescence is increased membrane permeability, expressed as increasing leakage of ions (THOMPSON, 1986; STANLEY, 1991). Increase ion leakage has been observed in senescing apples (LURIE & BEN-ARIE, 1983; LURIE et al., 1987), papayas (CHAN, 1991), tomatoes (PALMA et al., 1995) potatoes (KNOWLES & KNOWLES, 1989). In apples this leakage correlated with increased membrane viscosity and decreased degree of fatty acid unsaturation (LURIE & BEN-ARIE, 1983; LURIE et al., 1987). In cucumbers, elevated ethane productions (an indication of lipid peroxidation) was observed parallel to ion leakage (KUO & PARKIN, 1989). Increased phase transition temperatures of membrane lipids and a decline in fluidity have been described in the senescence of flowers; these events preceded enhanced ethylene production and ion leakage (FARAGHER et al., 1986). All these data suggest that compositional changes that determine the decreased fluidity of membranes are translated into leakage of ions, and therefore reduced functionality of membranes (MCCORMAC et al., 1993; MCKEGNEY et al., 1995). However, PALMA and co-workers

(1995) suggest that the increases in ion leakage observed for senescing tomato fruit are significantly correlated to losses in microsomal membrane K^+ stimulated H-ATPase activity and not to the saturation index of membrane lipids. Therefore, in that study at least, degradation of membrane lipids, as judged by the saturation index, was not the mechanism by which increased ion leakage occurred. However, ion leakage and ATPase activity could be correlated to losses in linolenic acid, a fatty acid particularly prone to oxidation. These findings agree with work by MARANGONI and co-workers (1996) who reported that sublethal freezing injury induced ion leakage was due to functional and/or structural changes in membrane associated H^+ ATPase.

Many of the theories on cold acclimation or cold injury are in some way centered on membranes, primarily the plasma membrane. It is also possible that the dramatic changes in sterol metabolism during chilling and after rewarming are involved in the loss of membrane function, particularly in the sterol-rich plasma membrane (WHITAKER, 1994). Apple tissues were found to have low permeability when transferred from harvest conditions (30–35 °C), to cold storage at 0 °C (LURIE et al., 1987).

Apple fruits (*Malus domestica* L. cv. Golden Delicious) were held at 20 or 38 °C for 4 days prior to storage at 0 °C for 5 months. Plasma membrane composition and function were examined before and after storage. While in fruits held at 38 °C membrane microviscosity and sterol content increased, phospholipid fatty acids became more saturated and there was an increased electrolyte leakage from apple fruit discs. ATPase activity increased during the first day of heating and decreased thereafter. After 5 months of storage at 0 °C, microviscosity and leakage were lower in heated fruit, and increased more slowly during ripening at 20 °C than in control apples. ATPase activity was similar in both treatments. Phospholipid content was higher in membranes from heated apples than in those from control fruit. Although fatty acid content in apples from both treatments was similar after storage, the loss of unsaturated fatty acids during ripening occurred more rapidly in control apples. Heated apples recovered rapidly from stress and acclimated more successfully to 0 °C than did control apples (LURIE et al., 1995).

Ultrastructural modification of Cherimoya fruit were studied after low temperature storage (4 °C for 6 days). The membrane system were severely damaged, resulting in a loss of cell compartmentalization. Cherimoya rewarmed to 22 °C after 9 days of low temperature storage was not able to recover, showing changes. In addition, disorganization of the internal lamella of chloroplasts, grana unstacking as well as a general swelling of plastids and mitochondria were also observed. The ultrastructural damage observed is explained in terms of membrane disruption (GUITIERREZ et al., 1992).

Inhibition of the pathway leads to increased levels of active oxygen species, supporting the hypothesis that altered mitochondrial respiration could pose an oxidative

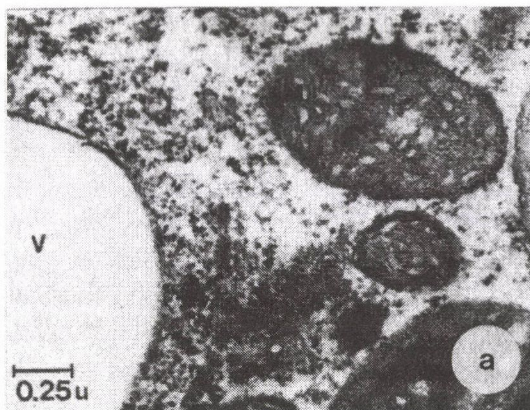
risk to other organelle membranes in the cell, without causing degradation of mitochondrial lipids (ROSARIO et al., 1995).

The responses of the tissues to halothene and methoxyflurane, which increase membrane fluidity, and to high pressures, which reduce membrane fluidity, are consistent with the hypothesis that cold-induced phase transition of membranes could be responsible for chilling injury. However, other cellular components may also be affected, e.g. low temperature, high pressures and anesthetics can alter protein conformation, affect ion channels, depolimerize microtubules and cause the release of Ca from membrane (SALVEIT, 1993).

High pressure He and N atmospheres of 12 MPa increased the severity of chilling injury (i.e. rate of ion leakage), in excised tomato pericarp discs increased the threshold temperature at which chilling occurred by 2–6 °C (SALVEIT, 1993).

Effects of chilling temperature on the activities of 4 lipid-degrading enzymes phospholipase D (EC 3.1.4.4), phosphatidate phosphatase (EC 3.1.3.4), lipolytic acyl hydrolase and lipoxigenase (EC 1.13.11.12) were studied using microsomal membranes isolated from the pericarp of mature green tomato fruit (*Lycopersicum esculentum* L. cv. Caruso). The activities of phospholipase and lipolytic acyl hydrolase remained essentially unchanged with decreasing temperature from 22 °C to 0 °C. In contrast, phosphatidate phosphatase and lipoxigenase were both less active at 0 °C than at 30 °C. There was also a reduction with decreasing temperature in bulk lipid fluidity of the microsomal membranes. Results suggest an accumulation of phospholipid catabolites in membranes during low temperature storage of tomato fruit (TODD et al., 1992).

Chill injury is defined as the physiological damage of special plants during exposure to low non freezing temperatures. Low temperatures (10 °C) have a drastic effect on cellular structure, such as the induction of microvesiculation of the endoplasmic reticulum, loss of ribosomes, nuclear chromatin, tonoplast degradation; swelling mitochondria, vesiculation, loss of cristae and Golgi apparatus. In addition, a general swelling and disruption of plastids, including degradation of starch granules, plastoglobuli degradation, grana unstacking, stromal dilation and disorganization of lamellae were observed. Further, the cytoplasmic membranes generally became vesiculated or formed myelin-like figures, lipid material appeared, and the cytoplasmic density seemed to increase. Lipid droplets appeared in the chloroplasts, cytoplasm and vacuoles between the cell wall and plasmalemma. The swelling of organelles due to the destruction of osmotic regulation, the disorganization of membranes, and the appearance of lipid bodies throughout the cell were general indications of membrane damage and degradation (MARANGONI et al., 1989). Chill sensitive tomato fruit cells showed signs of degradation, e.g. in starch granules, endoplasmic reticulum and ribosomes in 2 to 7 days of chilling. However, the characteristic features of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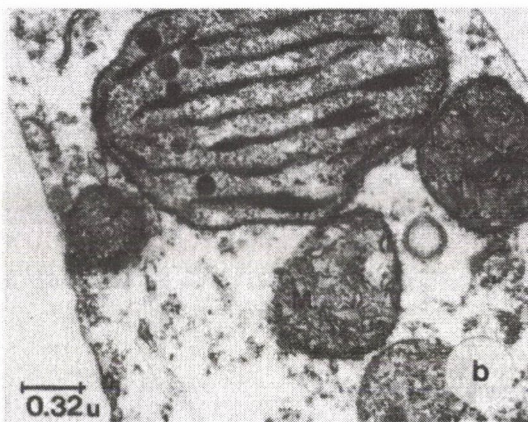
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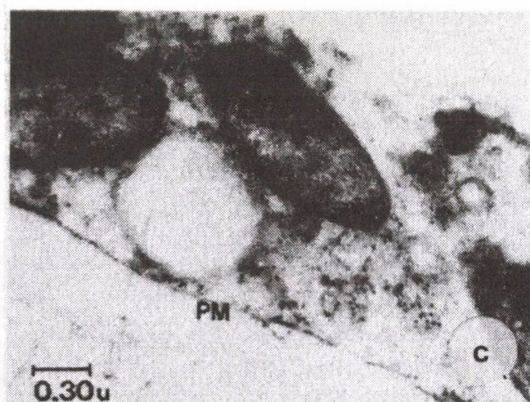
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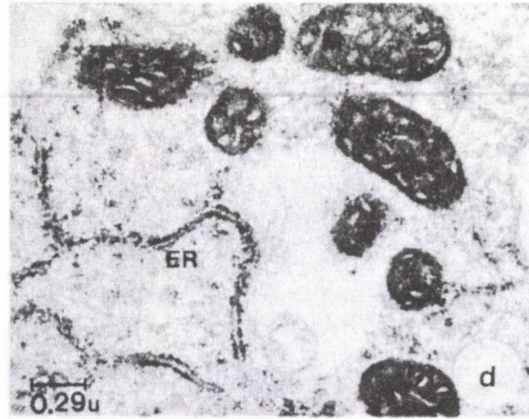
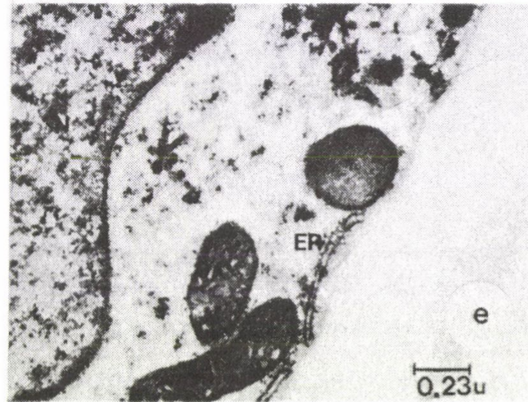
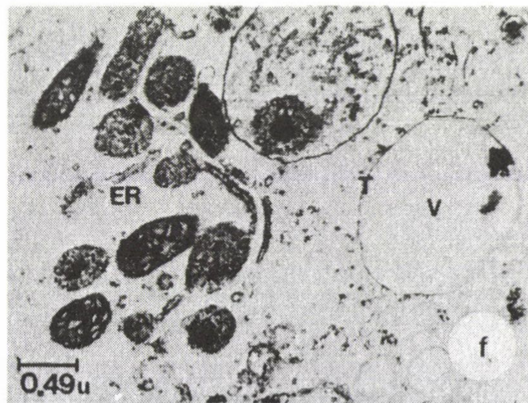
Fig. 1. Cross-sections of chill-sensitive (a,b,c) and chill-resistant (d,e,f) mature green tomato fruit pericarp. Tomatoes were stored for 2 days (a,d), for 7 days (b,e) and for 14 days (c,f). ER: endoplasmic reticulum; M: mitochondria; N: nucleus; PM: plasma membrane; T: tonoplast; V: vacuole. From: MARANGONI et al., 1989.







mitochondria remained unchanged. By 14 days of chilling, the mitochondria, as well as other cytoplasmic features, were not recognizable. The plasma membrane, however, remained intact. In contrast to sensitive tomato cells, resistant tomato cells showed little or no change in ultrastructure during chilling. The characteristic shape, cristae and double membrane of mitochondria remained unchanged throughout the chilling period (Fig. 1a–f) (MARANGONI et al., 1989).

Swollen mitochondria and ruptured tonoplasts were observed in chilled sweet potatoes (YAMAKI & URITANI, 1973). Mitochondria isolated from chill resistant apples indicated larger total lipid concentrations, larger ratios of phosphatidylcholine to phosphatidyl ethanolamine and higher concentrations of unsaturated fatty acids, than those isolated from chill sensitive apples. Significant differences were noted in calcium uptake pattern of chill resistant and sensitive apple cultivars during storage (LIN et al., 1985).

Effects of controlled atmosphere (CA) on membranes seem to be associated with their ability to regulate and delay senescence. Low O_2 and high CO_2 concentrations decrease ethylene production and other ripening-related changes (KADER, 1986) that in turn would delay the chain of events described earlier as natural senescence (MARANGONI et al., 1996).

High O_2 levels can be linked to peroxidation of fatty acids and free radical formation. Plants have enzymatic and non-enzymatic defense mechanisms to cope with these radicals. Superoxide dismutase, catalase and antioxidants protect plant cells from hyperoxia (SCANDALIOS, 1993). However, senescence, aging and other external stresses can alter this balance and consequently peroxidation of the membranes can occur (MARANGONI et al., 1996).

