ACTA ALIMENTARIA

An International Journal of Food Science

Editor

J. HOLLÓ

Volume 16 **March 1987** **Number 1**

Akadémiai Kiadó
Budapest

D. Reidel Publishing Company
Dordrecht & Boston

ISSN 0139—3006

CODEN ACALDI

ACTA ALIMENTARIA

An International Journal of Food Science

Sponsored by the Joint Complex Committee on Food Science of the Hungarian Academy of Sciences and Ministry of Agriculture and Food.

Editorial office:

Central Food Research Institute, H-1525 Budapest, Herman Ottó út 15, Hungary

Acta Alimentaria is a quarterly journal in English, publishing original papers on food science. The main subjects covered are: physics, physical chemistry, chemistry, analysis, biology, microbiology, enzymology, engineering, instrumentation, automation and economics of foods and food production.

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KULTURA, Hungarian Foreign Trading Company
P.O. Box 149, H-1389 Budapest 62, Hungary

For all other countries:

D. REIDEL PUBLISHING COMPANY
P.O. Box 17, 3300 AA Dordrecht, Holland

Publication programme, 1987: Volume 16 (4 issues).
Subscription prices per volume: Dfl. 266,—/$ 111.00 including postage.

Second class postage paid at New York, N. Y. USPS No. 756-270. U. S. Mailing Agent: Expediters of the Printed Word Ltd., 515 Madison Avenue (Suite 917), New York, N. Y. 10022, U. S. A.

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AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

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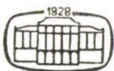
VOLUME 16

1987



AKADÉMIAI KIADÓ
BUDAPEST

D. REIDEL PUBLISHING COMPANY
DORDRECHT & BOSTON

PRINTED IN HUNGARY
Akadémiai Kiadó és Nyomda, Budapest

Acta Alimentaria, Vol. 16 (1), pp. 3–27 (1987)

OUTLINE OF A SYSTEM FOR THE SELECTION OF THE OPTIMUM STERILIZATION PROCESS FOR CANNED FOODS

I. CALCULATION METHODS

I. Körmendy

Department of Canning Technology, University of Horticulture,
H-1118 Budapest, Ménesi u. 45. Hungary
(Received: 5 October 1984; revision received: 3 September 1985;
accepted: 28 December 1985)

A system is presented for the selection of the best sterilization process from a finite number of process variations. The system has been planned for application to canned foods in industrial practice. Some new calculation methods for the determination of temperature and property fields within the processed food material are also described. Adequate computer programmes have been developed for practical use. The following fields are treated in detail: The transfer of heat in the food material solely by conduction. The transfer of heat dominantly by strong natural convection.

Some new methods are also included for the calculation of property changes. These enable a wide range of other than first order variations to be calculated. The concept of "slowest heating point" is also revised and substituted by a more exact term.

Keywords: heat transfer, optimum sterilization process, canned foods, computer programme

Many publications deal with the optimization of heat treatment (sterilization, pasteurization) processes (e.g. Lund, 1977, 1982; Thijssen & Kochen, 1980; Ohlsson, 1980). However, these only investigate property changes in the processed food and are not suitable for direct industrial use. Thus, the calculation of loads and stresses acting on the container are not included in these publications. Generally, first order kinetics are used, though in some cases other orders are also applied (Labuza, 1979; Saguy & Karel, 1980; Horak & Kessler, 1981). It therefore became unavoidable to develop and extend the methods currently applied for the calculation of property changes.

The optimization system proposed here applies to industrial heat treatment processes for food material preserved in cans. It is evident that certain previous results and concepts have also been included in the synthesis. Those parts of the system which can be regarded as more or less well elaborated are as follows:

a. Method for generating standardized process versions for the hydrostatic sterilizers used in Hungary. Among other things, the computer programme calculates the retort (ambient) temperature as a function of heat treatment time, for a selected transport velocity (Körmendy, 1985).

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b. Methods for the calculation of temperature and property fields for cylindrical containers, if heat is propagated exclusively by conduction in the foodstuff.

c. Methods for the calculation of temperature and property fields for cylindrical containers, if heat is transferred dominantly by strong natural convection in the container.

d. Methods for the evaluation of data measured for temperature—time relations during sterilization.

e. Development of methods for the measurement and calculation of deformations vs. load relations in containers (KÖRMENDY, 1982; DELI, 1982; FERENCZY & KÖRMENDY, 1984).

The above results, though designed to work inside a complete system, can be efficiently used separately and independently, too. It should be noted that relevant research activity can also be coordinated by applying the principles of the system. Points *b* and *c* from the above results are presented here in sections 2 and 3, while Point *d* will be treated in a subsequent publication (Part II of this series). Those parts of the system, which are related to the objective function (see Section 1) have not been elaborated so far and are only on a conceptual level.