Cauliflower (*Brassica oleracea* L. *Botrytis* group) were stored (5 days, 25 °C, in air and 3% O_2 + 15% CO_2). It was established, in air, postharvest senescence involved a 20% decrease in mitochondrial phospholipid content. A large reduction in the relative abundance of phosphatidylcholine (PC) and in the degree of unsaturation of PC and phosphatidyl unsaturation of PC and phosphatidyl ethanolamine (PE) was observed. The degree of unsaturation increased in cardiolipin (CL). Storage under 3% O_2 did not prevent phospholipid breakdown. Low O_2 prevented the relative decrease in PC observed during storage in air and the loss of linoleic acid from PC but not from PE. This relative protection offered by the low O_2 atmosphere was lost under 3% O_2 + 15% CO_2. The high CO_2 atmosphere caused twice the loss in phospholipids as that observed during storage in air. Extensive observations suggest structural alteration in the presence of high CO_2 (ROMO PARADA et al., 1991).

Ultrastructural features of mitochondria were not altered by storage of peppers for 5 days in 1.5% O_2. Examination of the micrographs revealed mitochondria of uniform size and shape, intact double membranes and highly distinct cristae (RAHMAN

et al., 1993). It is concluded that the residual respiration inhibition in whole peppers is due to reduction in mitochondrial oxidative capacity (RAHMAN, 1995).

Bell pepper fruit were exposed to 1.5% O_2 for 1 to 5 days at 20 °C to examine whether low O_2 induced poststorage respiratory suppression (PRS) in whole fruit could be due to initiations in mitochondrial oxidative capacity. Mitochondrial oxidative capacity was not affected after storing bell peppers for 1 day in 1.5% O_2 Extending the storage period from 1 to 5 days in 1.5% O_2 resulted in PRS of CO_2 production for about 55 h after transfer to air, and a marked reduction in the oxidative capacity of isolated mitochondria. Mitochondrial activity was suppressed for 10 h after transfer to air, but, within 24 h had recovered to values comparable to those of mitochondria from fruit stored continuously in air. They established, that cyanide-sensitive cytochrome and CN-insensitive pathways were suppressed after storing fruit in 1.5% O_2 for 5 days. After returning fruit from a low O_2 atmosphere to air, the alternative pathway recovered at a greater rate than the cytochrome pathway (RAHMAN et al., 1995).

Mitochondrial respiraton was altered in transgenic potatoe lines by overexpression of the alternative oxidase Aox1 gene. Overexpressing lines showed higher levels of Aox1 mRNA, increased levels of alternative oxidase protein(s). Induction of alternative pathway capacity by aging of potatoes appeared to be more dependent on increased levels of alternative oxidase protein than changes in its oxidation state (HISER et al., 1996).

The transcript expression is influenced by the physiological state of mitochondria, this phenomenon was observed, when the effects of inhibition of mitochondrial functions on the expression of 2 nuclear genes encoding the extra cellular cellobiohydrolase I and endoglucanase I of the cellulase system of the filamentous fungus *Trichoderma reesei* were investigated (ABRAHAO NETO et al., 1995).

3. The effect of irradiation on the mitochondria and membranes

Not all climacteric fruits respond to irradiation in the same way. For this reason each kind of fruit and, in some cases a particular variety, needs to be considered on an individual basis. Important to the nature of the response to irradiation also is the physiological stage of the fruit, particularly with reference to the climacteric phase. Application of irradiation after the start of the climacteric respiration increase generally is ineffective in influencing the ripening process effects upon the end result (URBAIN, 1986).

The respiration and ethylene production of fruits, as well as cell permeability and electrolyte leakage (KOVÁCS, 1995) increase after irradiation (JOSEPHSON & PETERSON, 1983; THOMAS, 1986; URBAIN, 1986). Special attention was paid to the structural and chemical changes of mitochondria and membranes. Earlier results proved, that the

mitochondria react to irradiation with a temporarily promoted activity of biological processes (PADWAL-DESAI et al., 1969) followed by a remarkable increase in resistance to irradiation and/or capacity for radiation repair.

Activities of cytochrome oxidase, catalase and acid phosphatase increased in mitochondrial fractions isolated 1 and 7 days after irradiation of pears at 2.5 kGy and 10 kGy (MILLER et al., 1967). The capacity of mitochondria to oxidize succinate and α-ketoglutarate increased as a function of radiation dose (up to 2.5 kGy), ripeness of pears (ROMANI et al, 1966).

The increase in respiration activity immediately after irradiation was also investigated by LEE and co-workers (1971). They studied the effect of treatment on the metabolism of ^{14}C labelled organic acids in the tricarboxylic acid (TCA) cycle by infiltrating disks of pericarp tissue of tomatoes of various stages of development with the labelled respiratory intermediates. Metabolism of the organic acids was increased, but it was not affected by the difference in developmental stage of the fruit. They concluded that the increase in respiration and metabolism of the organic acids in the TCA cycle could be explained by an increase in mitochondrial membrane permeability or a partial loss of respiratory control, and the apparent recovery from the damage to these processes is reflected in the restoration of the normal function of the mitochondria. They further stated that the independence of respiration and metabolism of the respiratory substrates from the physiological age of the fruit suggested that the delay in ripening of older irradiated fruit was a result of partially irreversible damage to some system controlling the ripening process and was not directly related with respiratory metabolism (JOSEPHSON & PETERSON, 1983).

According to MAXIE and co-workers (1968) only preclimacteric bananas were delayed in ripening, they observed that 0.35 kGy to 0.5 kGy inhibited ripening without harming the fruit quality, apparently by reducing the sensitivity of the fruit to the ripening action of C_2H_4 to reach a comparable stage of ripeness, irradiated bananas needed 12–24 h longer treatment with 1000 ppm C_2H_4 than did unirradiated bananas. The nature of the reduced sensitivity is not known. After the onset of the respiratory climacteric, the maximum tolerable dose of gamma radiation increased with increasing degree of ripening.

Separate and combined effects of high CO_2 storage and gamma-irradiation on cell membranes from cauliflower flowers (*Brassica oleracea* L. *Botrytis* group) were investigated. Storage of the florets (8 days, 13 °C) either under 15% CO_2 or in air after irradiation at 2 kGy, accelerated the deterioration of microsomal membranes during storage. Both treatments caused an early loss in lipid phosphate. Irradiation enhanced the free fatty acid content of the membrane during storage and caused an extensive protein loss. When irradiation and high CO_2 storage were combined, electrolyte leakage significantly increase while protein loss considerably reduced. Results indicate that high

CO_2 and irradiation accelerate membrane degradation through different mechanism. Combined effects of the treatments were not additive, but membrane yield was apparently reduced. The apparent increase in electrolyte leakage after irradiation may be caused by the release of ions following cell wall deterioration (VOISINE et al., 1993).

The effects of irradiation upon lipids are of interest almost entirely in term of their nutritional, functional properties. Lipids are subject to both direct and indirect action of radiation. The first stage of interaction with radiation leads to excitation of an ionization. The next stage is mainly the production of intermediates, mostly products. As can be anticipated, indirect action of radiation is influenced by environmental and other factors such as whether the lipid is solid or liquid, temperature of irradiation, the presence or absence of O_2, dose, and dose rate. Oxygen plays an unusually important role in that certain lipids readily undergo oxidation, which radiation can accelerate (URBAIN, 1986).

Irradiation did not cause any appreciable changes in the activities of oxidizing enzymes such as polyphenoloxidase and cytochromoxidase in crushed bud or pulp tissues. The activity of polyphenoloxidase and a peroxidase in the mitochondria of the buds decreased, as did the cytochromoxidase, particularly in the mitochondria, extracted from them. The increased sensitivity of the oxidizing enzymes in the mitochondria appeared to be due to changes in their structure caused by irradiation. The phospholipid and nucleic acid contents of the tuber pulp were not significantly affected by irradiation, while in the bud tissue irradiation caused an appreciable decrease in the nucleic acid content and a remarkable increase in the phospholipid content, especially in mitochondria. Thus, the radiation-induced suppression of bud-tissue respiration is connected with a slackening of the activity of oxidizing enzymes in the individual structural elements of the cell and especially mitochondria (JOSEPHSON & PETERSON, 1983).

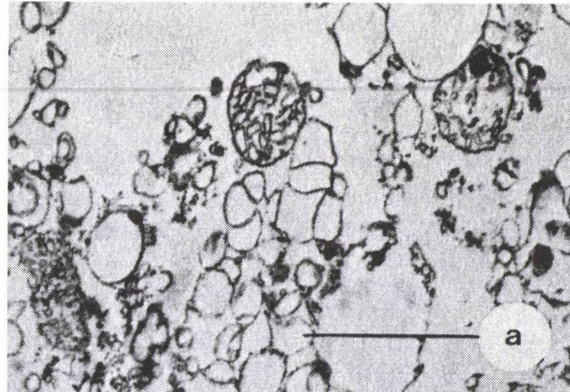
The primary effect of irradiation on mitochondria is focused on the lipids and/or proteins of membranes and membrane functions. There were surprisingly small effects of irradiation (2.5 kGy) on the protein distribution pattern of pear mitochondria (MILLER et al., 1967). Authors suggested, that structural and functional changes to membrane proteins were more probable sites of damage than lipids (GUILLE et al., 1987). In presence of oxygen, autooxidation of lipids occurs in addition to the radiolytic effects. Additionally, radiation treatment accelerates the autooxidation. Irradiation of unsaturated fatty acids leads to, besides the products of non-oxidative radiolysis such as the series of hydrocarbons, a number of oxygen containing products, such as hydroperoxide and carbonyl compounds (DELINCÉE, 1983). The mitochondrial inner membrane may well be capable of catalyzing self destruction by cytochrome oxidase of the respiratory chain. Treatment of membranes with free radicals induces lipid peroxidation (STANLEY, 1991; VOISINE et al., 1993), stimulates deacylation of membrane lipid, and promotes a phase separation within the bilayer resulting in a

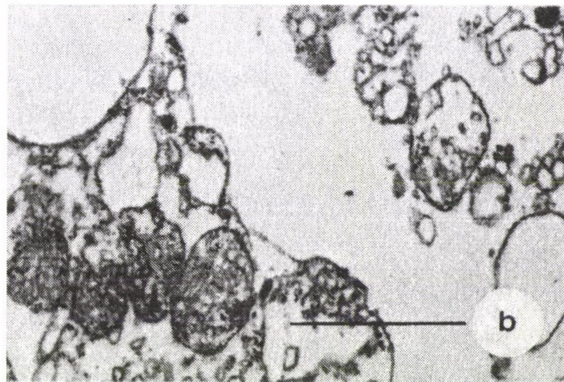
mixture of lipid-crystalline and gel phases. These symptoms of membrane deterioration are observed during natural aging, and thus it seems reasonable to propose that membrane deterioration in senescing plant tissues is mediated, at least in part, by free radical attack. Moreover, increased production of the superoxide radical with advancing senescence by what appears to be a membrane-associated enzyme was noted and correlated with progressive membrane rigidification (THOMPSON, 1986). Free radical-mediated lipid peroxidation is a characteristic feature of plant senescence, and large lipid phase changes in membranes accompanying senescence are attributable to specific neutral lipids, possibly long chain polymerized products of lipid peroxidation formed in the membranes with age. There is also evidence that the susceptibility of membranes to free radical attack is regulated by the degree of molecular order at the bilayer surface (THOMPSON, 1986).

Unsaturated fatty acids are prone to attack by lipoxygenase (DROILLARD et al., 1993) and increased free radical production has been observed in a variety of senescent tissues. LEGGE and co-workers (1986) reported decreases in the fluidity of senescing microsomal membranes in ripening tomato fruit, while PALMA and co-workers (1995) reported increases in the saturation index of ripening tomato fruit. Increased membrane peroxidation could be responsible for the increase in the saturation index observed in tomato fruit during ripening and senescence. This reaction is essentially autocatalytic since free radicals can continue their attack as long as O_2 and more substrate are available. Peroxidation leads to increased gel-phase formation and losses in membrane functionality (MARANGONI et al., 1996).