The explanation of a number of terms will be given before the more detailed descriptions. The term "process version" means basically a selected variation of the temperature and pressure of the heat transfer medium around the container as a function of time. The term "property of the food material" should be understood in a fairly general way: Microbe concentration, enzyme activity, vitamin content, mechanical and sensory properties, etc. "Property value" is a quantity attributed to or calculated for a specified property.

1. Description of the system

The structure of the system is presented in Fig. 1, and works in the following way:

Step 1: A finite number of process versions are generated. The versions are in conformity with the sterilizing apparatus, they satisfy capacity (number of containers per time) requirements, and are in harmony with previous treatments.

Step 2: Calculation of temperature and property values as functions of space and time coordinates (temperature and property field calculations) within the container. In addition, certain special tasks are carried out at the end of properly chosen time intervals: The determination and storage of extreme values in the temperature and property fields. The calculation and storage of temperature and property averages.

Step 3: Examination of the extreme values and averages of the properties. The question considered here is whether these remain within the acceptability domain specified by the proper limiting (PL) values. If *so,* then Step 4 follows. If *not,* then the version in question is dropped and the system returns to Step 1, where the next version will be generated.

Step 4: Calculation of the load on the container. For cans the load is typically the difference between the inside and outside pressures.

Step 5: Examination of the load on the container. If the load on the container is less than the previously stated limiting value (CL), then Step 6 follows. If *not,* then the version in question is dropped and the system returns to Step 1.

Step 6: Evaluation of the objective function. The objective function should mainly represent the quality of the food material.

Step 7: Comparison of the value of the objective function in the current version with the previous best value. If the new value proves to be better (re-

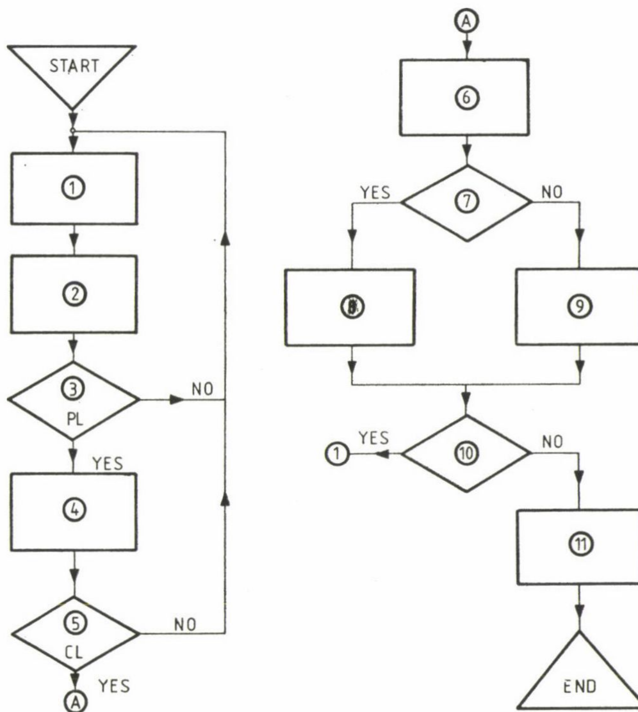

Fig. 1. Flow chart of a system for the optimization of sterilization processes for canned foods. 1: process version generation; 2: calculation of temperature and property fields; 3: examination of the property limits; 4: container load calculation; 5: examination of the container load limits; 6: evaluation of the objective function; 7: comparison of the current (new) and former (best) values of the objective function; 8: and 9: storage of the data of the better version; 10: answering the question, whether the process version in question is the last one; 11: printing out all data of the best version

presenting higher quality), then Step 8 follows. If *not*, then Step 9 will follow.

Steps 8 and 9: All necessary data belonging to the best version are stored, while those of the other are lost.

Step 10: The question raised is whether the current version is the last one. If *so*, then Step 11 follows. If *not*, the system returns to Step 1 for the next process version.

Step 11: All necessary data pertaining to the best version are displayed and printed. The procedure ends.

2. Heat propagation by conduction in cylindrical containers Calculation of temperature and property fields

2.1. Common principles

Two methods have been elaborated. One serves for temperature and property field calculations in infinite cylinders, the other for finite cylinders. Both methods include appropriate computer programmes and are based on an explicit version of elementary heat balances for cylindrical coordinates. Axial symmetry and convective boundary conditions are assumed for both methods. The basic concepts of the procedures were taken from the relevant literature. Recently more sophisticated (and presumably more accurate) methods have also been used (NAVEH et al., 1983); however, these are probably less convenient for practical application at present. The following principles are common to both methods.

The heat capacity of the container has been neglected, though the temperature variation through the container wall perpendicular to the inside surface is taken into account. Heat conduction in the wall in other directions is neglected. The Biot number is defined in the following manner:

$$Bi = \frac{\alpha E}{\lambda}, \qquad \text{Eqn. [1]}$$

where

$$\alpha = \left(\frac{1}{\alpha_R} + \frac{s}{\lambda_s}\right)^{-1} \qquad \text{Eqn. [2]}$$

In the case of an infinite cylinder, $E = R$, while for a finite cylinder $E = R$ at the cylindrical surface and $E = H/2$ for the end plates.

The arrangement of the nodal points is illustrated in Figs. 2 and 3. The distance between the nodes is constant in both the radial and axial directions ($\Delta r, \Delta h$). The cross-sections of the elementary bodies formed for the heat balances are indicated in the figures by hatching. The heat balance for an interior annulus results in the following equation, if only radial heat conduction

is present:

$$\Delta T'_{r,l,n} = \frac{1}{M_r}\left[(T_{l-1,n} - T_{l,n})\left(1 + 0.5\frac{\Delta r}{r_l}\right) - (T_{l,n} - T_{l+1,n})\left(1 - 0.5\frac{\Delta r}{r_l}\right)\right], \qquad \text{Eqn. [3]}$$

where $1 < l \leq L$.

The result for an elementary cylinder under the same conditions is:

$$\Delta T'_{r,L+1,n} = \frac{4}{M_r}(T_{L,n} - T_{L+1,n}) \qquad \text{Eqn. [4]}$$

In the above equations M_r is the radial module calculated by the formula

$$M_r = \frac{\Delta r^2}{a\Delta t'} \qquad \text{Eqn. [5]}$$

Obviously, the module is the reciprocal value of a special Fourier number.

Both methods are constructed in such a way that two subsequent paired values of heat treatment time-ambient (retort) temperature (t_j, $T_{R,j}$; t_{j+1}, $T_{R,j+1}$) are connected by a linear relation and the latter is approximated by a step-wise function as illustrated in Fig. 4.

Figure 4 shows a time—temperature relation, which is typical for a batch retort. The empty circles represent the time—temperature data introduced as expedient by the person carrying out the calculation. The time interval $\Delta t_j = t_{j+1} - t_j$ is always a multiple of the elementary time step $\Delta t'_j$. The Biot numbers can also be varied in each time interval. Thus, it is also possible to calculate cases where the container moves through various ambient mediums with differing heat transfer properties. The initial temperatures at the nodal

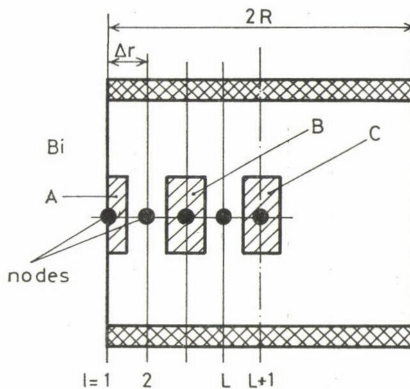

Fig. 2. Geometry and location of the nodal points for the "infinite cylinder". A: cross-section of the elementary annulus at the cylindrical surface; B: cross-section of an interior annulus; C: cross-section of the elementary cylinder

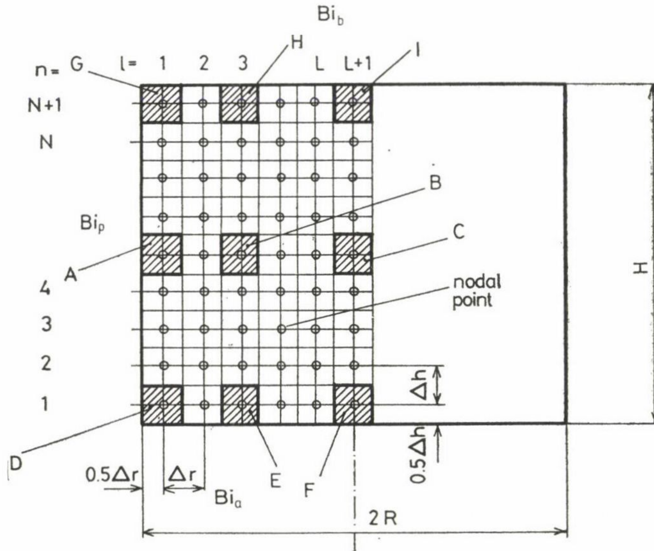

Fig. 3. Geometry and location of the nodal points for the "finite cylinder". A, B and C: see legend to Fig. 2; D and G: cross-sections of the elementary annuli located at the cylindrical surface and at one of the end planes; E and H: cross-sections of elementary annuli, interior in the radial direction, located at one of the end planes; F and I: cross-sections of the elementary cylinders at one of the end planes