Electron micrographs of cherry mitochondria isolated from control and irradiated (2.5 kGy) cherries are presented in Fig. 2a–2c and show a basic similarity in mitochondrial structures in both the control and the irradiated cherries. Perhaps the most distinguishable feature of mitochondria, from both irradiated and non-irradiated cherries is the prevalence of cristae that are closed and apparently self contained (ROMANI et al., 1967). However, the general observation that there are fewer particles in the pellets from isolates of irradiated cherries is consistent with earlier quantitative studies (ROMANI et al., 1966). In addition to mitochondria, there are other membrane-bound bodies devoid of internal structure. Such "empty bodies" appear to increase in number with increasing maturity (ROMANI et al., 1965) and irradiation dose (ROMANI et al., 1967).

Fresh and stored (Figs. 3a–b) control (0 kGy) and stored irradiated (2.5 kGy and 5 kGy) (7 days, 5–7 °C, 80–90% RH) (Figs. 3c–d) cherries were investigated (KOVÁCS et al., 1995). Mitochondria of 5 kGy irradiated cherries were less dense than the mitochondria of the 2.5 kGy irradiated and control cherries (Fig. 3). Five kGy induced disorganization of matrix and cristae of mitochondria. Our observations are similar to that of ROMANI and co-workers (1967).





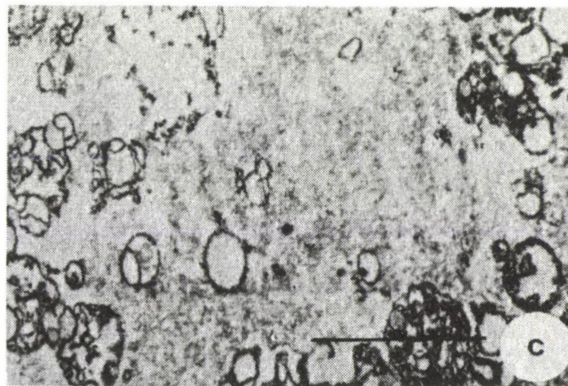


Fig. 2. Cherry fruit mitochondrial fraction from unirradiated control (a), 2.5 kGy (b) and 10 kGy (c) of ionizing radiation. Bars = 1 μm. From: ROMANI et al., 1967.

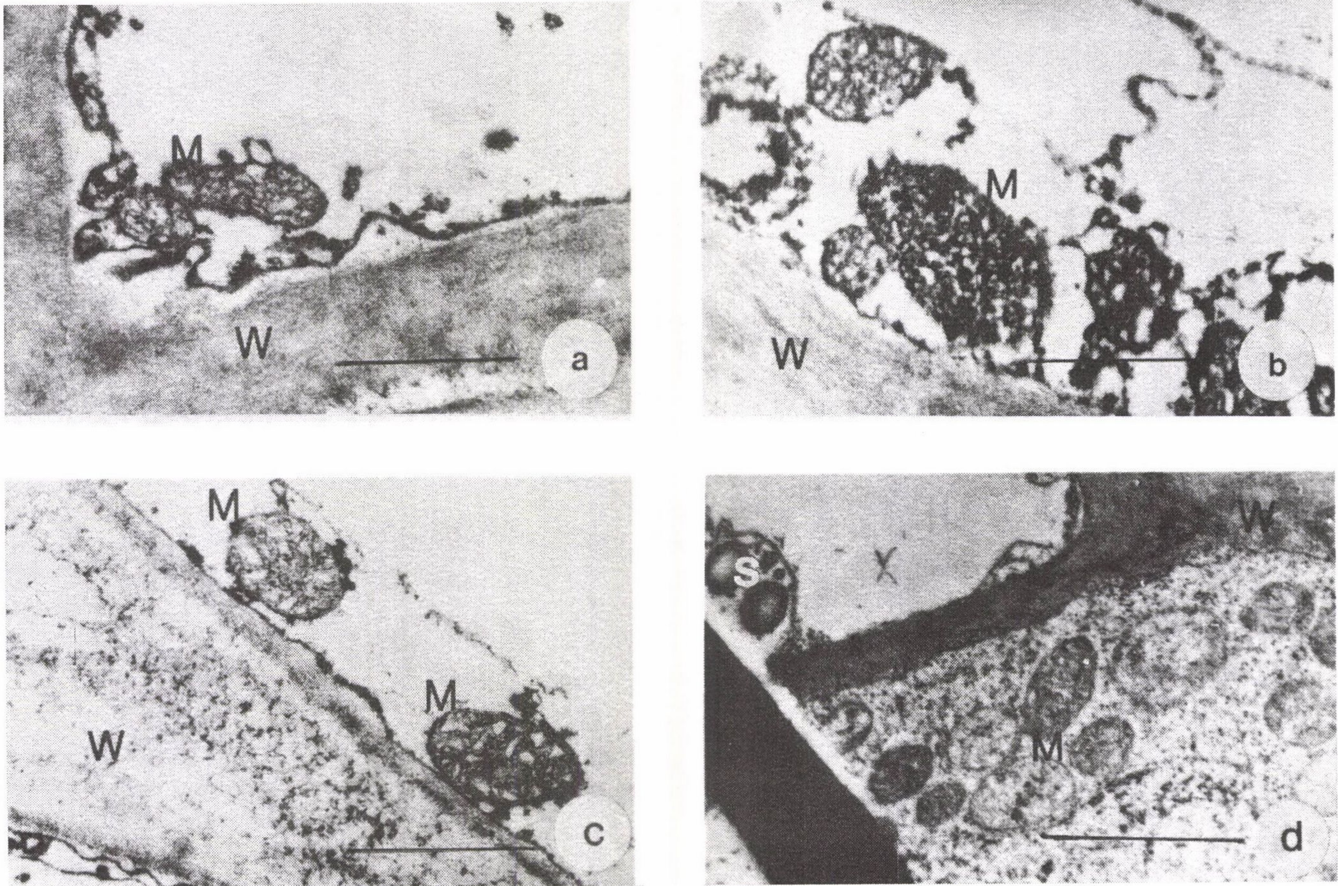


Fig. 3. Cherry fruit mitochondria from unirradiated, fresh control (a), unirradiated, stored control (b), irradiated and stored for 8 days: 2.5 kGy (c) and 5 kGy (d). M: mitochondria; S: starch; W: cell wall. Bars = 16 μm (a,d); 14 μm (b), 10 μm (c).

The mitochondria from irradiated algae (*Brachiomonas submarina*) were often swollen and distorted. The cristae of mitochondria commonly became circular or doughnut-shaped in profile. The cristae were located in the center of swollen mitochondria. A few cells, in which the cytoplasm was packed with numerous small mitochondria were observed (UNDERBRINK et al., 1969) (Figs. 4a–4b). VON WANGENHEIM (1969) observed less cristae in mitochondria of irradiated (660 kR X-ray is equivalent to 6.2 kGy) wheat seedlings than in the control (Figs. 5a–5b). Higher radiation doses (3.4–23.8 kGy) resulted in a dilution of the matrix and loss of cristae in mitochondria of root tissues of lettuce (*Lactuca sativa* L.) (SMITH, 1991).

Changes were also observed in the swelling rates of mitochondria when the fruits were irradiated (3 kGy) at the mature green or at the 2-day color breaker stage and during storage of tomatoes. Mitochondrial swelling was less evident during storage of either the control or irradiated 6-day breakers (AHMED et al., 1972). The decomposition was observed in the matrix and cristae of mitochondria mainly when irradiation doses were higher than the practical doses (up to 2.5 kGy).

4. Sugar

The protective role of sugars in preservation of the mitochondrial membranes and plant cells from the damaging effect of chill injury was discussed by WANG (1989). However, whether the accumulation of reducing sugars is an essential part of the chill resistance mechanism or is simply a consequence of chill stress is not clear. Soluble carbohydrates may influence the chill resistance mechanism in several possible ways: (a) carbohydrate contributes to the osmotic potential of the cell, thereby decreasing the cell water potential and reducing water loss from the tissue, (b) certain carbohydrates can stabilize cell membranes and enzymes by binding directly to the constitutive molecules, (c) carbohydrates serve as an energy source in the plant cell (WANG, 1989).

5. Calcium

Calcium delays senescence, in particular the onset of lipid peroxidation, in a manner that can be correlated with the ability of calcium to lend rigidity to the surfaces of lipid bilayers by acting as a divalent ligand (THOMPSON, 1986). Changes in cytosolic calcium concentration act as an intracellular signal and regulate various biochemical processes. Ca^{2+} may have a role as a regulator of cellular processes in plants. The inhibitory effects of Ca^{2+} on senescence are primarily extracellular, acting on the cell walls and the external surface of the plasma membrane. The calcium appeared to

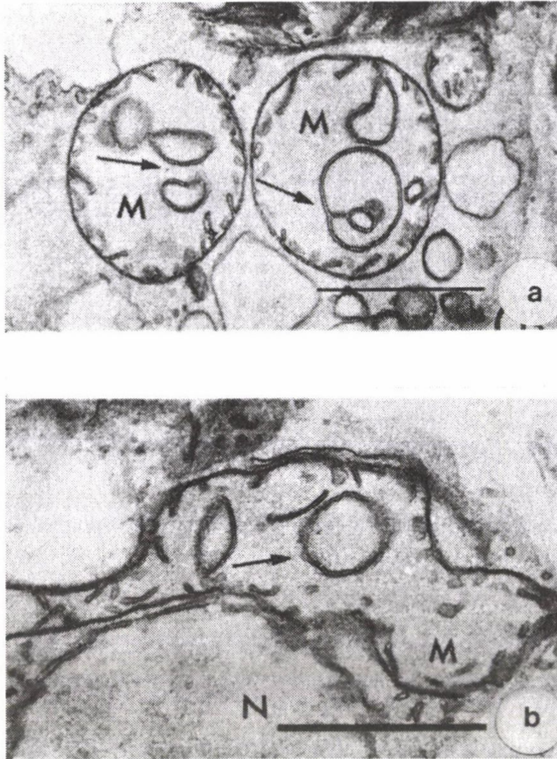
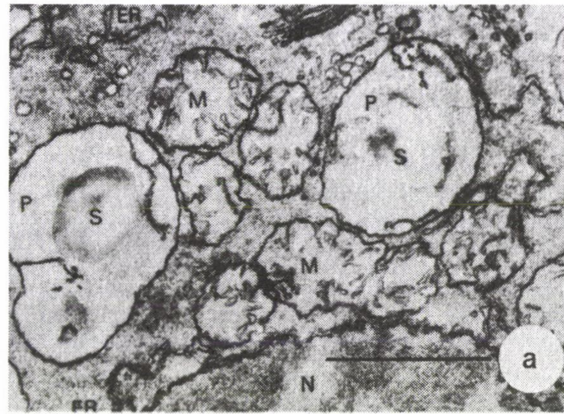


Fig. 4. The fine structure of green alga (*Brachiomonas submarina*), at various postirradiation times. Mitochondria fixed for 5 h: after 1.56 kR (a) and 4 kR (b) of X-rays. Bars = 3 µm (a) 4 µm (b). M: mitochondria; N: nucleus. From: UNDERBRINK et al., 1969.

reduce the increase in microviscosity in apple membranes which occurred when the apples were stored at room temperature. Therefore an effect of calcium in delaying senescence apparently involves loosening of the increase in microviscosity in membranes associated with senescence (LEGGE et al., 1982; 1986; PALIYATH et al., 1984). CHÉOUR and co-workers (1992) established that the catabolism of phospholipids was delayed by 0.05 M calcium, but accelerated by 0.25 M, as compared to the untreated control. Based on the levels of the lipid intermediates, phospholipase D, phosphatidyl acid phosphatase, lipolytic acyl hydrolase, and lipoxygenase appeared to be involved in the breakdown of phospholipids during senescence. Phospholipase D and phosphatidyl acid phosphatase may be directly influenced by calcium. The calcium treatment apparently did not affect the activity of acyl hydrolase. Lipoxygenase, responsible for the peroxidation of the polyunsaturated fatty acids, was probably indirectly influenced by calcium. They conclude that the delay of senescence of cabbage leaf discs by calcium treatment involved protection of membrane lipids from degradation. The calcium gradient across the membrane may have an important effect on membrane properties (FERGUSON, 1984). In general, non-specific effects of Ca^{2+} are

based on cross-linking of negative charges. Except for lecithin, sphingomyelin, and phosphatidyl ethanolamine, other natural phospholipids are negatively charged and, as such, are susceptible to cross-linking by Ca^{2+} (SHINITZKY, 1986). The inhibition of senescence by calcium may involve some protection of membranes from free radical or peroxidative attack when susceptible membranes have a large amount of calcium binding (FERGUSON, 1984). Mitochondrial structure of calcium treated and stored apple were normal and abundant. The internal structure of mitochondria was fairly well preserved (MAHANTY & FINERAN, 1975). FULLER (1980) did not observe differences in the mitochondrial structure as a function of calcium content of apples. The combined calcium and irradiation treatments of apples and pears prove that the calcium partly



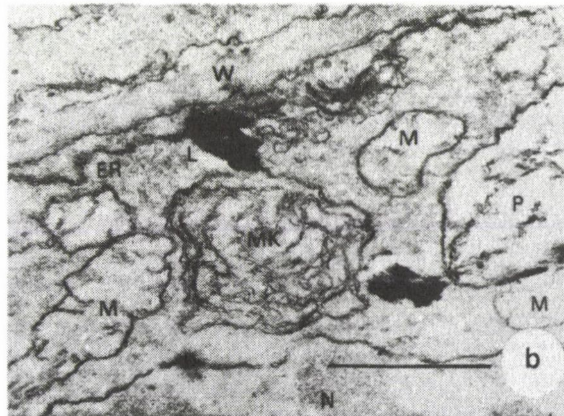


Fig. 5. Three hours after the exposure of wheat seedlings (5 mm) to X-rays [control (a) and 660 kR (b)]. ER: endoplasmic reticulum; G: Golgi apparatus; L: lipid; M: mitochondrion; MK: membranes; N: nucleus; P: plastid; S: starch; W: cell wall. Bars = 1 μm. From: VON WANGENHEIM, 1969.

prevents the unfavorable effects of irradiation. The calcium dipping and 1 kGy radiation dose preserved the ultrastructure of apple skin and flesh much better compared to the control or irradiated apples. The mobilization of calcium toward the skin was significantly greater in the irradiated than in the untreated apples (KOVÁCS et al., 1988). Similar findings were reported for carrots by SKOU (1963), ECHANDI and MASSEY (1970). Calcium prevented decomposition of structure of cell compounds (KOVÁCS & KERESZTES, 1989; KERESZTES et al., 1989; KERESZTES & KOVÁCS, 1991).

Calcium and antioxidants tend to protect membranes against irradiation and aging. Sugars have similar effect on the sensitivity to chill injury (WANG, 1989). The protective role of sugars in irradiation is not clear yet. The irradiation resulted in a stress effect, for starch converted into sugar in the potato and in the sweet potato protecting cells in tubers. Later, sugar converted into starch in the tubers, when the stress effect declined (about in three weeks after irradiation). The starch-sugar interconversion have positive effect on the physiological respect of tubers, but it has temporarily a negative effect in respect to processing of tubers, because of browning of the product. But this phenomenon decreased in three weeks after irradiation. More data are needed to understand the role of sugars (HAYASHI & KAWASHIMA, 1982a,b; 1983; HAYASHI & AOKI, 1985; ALWAKDI et al., 1991).

Mitochondria have a remarkable resistance to irradiation up to 2–3 kGy. Irradiation, aging and chilling induce the same visible structural changes in mitochondria, therefore, based on swelling properties of mitochondria only, a previous radiation treatment can not be identified.

6. Conclusions

During aging/senescence of fruits the composition of membrane lipids changes, phospholipids and fatty acid unsaturation levels decrease by oxidation. These changes lead to an increase of microviscosity, permeability of membranes and electrolyte leakage. During aging/senescence (natural physiological changes in fruits) saturation of fatty acids, microviscosity of membrane lipids, membrane lipid peroxidation increased. Electrolyte leakage increases in plasma membrane. Membrane protein degradation influences enzymic degradation. The effect depends on the physiological stage (pre- or postclimacteric) of fruits and the treatments (heat treatment, high and low O_2 and CO_2 atmosphere, irradiation). Chilling results in physical changes in the structure of membranes leading to functional changes in biological processes. The chill resistance, based on the chemical composition of fatty acid molecules is genetically determined. Ultrastructural features of mitochondria were not altered by storage of fruit in low O_2. Examination of micrographs revealed uniform size and shape, intact double membranes and highly distinct cristae. Low temperature (10 °C) have a drastic effect on chill

sensitive plants – swelling mitochondria, loss of cristae – but chill resistant plant showed characteristic shape, cristae and double membrane of mitochondria.

The mitochondria react to irradiation with a temporarily promoted activity of biological processes, followed by a remarkable increase in resistance to irradiation and/or capacity for radiation repair. After irradiation mitochondrial membrane permeability and disorganization of matrix and cristae of mitochondria increased. The mitochondrial oxidative capacity changes by an increase in mitochondrial membrane permeability or by a partial loss of respiratory control.

Irradiation increased electrolyte leakage and membrane permeability. Irradiation has an effect on the ultrastructure of mitochondria, which resulted in not only the structural changes of the mitochondria but the functionality of mitochondria, namely the mechanism of the biological oxidation. Controversial results could be found in respect of ripeness stage of fruits and the effectiveness of irradiation influencing shelf life of irradiated fruits. Probably, the differences could be interpreted on the basis of special properties of fruits, different ripeness stages, genetics etc. Calcium helped to preserve the structure of membranes of irradiated fruits. High CO_2 and high O_2 promoted the permeability of membranes and lipid peroxidation. It seemed, that the structural and functional properties of mitochondria are in good correlation, remarking, that biological oxidation is much more sensitive to irradiation than the ultrastructural changes in mitochondria. It would be interesting to compare chill resistant and chill sensitive fruits in the respect of lipid peroxidation. On the basis of these observations, it could be proposed, that irradiation of fruits should be carried out at low temperature, in low O_2 atmosphere, and during storage the CO_2 concentration in the storage room should be reduced.

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COMPARISON OF ANALYTICAL METHODS FOR DETERMINING THE ORANGE CONTENT OF PROCESSED JUICES

M. DOBOZI-KING[a,b], A. HALÁSZ[a] and ZS. SZABÓ[a]

[a] Central Food Research Institute, H–1022 Budapest, Herman Ottó út 15. Hungary
[b] Present address: Seminis Co., Wooland, California, USA

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Different methods (chemical analysis, comparison of UV spectra, SDS-PAGE and ELISA immunoassay) for determining the orange content of processed juices were studied. Characteristic differences were observed between fruit parts (albedo, flavedo, juice and pulp) and also between varieties in chemical composition (polyphenols, betaine, stigmasterin) and UV spectra. Thus it seems that these parameters may be used for detection of non-citrus additives and also the citrus by-products.

SDS-PAGE analysis of water soluble proteins and the ELISA assay using antisera raised in rabbits give only tentative results due to the low specificity of antigens and presence of other probably non-protein immunoactive components.

Keywords: orange, orange juice, orange content in juices, ELISA, SDS-PAGE, chemical composition of, UV-spectra

Due to the often increasing prices of oranges many processors introduce a great number of orange drinks containing cheaper components (sugar, citric acid, orange pulp, etc.) for dilution of original juice. For the protection of customers, the development of reliable routine quality control measures where information on the range of pure orange content in a juice sample will be adequate, is becoming increasible important (LEWIS, 1966; MOSHONAS & SHAW, 1986; KIMBALL, 1991; BELLIARDO & OOGHE, 1991; COCHET & DATAVERNIER, 1991). Complex chemical analysis of citrus composition results in valuable database of limited use in comparative studies, as the values depend on citrus variety, geographical conditions, analytical methods, etc. However, the complex analysis of citrus composition results in detection of minor components (such as e.g. limonoid glucosides) (OZAKI et al., 1995; FONG et al., 1990) which are difficult and uneconomical to forge, also in correlations among thoroughly tested parameters of high number, which are characteristic of a certain citrus variety.

The determination of pure fruit content in drinks with low juice percentage (10–12%) is even more difficult. Methods based on the high ion content characteristic of juices fail since ions could be added by water. Amino acids like glutamic acid and

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glycine or betaine (FLOYD & ROGERS, 1969) are easily complemented. More complex tests such as aminoacid analysis are too expensive. A simple, relatively inexpensive method, the immunodiffusion was applied by FIRON and co-workers (1979), using pure orange juice as antigen. Antibodies were successfully used for a qualitative test for orange content determination. Limonin, the intensely bitter component of processed juices of citrus varieties was used as antigen in a sensitive, quantitative immuntest by POY (1984). Compared to other analytical separation methods (TLC, HPLC), the values for limonin content obtained by immunobiochemical methods were found more reliable by CARTER and co-workers (1985).

In a collaborative study, the real values for limonin content were met with 94% and 111% precision by EIA or RIA, respectively, while TLC and HPLC methods resulted in only 53–70% and 65–100% accuracy. EIA methods using endogeneous cytokinins (zeatin riboside and isopentenyl adenosine) of citrus varieties as haptens conjugated to bovine serum albumin was described by BARTHE and STEWART (1985). The detectability is reported to be 50 pg of cytokinin.

The present work focuses on the reliability of different analytical methods for estimating pure orange content of commercial drinks using three different techniques: complex chemical analysis of citrus compounds (mediterranian-Greek and tropical-Cuban) orange and mediterranian (Cyprus) lemon, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and Enzyme Linked Immuno Assay (ELISA). This was a collaborative study involving two separate laboratories.

1. Materials and methods

1.1. Materials

Samples of 15 commercial orange concentrates from different Hungarian processing factories, declared to be of pure orange, and three controlled juices prepared from pure oranges (grown in Greece and Cuba) using a similar technology as the factories, were provided by the Analytical Chemical Department of the Central Food Research Institute, Budapest, Hungary. The yellow flavedo and white albedo parts of the orange peel, the pressed juice and the pulp of two orange cultivars (grown in Greece and Cuba) as well as lemon juice were also supplied. Pressed juice was obtained by vacuum filtering the juice of pressed orange halves through sintered glass funnel. As pulp, the precipitate of peeled and blended oranges was used after centrifugation at 3000 g for 10 min.

1.2. Methods

1.2.1. Chemical analysis and UV spectra of citrus compounds. The nitrogen content, the betaine content, the total polyphenol concentration, the stigmasterine content and the lipid content of the different botanical parts of two orange cultivars and lemon were determined by Hungarian standard methods (LÁSZTITY & TÖRLEY, 1987). Total amino acid content was measured by the Kjelfoss apparatus, calculated from nitrogen content. UV spectra of the polyphenols occurring in the samples were recorded in the range of 240–400 nm using a Beckman Model 165 UV detector (Beckman Instruments, Inc., Fullerton USA).

1.2.2. SDS-polyacrylamid electrophoresis. Water soluble protein of the samples were separated and their molecular weights were determined by SDS vertical slab electrophoresis according to WEBER and OSBORNE (1969). Proteins were denatured in the presence of urea and stained with the silver nitrate method. Slab gels were evaluated with a Shimadzu Model CS–930 dual-wavelength thin-layer chromato-scanner.

1.2.3. Antisera. Water soluble protein from 1 ml orange samples after ammonium sulfate precipitation and dialysis against distilled water was used as antigen. Antisera were raised in rabbits inoculated every two weeks by the protocol described by JOURDAN and co-workers (1984). Serum was collected after twelve weeks and stored at 10 °C. IgG was purified following ammonium sulfate precipitation according to STEINBUCH and AUDRAN (1969).

1.2.4. ELISA immunoassay. Sample aliquots of 100 microliter were applied in 50× and 100× dilutions to microtiter plates using 40 nm $NaHCO_3$ at pH 9.6. After overnight incubation at 4 °C, the solution was discarded and the wells were washed with distilled water. To saturate the uncoated adsorption sites, the wells were incubated at RT for 60 min. with a 0.01% solution of BSA in Tris-HCl buffered saline (TBS), and washed as before. To each antigen coated well was added a 5 microgram/ml solution of anti-orange IgG (200 microgram/well). The plate was scaled and incubated at 24 °C for 2 h. The liquid was then decanted off and the wells were rinsed several times with PRS. To each well, goat anti-rabbit IgG, labelled with peroxidase, was added in 500× dilution and the plate was incubated for an other hour at 37 °C. Excess reagents were rinsed off and washed with PBS contaning 0.05% Tween 20. For the detection of POX-activity, 100 microliter of substrate (1 mg/mg of *o*-phenylene-diamine) was added to each well and the plate was incubated for 10 min. at 38 °C. The substrate reaction was stopped by addition of 50 microliter of $2N$ H_2SO_4 and the plate was read at 405 nm.

2. Results and discussion

2.1. Chemical analysis and UV spectra of the citrus compounds

Chemical composition varied between the different morphological parts of fruits. The nitrogen content shows increasing tendency in the order of juice pulp, albedo and flavedo (Table 1). The total amino acids content shows similar tendency. However, the betaine content accumulates mostly in the juice and the pulp. The polyphenol concentration increases towards the peel, accumulating in the flavedo. In all the samples, the stigmasterine concentrates in the pulp and albedo. The lipid content varies according to the fruit, which helps in detecting the presence of lipid additives of non-citrus origin.