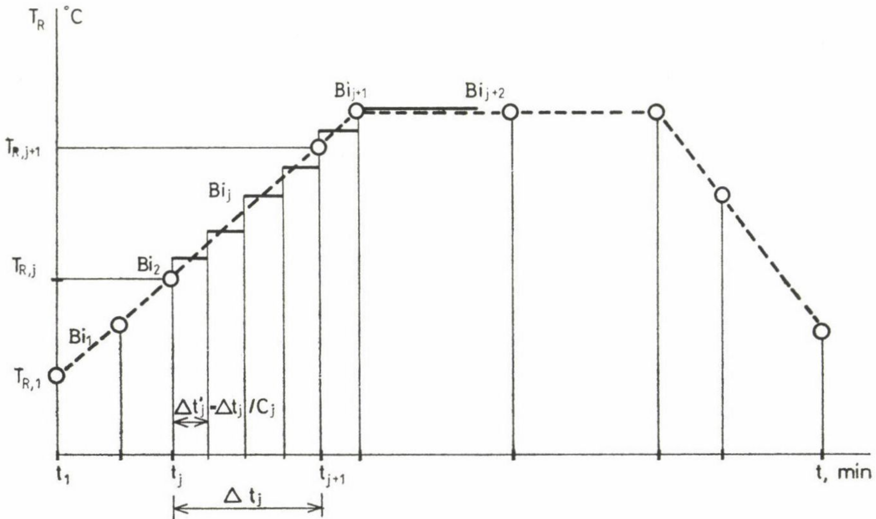

Fig. 4. Illustration of retort temperature vs. heat treatment time and its approximation by a step-wise function within a time interval. Circles (○) illustrate paired data to be entered by the operator

points must be known and included in the calculations. The programmed calculation of the temperature and property fields requires the input of the following data:

- Integers (L, N) for the calculation of nodal distances $(\Delta r, \Delta h)$
- Geometric parameters of the container (R, H, s)
- Thermodynamic properties of the food and the container (a, λ, λ_s)
- Reaction kinetic properties of the food material
- Initial temperatures of the food
- Retort temperatures at appropriate heat-treatment times
- Biot numbers or convective heat transfer coefficients for the outside surface of the container

The $Bi = 0$ or $\alpha_R = 0$ indicates heat insulation at the surface in question. It is therefore also possible to calculate the temperature equalization and the associated property variations within insulated containers.

So-called dimensionless temperatures can also be calculated by substituting the appropriate Fourier number (e.g. $Fo = at/R^2$) for time and the appropriate values of T_R and T_i (e.g. $T_R = 1$ and $T_i = 0$).

The property values or their equivalents (e.g. F-values) are also calculated at the nodal points immediately after the determination of the temperature increment at the same point for the $\Delta t'$ elementary time step. However, the results are not displayed until the previously stipulated (input) process times. These results include the extreme values of temperature and properties (together with their locations) and the averages based on the total volume (or mass).

2.2. The infinite cylinder

This method can be used to solve industrial problems where the cylinder is sufficiently long compared to its diameter, or heat insulation can be assumed at both end surfaces.

The nodal points and elementary bodies are illustrated in Fig. 2. The number of divisions (L) must be at least 2. The heat balances are grouped in three types, and the relevant elementary bodies (their cross-sections) are marked by A, B and C in Fig. 2. Equation [3] is valid for interior annuli (B), while Eqn. [4] is valid for the elementary cylinder (C). The subscripts of the temperatures "r" and "n" have been omitted, as they are superfluous here. The heat balance for the superficial annulus results in

$$\Delta T'_s = T'_s - T_s = \frac{8}{3M_r(1 - 0.25\,\Delta r/R)}[Bi(T_R - T_s)\Delta r/R - (T_s - T_2)(1 - 0.5\Delta r/R)] - \frac{1}{3}\Delta T'_2 \qquad \text{Eqn. [6]}$$

Here the surface temperature (associated with $l = 1$)is denoted by T_s instead of T_1. The $\Delta T'_2$ in Eqn. [6] is calculated from Eqn. [3] by substituting $l = 2$.

The value of the module (M_r) is calculated separately for each time interval (Δt_j) in a programmed manner. The calculation takes into account the stability criterion according to CROFT and LILLEY (1977). As a result two (lower) limits are observed:

$$M_r \geq 4, \tag{7}$$

and

$$M_r \geq \frac{(8Bi - 4.5)\Delta r/R + 10}{3(1 - 0.25\Delta r/R)} \tag{8}$$

Relation [7] is deduced from Eqn. [4], and Relation [8] from Eqn. [6] by selecting temperatures without an upper comma according to Fig. 7 (A) and Fig. 5.

However, Relation [7] has been replaced by the criterion $M_r \geq 6$, which results in smaller elementary times steps ($\Delta t'$) and a better approximation to the "real temperature".

The programmed calculation of the module is now carried out in the following way: The known data are R; L or Δr as $\Delta r = R/L$; a, Bi, Δt.

The first approximate value of the module is denoted by M'_r. The first step is to calculate the value of the right hand side of Relation [8]. If this value is less than 6, then further calculations will proceed with $M'_r = 6$. If not, then the actual value of the right hand side of Relation [8] will be denoted as M'_r and used for further calculations.

The next step is to introduce the value of M'_r into the left hand side of Eqn. [5] and to calculate the first approximative value of the elementary time

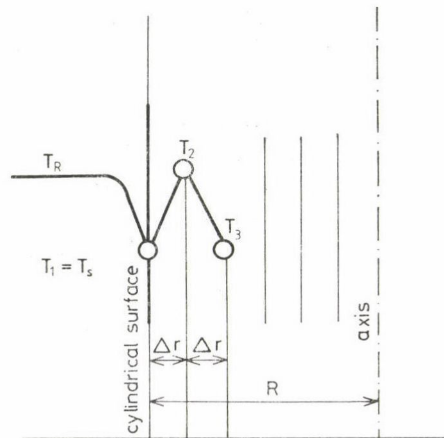

Fig. 5. Set of temperatures for the deduction of Relation [8] from Eqn. [6] according to CROFT and LILLEY (1977). Critical elementary body: Surface annulus A in Fig. 2

step. Next, the time interval (Δt) will be divided by the approximative value of the elementary time step and the result will be rounded up to the next higher integer (C). These steps are integrated in the following formula:

$$C = INT\left(\frac{a\Delta t}{\Delta r^2} M'_r + 1\right) \qquad \text{Eqn. [9]}$$

The final values of the elementary time step and the module are then:

$$\Delta t' = \frac{\Delta t}{C} \qquad \text{Eqn. [10]}$$

and

$$M_r = \frac{\Delta r^2}{a\Delta t} C\,. \qquad \text{Eqn. [11]}$$

The time interval is thus a whole number multiple of the elementary time step. For example, if $R = 0.05$ m, $L = 5$, $\Delta r = 0.01$ *m*, $\Delta r/R = 0.2$, $a = 8.4 \times 10^{-6} \text{m}^2 \text{min}^{-1}$, $Bi = 8$ and $\Delta t = 5$ min, then

$$\frac{(8Bi - 4.5)\Delta r/R + 10}{3(1 - 0.25\,\Delta r/R)} = 7.68 > 6 \quad \text{and} \quad M'_r = 7.68$$

$$C = INT\left(\frac{8.4 \times 10^{-6} \times 5}{0.01^2} 7.68 + 1\right) = INT\ 4.23 = 4$$

$$\Delta t' = 5/4 = 1.25 \text{ min}$$

$$M_r = \frac{0.01^2}{8.4 \times 10^{-6} \times 5} 4 = 9.5238$$

It follows from Relation [8] that at above $M'_r = 6$, there is a linear relation between M'_r and Bi and higher M'_r values are associated with higher Biot numbers. Therefore, the computer time for high Biot numbers is unfavourably long and the case $Bi \to \infty$ cannot be treated. However, one advantage of this arrangement is that one of the nodal points is located on the surface of the cylinder ($T_s = T_1$).

The method is only valid for property changes which have first order reaction kinetics. Property values are calculated in the following way: Each property needs the knowledge of four reaction kinetic parameters, which are the reference temperature (T_r), the z-value (z), the lower limit temperature (T_0) and the decimal reduction time at T_r (D_r). The programmed calculation results in equivalent heat treatment times, e.g. F-values, and these have to be transformed into property values. For a given nodal point, first the temperature increment and the new temperature are calculated, immediately followed by the calculation of the increment of the heat treatment equivalent, for each

previously stated property. The approximate formula

$$\Delta F' = \frac{1}{2}\left(10^{\frac{T'-T_r}{z}} + 10^{\frac{T-T_r}{z}}\right)\Delta t' \qquad \text{Eqn. [12]}$$

is applied and the increment is added to the previous equivalent for the nodal point. If

$$\frac{T'+T}{2} < T_0 \,, \qquad [13]$$

then $\Delta F' = 0$.