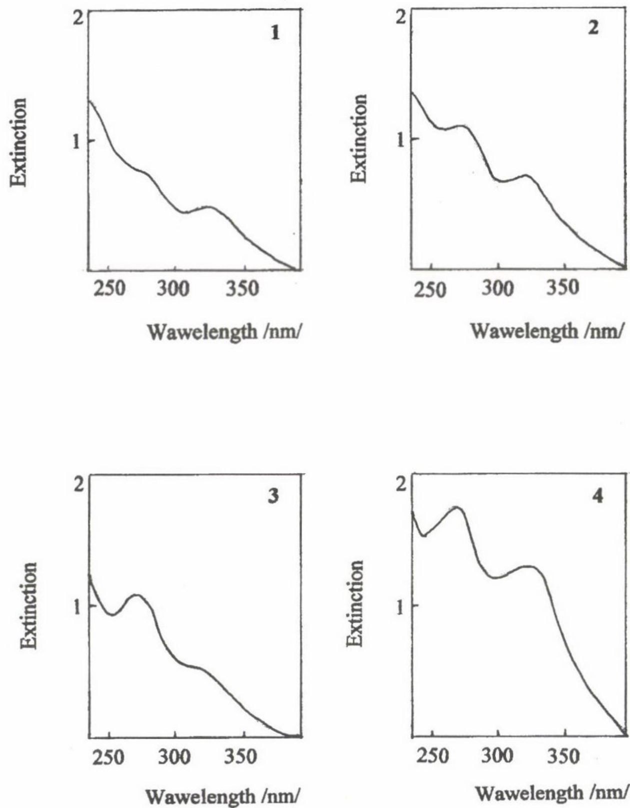

Fig. 1. UV-spectra of samples produced from Greek orange 1: pressed, filtered juice; 2: pulp; 3: albedo; 4: flavedo

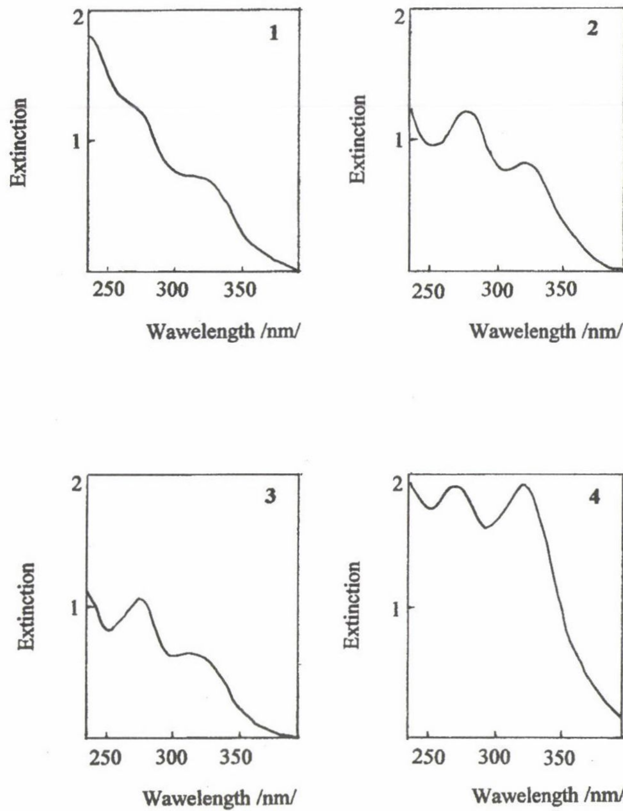

Fig. 2. UV-spectra of samples produced from Cuban orange 1: pressed, filtered juice; 2: pulp; 3: albedo; 4: flavedo

The UV-spectra of the botanical parts of the investigated orange cultivars and lemon sample show characteristic differences (Figs. 1, 2, 3). The spectra of albedo and the pulp from Greek and Cuban oranges are quite similar. However, the flavedo curves of the two oranges are different. Similarities may be observed between flavedo of lemon and Greek orange. Also the spectra of juices from lemon and Greek orange are similar. The highest polyphenol content was found in the juice of Cuban orange. The lemon pulp and albedo are the richest in polyphenols. The data of UV-spectra support the results of chemical analysis data shown in Table 1.

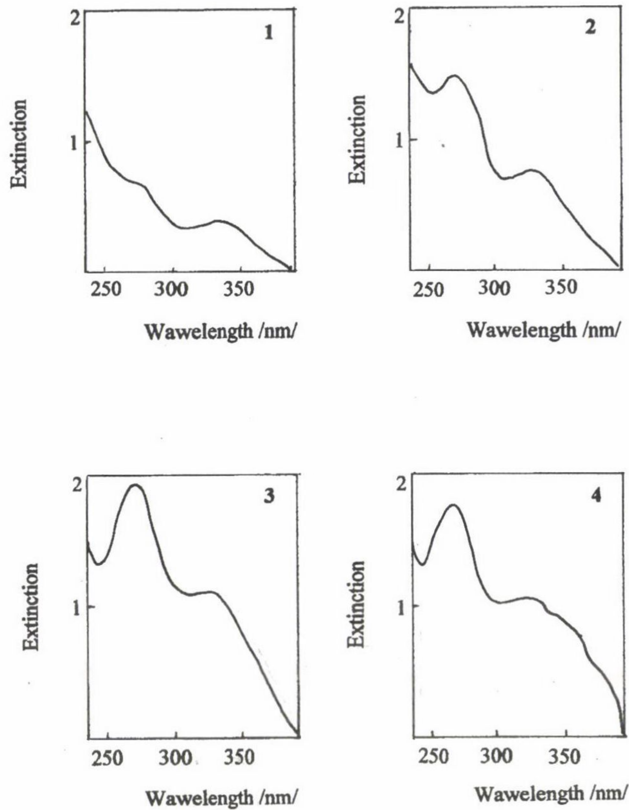

Fig. 3. UV-spectra of samples produced from lemon 1: pressed, filtered juice; 2: pulp; 3: albedo; 4: flavedo

In summary it would be concluded that the differences in chemical composition (lower polyphenol content and higher free amino acid content of the juices) and UV-spectra of different parts (albedo, flavedo pressed juice, pulp) of investigated fruits suggest a possibility of detection of citrus by-products and non citrus additives in orange concentrates.

2.2. SDS-PAGE of proteins

Purification of the water soluble protein by ammonium sulfate precipitation and subsequent dialysis allowed a good separation of proteins. As shown in the scheme of electrophoretic patterns (Fig. 4) 6 to 10 protein components were separated and visualized as distinct bands. High similarity was found for samples 1 through 9, 10, 11 13.

Similar observation could be made in relation of albedo, flavedo and pulp samples of investigated oranges. A lower degree of similarity is characteristic for the pressed juices. A double band in the region of 12–14 kD was observed in all orange products and botanical parts of orange. The lemon juice appeared to be characteristically different with double bands in the higher molecular weight range. Some typical electropherograms are shown in Fig. 4.

Table 1

The nitrogen, polyphenol, stigmasterine, volatile oil and lipid content of Greek orange, Cuban orange and lemon juice samples

Sample	Nitrogen content (g/100 g)	Betaine HCl (mg/100 g)	Total amino acid (mg/100 ml)	Total polyphenol (E/100 ml)	Total amino acid/total polyphenol	Stigma-sterine (mg/100 g)	$CHCl_2$/MeOH soluble (%)
Greek orange							
juice	0.48	576	1.52	0.76	2.00	0	0.53
Pulp	0.54	577	1.93	1.46	1.32	44	1.08
Albedo	0.40	165	1.71	1.68	1.02	58	3.05
Flavedo	0.70	173	3.23	8.76	0.37	14	0.00
Cuban orange							
juice	0.49	423	1.74	0.76	2.30	24	0.20
Pulp	0.55	1320	2.04	1.60	1.28	133	0.31
Albedo	0.78	762	3.33	5.26	0.63	58	0.00
Flavedo	0.78	260	3.10	14.96	0.21	27	1.28
Lemon							
juice	0.32	408	2.74	0.64	4.30	51	0.22
Pulp	0.49	467	3.51	1.42	2.47	163	0.19
Albedo	0.80	25	2.72	3.37	0.81	44	1.24
Flavedo	0.72	0	5.22	8.06	0.65	24	0.62

2.3. ELISA analysis

In Table 2 characteristic results based on the ELISA absorbancy are collected. The average value of the three controlled orange juices was used as the control value (100%). The differences between three control juices did not exceed 5%, the confidence limit based on three replications was ±2.1%. As seen, majority of the commercial samples showed values being near the control value, only samples 4, 8 and 9 had quite different absorbancy data.

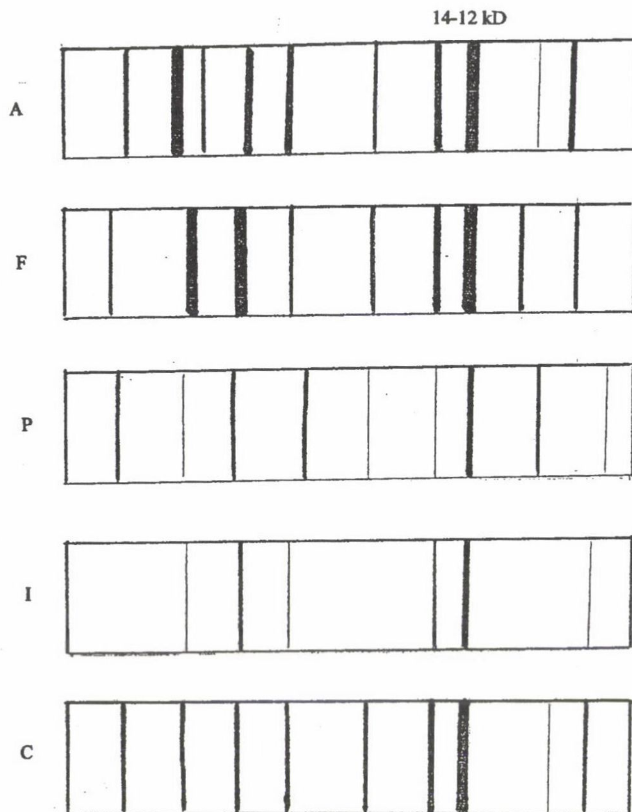

Fig. 4. Characteristic PAGE electropherogram schemes of samples. A: albedo; F: flavedo; P: pulp; J: pressed, filtered juice; C: orange juice concentrate

In some cases differences were observed between the data obtained using 50× dilution and that of 100× dilution. This may be probably explained so that the increased amount of alkaline buffer used for dilution may enhance the adsorption of orange samples antigens.

The immunreaction of lemon proteins with anti-orange IgG shows that the antibody is not specific for orange immunoreactions of albedo, flavedo and pulp exclude the possibility of quantitating the pure orange content of high quality drinks. The very low values of pressed filtered juice suggest that by filtration great part of immunochemically active components were removed. Some values over 100% suggest that there could be present immunoactive components other than the orange proteins.

Table 2

*Calculated amounts of pure orange content of some commercial juice samples based on the immunoassay values**

No	Sample	Calculated amount of pure orange juice content (%)	
		Determined by ELISA applying	
		50×dilution	100×dilution
1	Orange concentrate	96.2±2.5	99.8±12.6
2	Orange concentrate	114.1±4.1	123.4±2.8
3	Orange concentrate	95.8±2.3	87.0±4.2
4	Orange concentrate	67.6±6.3	103.8±8.6
5	Orange concentrate	90.1±2.7	81.8±5.1
6	Orange concentrate	80.3±1.8	125.9±20.1
7	Orange concentrate	90.1±5.1	136.3±11.2
8	Orange concentrate	56.3±1.7	101.3±15.8
9	Orange concentrate	70.4±2.1	71.4±1.6
10	Orange concentrate	100.0±6.5	114.2±5.5
11	Orange concentrate	91.9±1.2	76.6±3.2
12	Orange concentrate	89.4±1.3	75.3±2.1
13	Orange concentrate	116.2±0.5	94.8±1.3
14	Orange concentrate	114.1±1.3	103.8±2.7
15	Orange concentrate	89.4±2.1	90.9±1.8
16	Control pure orange juice	100.0	100.0
17	Albedo	53.5±3.3	63.6±7.1
18	Flavedo	51.7±1.4	56.5±1.3
19	Pressed filtered juice	36.6±1.2	41.6±2.7
20	Pulp	22.2±1.1	30.1±3.3
21	Pure fresh lemon juice	60.8±4.2	50.0±5.5

* Means of three replicates and confidence limits

As conclusion it could be stated that the ELISA method based on antibodies of orange proteins is not suitable for control of orange content of drinks. The eventual use in detection of non-citrus components needs further investigations.