Results are only displayed at heat treatment times where time-temperature data are introduced into the programmed calculation. The results are as follows:

Surface temperature (T_s)
Mean temperature (T_m)
Central (axis) temperature (T_c)

For the 1st property:

Surface equivalent (F_{s1})
Mean equivalent (F_{m1})
Central (axis) equivalent (F_{c1})

For the second property:

Surface equivalent (F_{s2})
Mean equivalent (F_{m2})
Central (axis) equivalent (F_{c2})

.
.
.
etc.

The calculation of the mean (or average) property value is done by first transforming the local equivalents into relative property values using the relation

$$Y = 10^{-F/D_r} \qquad \text{Eqn. [14]}$$

The concept "relative property value" is explained in Section 2.3. This is followed by the calculation of the average relative property value (Y_m), using the same simple algorithm with which the mean temperature was calculated. The next step is inverse transformation using the relation

$$F_m = -\, D_r \log Y_m \qquad \text{Eqn. [15]}$$

For the first treatment time (usually $t = 0$) the initial temperature at each nodal point must be given by the operator. The mean temperature is calculated in this case, too, and all the values usually printed out at the end of the time intervals are displayed, though in this case all the equivalents are equal to

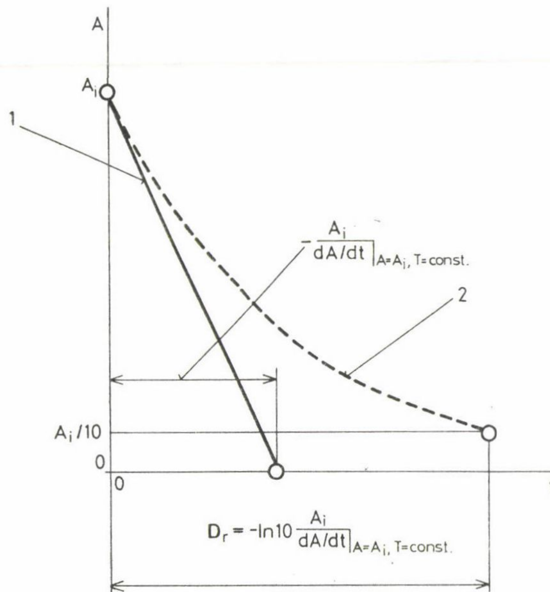

Fig. 6. Illustration of the fictitious decimal reduction time. 1: zero order variation of property A; 2: fictitious first order variation of property A, when the initial rate of change is equal to that for the zero order variation

zero. Property changes following zero order kinetics can be also treated, if the changes are moderate, by introducing a fictitious decimal reduction time, given by the equation

$$D_r = -\ln 10 \frac{A_i}{\left.\frac{dA}{dt}\right|_{A=A_i, T=T_r}} . \qquad \text{Eqn. [16]}$$

The zero order variation is approximated here by first order kinetics as illustrated in Fig. 6.

If D_r is positive, then the relative property value diminishes in time, while negative D_r results in increasing relative property values, as illustrated in Fig. 8 for $p = 1$ (or $p = 0$). The F/D_r is found on the abscissa and the F equivalent is equal to the heat treatment time at the reference temperature.

2.3. *The finite cylinder*

The method for the finite cylinder has been planned to solve industrial tasks where different convective heat transfer coefficients exist at the cylindrical surface and at the lower and upper end plates. If any of the three heat transfer coefficients is zero, then heat insulation is assumed at the surface in

question. For example, if $\alpha_R = 0$ at the end plates, then the case of the infinite cylinder can be treated. If $\alpha_R = 0$ at the cylindrical surface, then we face the case of the infinite slab. If there are different heat transfer coefficients at the lower and upper end plates, then the temperature distribution in the axial direction will be asymmetrical to the bisectional plane of the cylinder.

The nodal points and elementary bodies are illustrated in Fig. 3. The number of divisions, both in the radial (L) and axial (N) directions must be at least 2.

No nodal points are located at the surfaces and the distance of the nearest nodal point to the relevant surface is half the nodal point distance in both the radial and axial directions ($\Delta r/2$, $\Delta h/2$). The heat balances can be grouped in six versions and the related elementary bodies (their cross-sections) are denoted by $A, \ldots, F$ in Fig. 3. The elementary bodies marked G, H, I belong to the same version as the bodies D, E, F.