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IDENTIFICATION OF INDUSTRIAL YEAST STRAINS OF INDIAN ORIGIN

KALYANI MANI, G. PÉTER and T. DEÁK

University of Horticulture and Food Industry, H–1118 Budapest, Somlói út 14–16. Hungary

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Nine yeast strains were isolated from red wine, beer and commercial baker's yeast, originating from India. They were subjected to characterization using classical method of identification as proposed by KREGER-VAN RIJ (1984), whereupon they were identified as members of *Saccharomyces sensu stricto* group (7), *Pichia fabianii* (1) and *Issatchenkia orientalis* (1). The key proposed by VAUGHAN-MARTINI and MARTINI (1993), was used for species level assignment within *Saccharomyces sensu stricto* group, and all isolates were found to be *Sacch. cerevisiae,* although they differed in the vigorous fermentative characteristics exhibited by two of the strains, inspite of their sluggish growth.

Keywords: *Saccharomyces sensu stricto,* characterisation, identification, wine yeast.

The genus *Saccharomyces* has undergone innumerable changes during the last 150 years of its history. Since VAN DER WALT (1970) accepted 41 species of *Saccharomyces,* several studies have reported that phenotypic criteria such as fermentative and assimilative activities traditionally used in yeast taxonomy may lead to faulty identification of *Saccharomyces* species (ROSINI et al., 1982). Based on hybridization studies, serological observations and base composition of the nuclear DNA, only seven species remained in the genus *Saccharomyces* (YARROW, 1984). nDNA/nDNA homology studies within the amalgamated species reestablished three further species, namely, *Sacch. pastorianus, Sacch. paradoxus* and *Sacch. bayanus*. According to the current classification four biological species *Sacch. cerevisiae, Sacch. paradoxus, Sacch. bayanus* and *Sacch. pastorianus* are recognized within the *sensu stricto* complex (VAUGHAN-MARTINI & KURTZMAN, 1985; VAUGHAN-MARTINI & MARTINI, 1987; VAUGHAN-MARTINI, 1989). However the adventures of the genus *Saccharomyces* are far from over. Recently, a genetically isolated population of *Saccharomyces sensu stricto* complex has been reported to have been found in Brazil, which probably represents a new species within the complex (NAUMOV et al., 1995).

As the Indian Peninsula has some geographic isolation, it was interesting to study the yeast flora of this region. We focused our attention on *Saccharomyces sensu stricto*

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yeasts isolated from red wine, beer and commercial baker's yeast, all of them originating from peninsular India, with a view to identify the strains which may perhaps exhibit interesting characteristics worthy of further investigation. As a part of this initiative, here, we present our results of conventional method of identification.

1. Materials and methods

1.1. Yeast strains

Yeast strains were isolated from beer, red wine and two brands of commercial baker's yeast, originating from India. The strains isolated and sources are listed in Table 1.

Table 1

Strains isolated and their sources

Strains	Source of isolation
Sh 1	Red wine
Sh 2	Beer
Sh 3	Beer
Sh 4	Red wine
Sh 5	Red wine
Sh 6	Beer
Sh 7	Active baker's yeast
Sh 8	Active baker's yeast
Sh 9	Active baker's yeast

1.2. Morphological and physiological examinations

Complete morphological and physiological profiles for the strains studied were determined according to standard methods for yeast identification (VAN DER WALT & YARROW, 1984). Unless otherwise indicated all tests were done by auxanographic method at 25 °C. Fermentation was carried out in static culture using Durham tubes to demonstrate production of CO_2.

1.3. Measurement of fructose transport

Detection of active fructose transport system was performed according to the method of RODRIGUES DE SOUSA and co-workers (1990). Proton symport activity was tested by recording the alkalinization of an aqueous cell suspension upon addition of fructose to a final concentration of 6–10 mmol l^{-1} using a RADELKIS OP-211/1 pH

meter (precision ± 0.05 pH) with a pH sensitive combination glass electrode (OP–0808P) attached to a potentiometric type recorder (RADELKIS OH–814/1).

2. Results and discussion

The results of traditional carbon and nitrogen assimilation tests; morphological and reproductive characteristics; growth characteristics and fermentation characteristics are presented in Tables 2, 3, 4 and 5, respectively. Based on the results obtained and taxonomic key of YARROW (1984) and Barnett's computer program (BARNETT, 1995), the strains Sh 1–Sh 7 were identified as *Sacch. cerevisiae* (sensu YARROW, 1984) which is currently termed as *Saccharomyces sensu stricto.* Strain Sh 8 was identified as *Pichia fabianii* and strain Sh 9 as *Issatchenkia orientalis.*

Strains Sh 1–Sh 7 were not identical in the morphological traits of vegetative cells, (such as formation of pseudo-mycelium) and production of ascospores (Table 3). Similarly the strains showed differences in fermentation and assimilation of various carbon sources (Table 5&2), and growth at 37 °C (Table 4). However, these differences are within the limits expected for species in *Saccharomyces sensu stricto.* Strains Sh 1–Sh 7 were further analyzed for those phenetic characteristics that can distinguish the four taxons of *Saccharomyces sensu stricto* (VAUGHAN-MARTINI & MARTINI, 1993; RODRIGUES DE SOUSA et al., 1990).

In their recent taxonomic key using traditional physiological tests, VAUGHAN-MARTINI and MARTINI (1993) stated that it was possible to separate the four taxa of *Saccharomyces sensu stricto.* According to this key *Sacch. bayanus* was the only species within genus *Saccharomyces* to be able to grow in vitamin free medium. Concurrently, maximum temperature of growth (T_{max}) immediately separates *Sacch. bayanus* and *Sacch. pastorianus* which never grow above 34 °C, from *Sacch. paradoxus* which grow at 37 °C and even at much higher temperatures (40–43 °C). The T_{max} of *Sacch. cerevisiae* was found to be variable. In addition, *Sacch. pastorianus* and *Sacch. bayanus* can be delimited from *Sacch. paradoxus* and *Sacch. cerevisiae* by the presence of an active fructose transport mechanism. *Sacch. cerevisiae* and *Sacch. paradoxus* can be separated on the basis of the ability of the latter to grow on D-mannitol as a sole carbon source, low to nil fermentation of maltose and ecology: *Sacch. paradoxus* is found exclusively in natural habitats. However this key has run into criticism, since *Sacch. bayanus* was shown to be variable for its ability to grow in vitamin free medium (KISHIMOTO & GOTO, 1995; TORNAI-LEHOCZKI et al., 1996). As a result, *Sacch. bayanus* and *Sacch. pastorianus* cannot be distinguished by classical techniques of identification. Nevertheless, *Sacch. cerevisiae* and *Sacch. paradoxus* can be readily identified using classical techniques. With this background, we tried to interpret our results.

Table 2

Carbon and nitrogen assimilation characteristics of isolated yeast strains

Source	Strains								
	1	2	3	4	5	6	7	8	9
Glucose	+	+	+	+	+	+	+	+	+
Galactose	–	+	+	–	–	+	+	+	+
L-sorbose	–	–	–	–	–	–	–	–	–
Saccharose	+	+	+	+	+	+	+	–	–
Maltose	+	+	+	+	+	+	+	+	–
Cellobiose	–	–	–	–	–	–	–	+	–
Trehalose	+	+	+	–	–	+	+	–	–
Lactose	–	–	–	–	–	–	–	–	–
Melibiose	–	–	–	–	–	–	–	–	–
Raffinose	+	+	+	+	+	+	+	–	–
Melecitose	–	+	–	–	–	+	+	+	–
Inulin	–	–	–	–	–	–	–	–	–
Starch	–	–	–	–	–	–	–	+	–
Xylose	–	–	–	–	–	–	–	+	+
Rhamnose	–	–	–	–	–	–	–	–	–
Glycerol	–	–	–	–	–	–	–	+	+
Erythritol	–	–	–	–	–	–	–	–	–
Ribose	–	–	–	–	–	–	–	–	–
Succinate	–	–	–	–	–	–	–	+	+
D-mannitol	–	–	–	–	–	–	–	+	–
α-methyl D-glucoside	–	–	–	–	–	–	+	+	–
Citrate	–	–	–	–	–	–	–	+	–
Inositol	–	–	–	–	–	–	–	–	–
Nitrate	–	–	–	–	–	–	–	+	–
Lysine	–	–	–	–	–	–	–	+	+
Cadaverine	–	–	–	–	–	–	–	+	+
Ethylamine	–	–	–	–	–	–	–	+	+
Peptone	+	+	+	+	+	+	+	+	+

Table 3

Morphological and reproductive characteristics of isolated yeast strains

Character	Strains								
	1	2	3	4	5	6	7	8	9
Budding	+	+	+	+	+	+	+	+	+
Pseudo mycelium	–	–	–	–	–	–	+	–	+
True mycelium	–	–	–	–	–	–	–	–	–
Pellicle formation	–	–	–	–	–	–	–	+	+
Sporulation	–	+	+	–	–	+	+	–	–

Table 4

Growth characteristics of isolated yeast strains

Character	Strains								
	1	2	3	4	5	6	7	8	9
In vitamin free medium	–	–	–	–	–	–	–	–	–
At 37 °C	–	+	+	–	–	+	+	+	+
In 0.01% actidione	–	–	–	–	–	–	–	–	–
In 50% glucose	+	+	+	+	+	+	+	+	–
At 34 °C	+	+	+	+	+	+	+	+	+

Table 5

Fermentation characteristics and urease test results of isolated strains

Source	Strains								
	1	2	3	4	5	6	7	8	9
Glucose	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	–	–	+	+	–	–
Saccharose	+	+	+	+	+	+	+	+	–
Maltose[a]	+	+	+	+	+	+	+	+	–
Raffinose	–	+	+	+	+	+	+	–	–
Melibiose	–	–	–	–	–	–	–	–	–
Trehalose	–	–	–	–	–	–	–	–	–
Urease test	–	–	–	–	–	–	–	–	–

[a] Within 24–28 h

2.1. *Growth temperature*

Since strains Sh 2, Sh 3, Sh 6 and Sh 7 showed positive growth at 37 °C, we can safely eliminate the possibility of their being *Sacch. bayanus* or *Sacch. pastorianus,* and they should hence be either *Sacch. paradoxus* or *Sacch. cerevisiae.* Strains Sh 1, Sh 4 and Sh 5, however, did not grow at 37 °C and hence cannot be *Sacch. paradoxus* which is known to grow readily at this temperature. This can mean that Sh 1, Sh 4 and Sh 5 can be either *Sacch. pastorianus* or *Sacch. bayanus* or *Sacch. cerevisiae.*

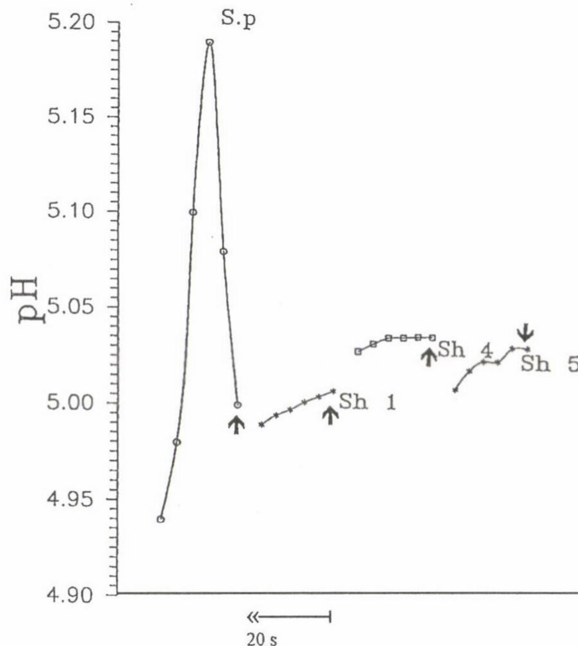

Fig. 1. Effect of fructose on the extracellular pH of aqueous suspensions of fructose grown cells of *Sacch. pastorianus*, Sh 1, Sh 4 and Sh 5; Arrows indicate addition of fructose. S.p. = *Sacch. pastorianus*

2.2. *Active fructose transport*

Strains Sh 1, Sh 4 and Sh 5 were tested for the presence of active fructose transport. Other strains (Sh 2, Sh 3, Sh 6 and Sh 7) were not subjected to active fructose transport test, in view of elimination of rest as belonging to either *Sacch. bayanus* or *Sacch. pastorianus.* The type strain of *Sacch. pastorianus* (CBS–1503) was used as a

positive control and the results presented in Fig. 1, are in accordance with literature values, indicating the robustness of the experimental conditions (RODRIGUES DE SOUSA et al., 1990).

On all three sample strains tested, i.e., Sh 1, Sh 4 and Sh 5, the active fructose transport response were negative, extrapolating the results of RODRIGUES DE SOUSA et al., (1990), the three strains should be either *Sacch. paradoxus* or *Sacch. cerevisiae*. The possibility of their being *Sacch. paradoxus* has already been eliminated for their inability to grow at 37 °C. Therefore, strains Sh 1, Sh 4 and Sh 5 can be considered to belong to *Sacch. cerevisiae.*

2.3. Mannitol assimilation

Strains Sh 1–Sh 7 failed to grow in a medium containing D-mannitol as a sole source of carbon (Table 2). *Sacch. paradoxus* and *Sacch. cerevisiae* can be distinguished from one another on the basis of their ability and the lack of their ability to assimilate D-mannitol, respectively (VAUGHAN-MARTINI & MARTINI, 1993). Hence, strains Sh 1–Sh 7 do not belong to *Sacch. paradoxus*. In conjunction with the results of growth at 37 °C, wherein, the strains Sh 2, Sh 3, Sh 6 and Sh 7 were inferred to be either *Sacch. paradoxus* or *Sacch. cerevisiae,* the results of the D-mannitol assimilation lead to the conclusion that these strains belong to *Sacch. cerevisiae.* The strains Sh 1, Sh 4 and Sh 5, in which, on the basis of growth at 37 °C, *Sacch. paradoxus* was excluded, and subsequently, active fructose transport test pointed to the fact that these strains belong to *Sacch. cerevisiae,* D-mannitol assimilation studies further prove that these strains, in fact, are not *Sacch. paradoxus* but *Sacch. cerevisiae.*

2.4. Maltose fermentation

Strains Sh 1–Sh 7 showed rapid fermentation of maltose, within a time range of 24–28 h (Table 5). Such behavior is known to be characteristic of *Sacch. cerevisiae,* while *Sacch. paradoxus* on the other hand shows either no maltose fermentation, or if at all, rather very slowly (VAUGHAN-MARTINI & MARTINI, 1993). This result supports earlier conclusion that Sh 1–Sh 7 are *Sacch. cerevisiae* and not *Sacch. paradoxus*.

2.5. Origin of the strain

Strains Sh 1–Sh 7 were all isolated from man made environments, namely, from red wine, beer and baker's yeast. So far, *Sacch. paradoxus* are known to be isolated only from natural environments while *Sacch. cerevisiae* though originally claimed to be isolated from man made environs, was later isolated from natural environs as well (VAUGHAN-MARTINI & MARTINI, 1993). Thus the possibility of strains Sh 1–7 belonging to *Sacch. paradoxus* is further excluded.

Hence on the basis of key characteristics, we are unequivocally able to conclude that the strains Sh 1–Sh 7 belong to *Sacch. cerevisiae* while Sh 8 and Sh 9 belong to *Pichia fabianii* and *Issatchenkia orientalis,* respectively. Additional specific aspects such as maltose fermentation and origin of strains are in line with the above conclusion.

Therefore, despite certain geographic isolation of the Indian Peninsula, no new *Saccharomyces* species have been found among the strains studied. However, it is worth mentioning that strains Sh 1 and Sh 4 showed very sluggish growth, though they exhibited vigorous fermentation characteristics. Since these strains were isolated from the industrial environment little is known about their genetic history; investigation of the above strains would shed light for the cause of this behaviour. The identity of these strains were further subjected to molecular techniques of characterization and these results are to be published subsequently.

3. Conclusion

Of the nine yeast strains isolated, only seven belonged to *Saccharomyces sensu stricto* group. The other two were *Pichia fabianii* and *Issatchenkia orientalis.* All the seven *Saccharomyces sensu stricto* species were identified using the key of VAUGHAN-MARTINI and MARTINI (1993), as *Sacch. cerevisiae.* Two of the isolated species, Sh 1 and Sh 4, showed vigorous fermentative characteristics although they showed sluggish growth.

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KEEPING QUALITY AND SPOILAGE

A MATHEMATICAL APPROACH[a]

In memory of Prof. Varsányi

L. M. M. TIJSKENS, L. G. M. GORRIS and M. L. A. T. M. HERTOG

ATO-DLO, P.O. Box 17, 6700 AA, Wageningen. The Netherlands

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The effect of the presence and growth of spoilage bacteria on the keeping quality of perishable products was modelled on theoretical premises. Growth was modelled as simply as possible, to direct attention to effects on quality and to detect how quality could be affected by the bacteria. Two approaches were investigated. Data on quality behaviour, obtained by a Monte Carlo type of simulation, based on the full model description in differential equations, were analysed with simplification of the model formulation, one mathematical and one mechanistic. Although a perfect fit with multivariate nonlinear regression was not obtained, the results of the analyses indicate practical solutions to be possible.

Keywords: keeping quality, microbial spoilage

The keeping quality, shelf life or storage potential of a large number of food products, ranging from fruits and vegetables to fish and meat, is often determined by microbial spoilage (DALGAARD, 1996). Whereas the microorganisms themselves bear no threat to the consumers (non pathogenic) the food is rightly rejected for further trade or consumption. Many models exist, verbal as well as mathematical, which describe the growth of bacteria as a function of level of infection, time, temperature, pH and other external factors (ZWIETERING, 1996; ROBERTS, 1995; BARANYI & ROBERTS, 1994; 1995). A stochastic approach to bacterial modelling was presented by NICOLAÏ (1995). These models only take the growth itself into account, without considering the consequences for the quality of the infected food and for the resulting keeping quality.

Since a fundamental model for keeping quality has been developed (TIJSKENS & POLDERDIJK, 1994; 1996; TIJSKENS, 1995) based on those fundamental reactions in the

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product (enzymatic as well as chemical) that change the intrinsic quality, also the effect of bacterial infection and growth on quality and keeping quality can, on a theoretical basis, be taken into account.

1. Bacterial growth

1.1. General bacterial growth

In analogy to a chemical reaction mechanism, the growth of bacteria can be depicted as:

$$S + B \xrightarrow{k_b} 2B \qquad (1)$$

with S = the concentration of substrate, B = the number of bacteria and k_b = the specific growth rate of bacteria.

Based on the fundamental laws of chemical kinetics, the differential equation can be derived as:

$$\frac{\delta B}{\delta t} = k_b \cdot B \cdot S \qquad (2)$$

with t = time.

The maximum population size is equivalent to or determined by the concentration of available substrate. This relation can be used to express bacterial growth completely in terms of number of bacteria present as shown in Eq. (3),

$$\frac{\delta B}{\delta t} = k_b \cdot B \cdot (B_{max} - B) \qquad (3)$$

with B_{max} = the maximum number of bacteria possible for a given type of substrate. Solving this differential equation at constant temperature, results in the well known logistic equation, frequently applied for bacterial growth:

$$B = \frac{B_{max}}{1 + \left(\frac{B_{max}}{B_0} - 1\right) e^{-k_b \cdot B_{max} \cdot t}} \qquad (4)$$

with B_0 = the initial number of bacteria present. In bacteriology, the term $-k_b \cdot B_{max}$ is usually called μ. The growth rate k_b depends on temperature, assumed according to Arrhenius' law:

$$k = k_{ref} \cdot e^{\frac{Ea}{R} \cdot \left(\frac{1}{T_{ref}} - \frac{1}{T_{abs}}\right)} \qquad (5)$$

with k_{ref} = the reaction rate at reference temperature, Ea = the energy of activation, T_{abs} = the experimental temperature in K and T_{ref} = the fixed reference temperature in K.

Equation (4) and (5) describe the dynamic behaviour of bacterial growth at any constant temperature and initial infection. In Fig. 1 a three dimensional example as a function of time and temperature is shown, in Fig. 2 the same example is shown on a logarithmic scale as is usually applied in microbiology.

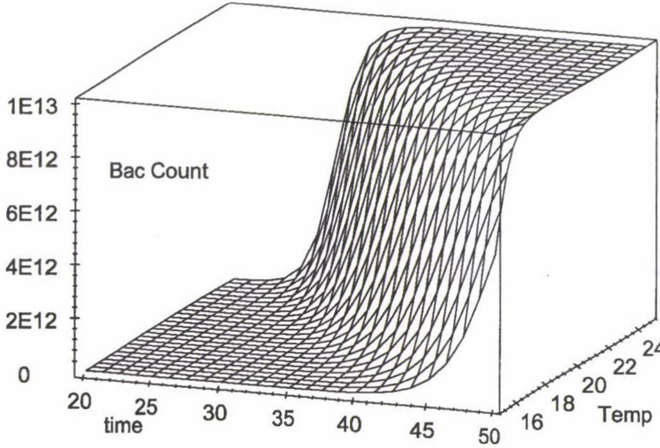


Fig. 1. Bacterial count during logistic growth at temperatures ranging from 15 to 25 °C

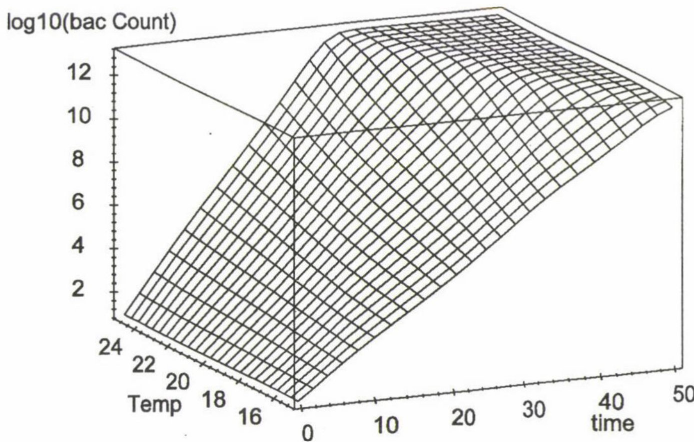


Fig. 2. $\log_{10}$ (Bact. count) during logistic growth at temperatures ranging from 15 to 25 °C

1.2. Simple bacterial growth

As long as the number of bacteria (bacterial count) does not increase above the value of about 50% of the maximum possible number of bacteria (about $5 \cdot 10^{12}$, see Fig. 1), the bacterial growth curve can safely be described using an exponential equation (Eq. (6)):

$$B = B_0 \cdot e^{k_b \cdot B_{max} \cdot t} \tag{6}$$

DALGAARD (1996) even suggested a count limit of about 10^7 compared to a maximum count of 10^{13}. This is only a few ten thousands of a percent and far less then the 50% allowed.

2. Quality decay by bacteria

Whether or not bacteria are present in or at the surface of the product, quality of the product will deteriorate during its lifetime. This can be represented in a simple scheme as Eq. (7):

$$Q \xrightarrow{k_q} \text{decay products} \tag{7}$$

with decay products = some unwanted quality attribute or absence of quality.

When bacteria are present, which is usually the case, and when these bacteria affect the quality of the product, quality will deteriorate the faster the more bacteria are present. This can be represented by Eq. (8):

$$Q + B \xrightarrow{k_{qb}} \text{decay products} \tag{8}$$

with B = number of bacteria and k_{qb} = the rate constant of the bacterial induced quality decay.

The reaction mechanisms shown in Eq. (7) and (8) can be converted into a differential equation (Eq. (9)):

$$\frac{\delta Q}{\delta t} = -\left(k_q + k_{qb} \cdot B\right) \cdot Q \tag{9}$$

Based on the differential equations (3) and (9), simulated data on bacterial count, quality and keeping quality are generated with a range of imaginary (arbitrarily chosen) parameters (Monte Carlo simulation). In Table 1 an overview is given for the parameters applied. The total number of Monte Carlo simulations was 768. The parameter ranges applied are arbitrarily chosen to represent, as nearly as possible, combinations that could occur in practice. The generated data were first analysed as a function of temperature for each combination of the other input parameters separately,

using the standard model for keeping quality as presented by TIJSKENS and POLDERDIJK (1994, 1996) and TIJSKENS (1995). Assuming no previous knowledge on bacterial infection and growth, the parameters estimated, that is the keeping quality at reference temperature (KQ_{ref}) and its activation energy (Ea_{KQ}), show quite different values for different values of input parameters as can be taken from Fig. 3 and Fig. 4. The explained part or percentage variance accounted for (R^2_{adj}) was, however, 100% for each analysis, with temperature as the only explaining variable. This implies that the formulation for the keeping quality is fundamentally correct, but that bacterial spoilage exerts a major effect on the level of the parameters. In other words the parameters in the model of Keeping Quality represent an unknown combination of product information and information with respect to bacterial properties.