The heat balance for the annuli at the cylindrical surface (A, D and G in Fig. 3) results in the following equation, if heat is propagated only in the radial direction:

$$\Delta T'_{r,1,n} = \frac{1}{M_r}\left[\frac{2Bi_p\Delta r/R}{(1-0.5\Delta r/R)(2+Bi_p\Delta r/R)}(T_R - T_{1,n}) - (T_{1,n} - T_{2,n})\frac{1-\Delta r/R}{1-0.5\Delta r/R}\right] \qquad \text{Eqn. [17]}$$

The corresponding equation for the elementary bodies at the end surfaces (D, E, F, G, H and I in Fig. 3), if heat is propagated only in the axial direction, is:

$$\Delta T'_{h,l,n} = \frac{1}{M_h}\left[\frac{2Bi_h\Delta h/H}{1+Bi_h\Delta h/H}(T_R - T_{l,n}) - (T_{l,n} - T_{l,d})\right] \qquad \text{Eqn. [18]}$$

At the lower end surface: $n = 1$, $d = 2$ and $Bi_h = Bi_a$.
At the upper end surface: $n = N+1$, $d = N$ and $Bi_h = Bi_b$.

The following equation is obtained for elementary bodies which are not located at the end surfaces (A, B and C in Fig. 3), if heat is propagated only in the axial direction:

$$\Delta T_{h,l,n} = \frac{1}{M_h}(T_{l,n-1} - 2T_{l,n} + T_{l,n+1}) \qquad \text{Eqn. [19]}$$

Here $1 < n \leq N$.
The temperature increment at the nodal points is calculated from the following equations, if heat is propagated in both the radial and axial directions:
For elementary body A ($l = 1$, $1 < n \leq N$), by adding Eqns. [17] and [19]:

$$T'_{1,n} - T_{1,n} = \Delta T'_{r,1,n} + \Delta T'_{h,1,n} \qquad \text{Eqn. [20]}$$

For the elementary body B $(1 < l \leq L,\ 1 < n \leq N)$, by adding Eqns [3] and [19]:

$$T'_{l,n} - T_{l,n} = \Delta T'_{r,l,n} + \Delta T'_{h,l,n} \qquad \text{Eqn. [21]}$$

For the elementary body C $(l = L + 1,\ 1 < n \leq N)$, by adding Eqns [4] and [19]:

$$T'_{L+1,n} - T_{L+1,n} = \Delta T'_{r,L+1,n} + \Delta T'_{h,L+1,n} \qquad \text{Eqn. [22]}$$

For the elementary bodies D and G $(l = 1,\ n = 1 \text{ or } n = N + 1)$, by adding Eqns [17] and [18]:

$$T'_{1,n} - T_{1,n} = \Delta T'_{r,1,n} + \Delta T'_{h,1,n} \qquad \text{Eqn. [23]}$$

For the elementary bodies E and H $(1 < l \leq L,\ n = 1 \text{ or } n = N + 1)$ by adding Eqns [3] and [18]:

$$T'_{l,n} - T_{l,n} = \Delta T'_{r,l,n} + T'_{h,l,n} \qquad \text{Eqn. [24]}$$

For the elementary bodies F and I $(l = L + 1,\ n = 1 \text{ or } n = N + 1)$, by adding Eqns [4] and [18]:

$$T'_{L+1,n} - T_{L+1,n} = \Delta T'_{r,L+1,n} + \Delta T'_{h,L+1,n} \qquad \text{Eqn. [25]}$$

In the above equations Bi_p is the Biot number for the cylindrical surface. This can be calculated by substituting $\alpha = \alpha_p$ and $E = R$ into Eqn. [1]. Bi_a and Bi_b are the Biot numbers for the lower and upper end plates and are calculated by substituting $\alpha = \alpha_a$ or $\alpha = \alpha_b$ and $E = H/2$ into Eqn. [1].

The radial module M_r is the same as for the infinite cylinder, while the axial module M_h is calculated by the equation

$$M_h = \frac{\Delta h^2}{a \Delta t'} \qquad \text{Eqn. [26]}$$

It is obvious that the ratio of the two modules depends only on the values of Δr and Δh. The test of stability resulted in the relation:

$$M_r \geq 4 + 3\left(\frac{M_r}{M_h}\right) = 4 + 3\left(\frac{\Delta r}{\Delta h}\right)^2 \qquad [27]$$

when the same principles were applied as for the infinite cylinder (CROFT & LILLEY, 1977).

Relation [27] was deduced from Eqn. [25] and refers to elementary bodies F or I in Fig. 3 when temperatures without a comma are chosen in accordance with Fig. 7. Bi_a or $Bi_b \to \infty$ was also introduced in Eqn. [25].