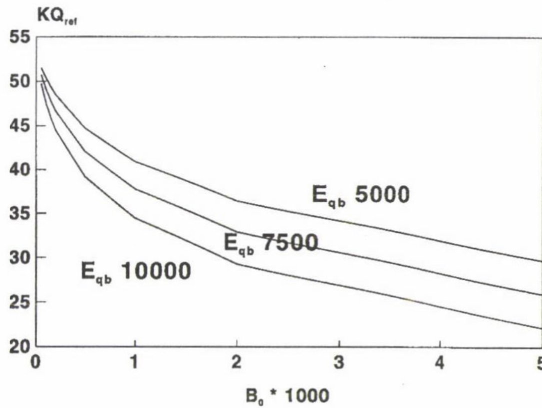


Fig. 3. Keeping quality at reference temperature versus initial bacteria count for three levels of E_{qb}, see Table 1

3. Formulating keeping quality with bacterial spoilage

Substituting the solution for B (Eq. (6)) in Eq. (9) and solving at constant temperature gives the description of quality at all times and all temperatures:

$$Q = Q_0 \cdot e^{-k_q \cdot t - k_{qb} \cdot B_0 \cdot \left(\frac{e^{k_b \cdot t} - 1}{k_b} \right)} \tag{10}$$

Table 1

Parameter combinations used in Monte Carlo simulation

T	[°C]	0 2 4 6 8 10 12 14 16
		18 20 22 24 26 28 30 32
Q_0	[%]	100
Q_l	[%]	70
B_0	[–]	50 100 150 200
		500 1000 2000 5000
B_{max}	[–]	10^{13}
k_{qbref}	[day^{-1}]	10^{-7}
E_{qb}/R	[K]	5000 7500 10000
k_{qref}	[day^{-1}]	0.001
E_q/R	[K]	10000
k_{bref}	[day^{-1}]	0.01
E_b/R	[K]	11000
T_{ref}	[°C]	15

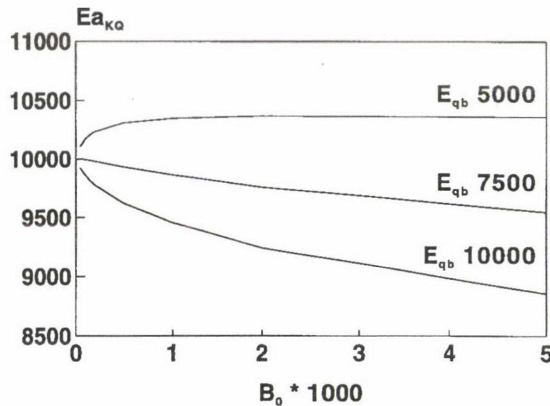


Fig. 4. Energy of activation of keeping quality versus initial bacteria count for three levels of E_{qb}, see Table 1

Keeping quality KQ is now the time to elapse for the quality Q to become unacceptable with respect to the quality limit Q_l imposed by the buyer/consumer. The obtained equation, however, cannot be solved for the keeping quality KQ, and hence, no mathematical equation is available. To obtain an acceptable expression for keeping quality with bacterial spoilage included, we have to make some simplifications and

assumptions. Let us assume that bacteria are always present in the product or at its surface, which is most often the case, and that almost all quality decay, even at normal conditions, are bacterially induced decay, which is most probably not always the case, k_q will always be smaller than the product of B_0 and k_{qb} (see Eq. 9)). The rate constant k_q can then be neglected. Substituting KQ for the time that quality reaches the quality limit Q_1, Eq. (10) reduces to:

$$Q_1 = Q_0 \cdot e^{-\left(\frac{k_{qb} \cdot B_0 \cdot \left(e^{k_b \cdot KQ} - 1\right)}{k_b}\right)} \tag{11}$$

This equation can be solved for KQ as shown in Eq. (12):

$$KQ_B = \frac{\log_e\left(\frac{\log_e\left(\frac{Q_1}{Q_0}\right) \cdot k_b + k_{qb} \cdot B_0}{k_{qb} \cdot B_0}\right)}{k_b} \tag{12}$$

Again we see that keeping quality can be expressed as a quality function depending on the mechanism (numerator in Eq. (12)) divided by the (overruling) reaction rate. This general structure is the same as deduced for the simple model on keeping quality without bacterial spoilage. The quality function is, however, more complex, and resembles in itself the general structure: a quality function divided by a reaction rate.

4. Combined statistical analysis

With the simplified and hence incorrect solution for keeping quality (Eq. (12)), the simulated data were analysed in their entirety with temperature and the different parameters chosen in the Monte Carlo simulation as simultaneous explaining variables. As E_{qb} had three levels of input (see Table 1), this parameter was also estimated. All other parameters were taken from the input for the Monte Carlo simulation. In Table 2 the results of the analysis are shown. Although the explained part (R^2_{adj}) is high (99%), and the standard errors of estimates (s.e.) for the individual parameters are small, a consistent deviation between input and output data can be observed (see Fig. 5 and Fig. 6), except at those conditions where keeping quality is low, that is, at a high bacterial load (B). This is not at all surprising as in the regions of high to moderate keeping quality the assumption made that (almost) all quality decays by bacterial effects, is definitely not true.

Table 2

Result of combined analysis of simulated data with the simplified function (Eq. 12)

		estimate	s.e.
E_{qb}	[K]	7184	113
KQ_{ref}	[day^{-1}]	0.202913	$5.28 \cdot 10^{-4}$
T_{ref}	[C]	15	
N_{obs}	[-]	768	
R^2_{adj}	[%]	99.1	

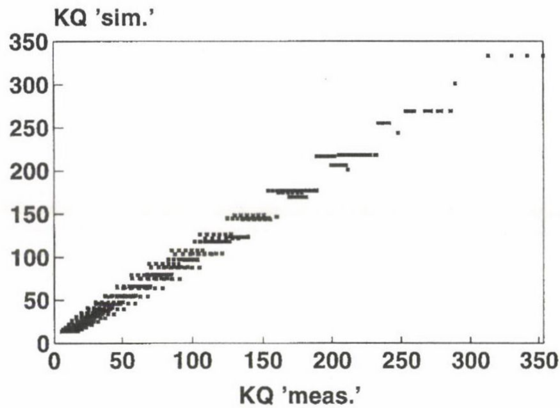


Fig. 5. Scatter plot simulated versus measured keeping quality for all Monte Carlo combinations simultaneous, analysed according to Eq. (12)

Nevertheless, it is encouraging that a very complex behaviour can be simplified into a manageable formulation, that enables the direction of further research with respect to bacterial growth, quality and keeping quality or shelf-life. When applying this line of reasoning for practical applications, the model has to be extended among other with lag phases in bacterial growth, possibly induced by dynamic changes in storage conditions, extra infection during storage, changes in susceptibility of the product, caused by mechanical and chemical damages occurring during its lifetime. The model has also to be extended to more and different but quality relevant bacterial species growing at the same time or at different temperatures as indicated by DALGAARD (1996).

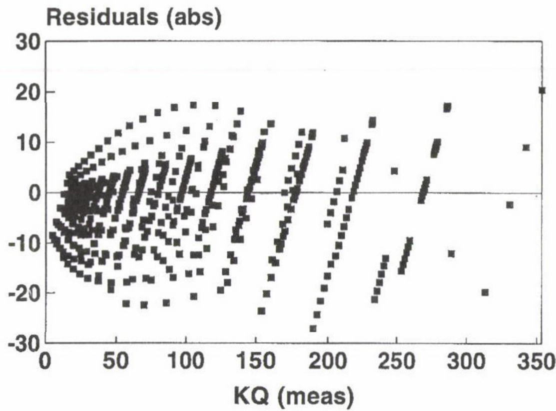
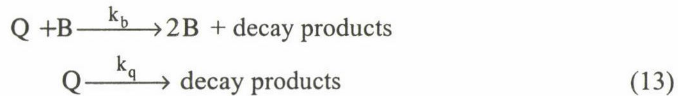


Fig. 6. Residual versus measured keeping quality for all Monte Carlo combinations simultaneous, analysed according to Eq. (14)

5. Alternative approach

In the simplified expressions (Eqs (11) and (12)), the simplifications were sought, although fundamentally initiated, in the mathematical expressions. In the approach now elaborated, the simplifications are sought in the applied mechanism. Let us assume that in the growth of bacteria the quality is directly consumed. In other words, quality decay is directly linked to bacterial growth as can be taken from the reaction mechanism:



$$Q + B \xrightarrow{k_b} 2B + \text{decay products}$$

$$Q \xrightarrow{k_q} \text{decay products} \qquad (13)$$

This reaction mechanism can also be converted into a set of differential equations (not shown), that can be solved at constant external conditions e.g. temperature. This results for both the bacterial count and the quality in a logistic function, with a structure similar to Equation (4). The main differences with the former expression is that this function can be solved for the time (KQ) to reach a certain quality limit (Q_l):

$$KQ = \frac{\log_e\left(\frac{Q_0 \cdot (B_0 + Q_0 - Q_1) \cdot k_b + k_q}{Q_1 \cdot (k_b \cdot B_0 + k_q)}\right)}{(B_0 + Q_0) \cdot k_b + k_q} \tag{14}$$

This equation combines, based on the simplified mechanism, the effects that quality decay processes, with or without bacterial influence, exert on the observed keeping quality. The unusual situation now occurs that the quality function (numerator or Eq. (14)) needs information about the rates of the constituting reactions.

With this equation, the data generated by the complex model were analysed, estimating the four kinetic parameters ($k_{b,ref}$, $k_{q,ref}$, E_b and E_q). In Table 3 the results of this analysis are shown. The obtained explained part is somewhat higher than in the previous analysis, the standard errors are considerably less. Consequently, this approach will be more reliable. This can also be taken from the plot of the residual values (measured – estimated) as shown in Fig. 7.

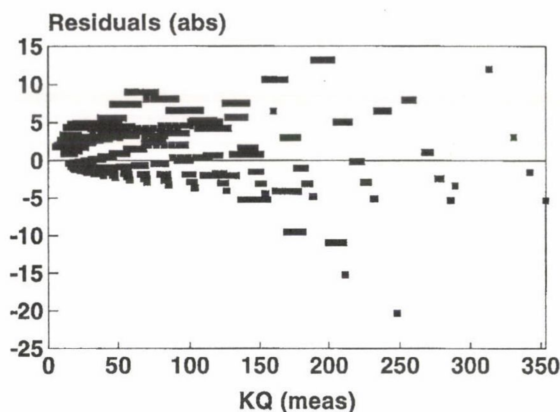


Fig. 7. Residual versus measured keeping quality for all Monte Carlo combinations simultaneous, analysed according to Eq. (14)

Table 3

Results of analysis with the simplified mechanism (Eq. 14)

	Dim.	estimate	s.e.
$k_{b,ref}$	[day^{-1}]	$0.87 \cdot 10^{-6}$	$0.31 \cdot 10^{-7}$
E_b/R	[K]	5584	228
$k_{q,ref}$	[day^{-1}]	$4.7031 \cdot 10^{-3}$	$2.55 \cdot 10^{-6}$
E_q/R	[K]	7877.1	36.1
R^2_{adj}	[–]	99.4	

6. Conclusions

The initial level of infection has, as expected, a marked effect on the keeping quality of the infected product and on the model formulation.

Comparison of both model simplifications applied makes very clear that, although different model formulations can deliver about the same predictive power and accuracy, the structural validity of the model, that is using the correct mechanisms for generating model formulations, is of utmost importance to arrive to generic and general applicable models and data analysis.

Applying proper problem decomposition, it is possible to formulate a model on keeping quality and bacterial spoilage provided sufficient information is available about the growth and infection characteristics of the bacteria involved. These generic models can then be used for statistical analysis of experimental data concerning bacterial growth and quality decay.

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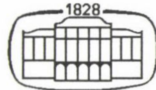

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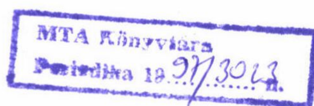
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