The programmed calculation of the module rests on the same principles as that for the infinite cylinder and Eqn. [9], [10] and [11] are valid in this

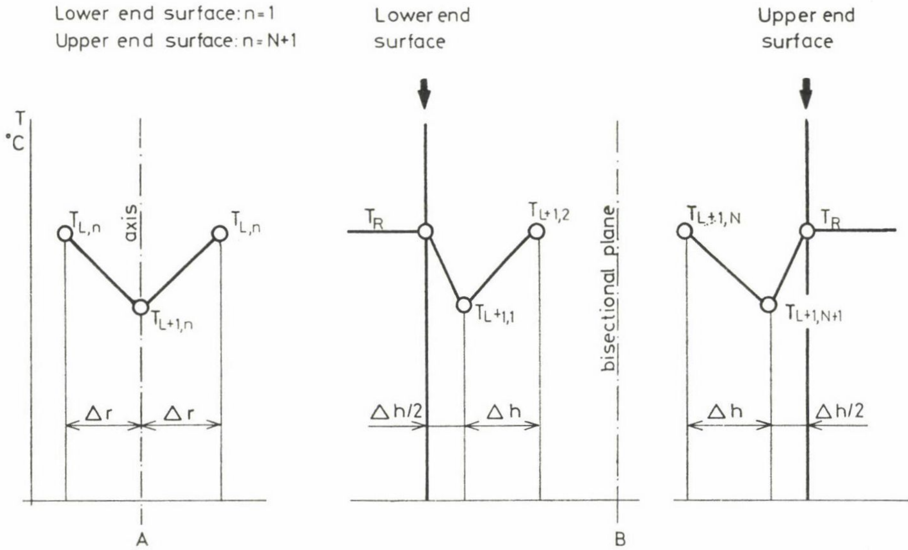

Fig. 7. Set of temperatures for the deduction of Relation [27] from Eqn. [25] according to CROFT and LILLEY (1977). Critical elementary body: Elementary cylinder at one of the end planes, marked by *F* or *I* in Fig. 3. A: variation of temperature in the radial direction; B: variation of temperature in the axial direction

case, too. The axial module has the value:

$$M_h = \left(\frac{\Delta h}{\Delta r}\right)^2 M_r \qquad \text{Eqn. [28]}$$

This method, in contrast to the method for the infinite cylinder, can be applied without difficulty to high Biot numbers. Thus, any of the cases $Bi_p \to \infty$, $Bi_a \to \infty$ or $Bi_b \to \infty$ can easily be approximated and handled.

The calculation of the property values is carried out using a more advanced method. Each property needs five reaction kinetic constants (T_r, z, p, T_0, D_r). Three of these (T_r, z, T_0) have the same meaning as in the method for the infinite cylinder. p is the dimensionless constant for the reaction order. D_r can only be interpreted as the decimal reduction time in the case of first order reactions ($p = 1$). Otherwise it has a somewhat different meaning and will therefore be called the "time constant" at the reference temperature (see later).

The method is suitable for types of property variations, which are described in the case of first order kinetics ($p = 1$) by the differential equation

$$\frac{dY}{dt} = -\frac{\ln 10}{D} Y \,, \qquad \text{Eqn. [29]}$$

or, if $p \neq 1$, according to

$$\frac{dY}{dt} = -\frac{1}{(1-p)D} Y^p \qquad \text{Eqn. [30]}$$

p can be called the constant for the reaction order and has values in the range $-\infty < p < +\infty$. Y is the relative property value, according to

$$Y = \frac{A - A_p}{A_i - A_p}, \qquad \text{Eqn. [31]}$$

where A_i is the initial value of the property and A_p is an expediently chosen constant. The time constant D depends only on the temperature. The solution of Eqn. [29] at constant reference temperature (T_r), the initial condition being $t = 0$, $A = A_i$ and $Y = 1$, and substituting the symbol t by F, results in Eqn. [14]. If $p \neq 1$ the solution of Eqn. [30] is

$$Y^{1-p} = 1 - \frac{F}{D_r} \qquad \text{Eqn. [32]}$$

The above solutions are illustrated in Fig. 8 for different values of p. Negative F/D_r indicates a negative value of D_r, which is the time constant at the reference temperature. Figure 8 also shows that very different types of property changes can be described in this way. If $p = 1$ and $D > 0$, the time constant is the well-known "decimal reduction time". If $p = 1$ and $D < 0$, D can be called the "decimal multiplication time". In other cases ($p \neq 1$), taking a time interval having the absolute value of D, the absolute value of the change in Y^{1-p} will be unity at constant temperature.

The calculation of the time constant—temperature relation or of the decimal reduction time follows the Bigelow theorem:

$$D = D_r 10^{-\frac{T-T_r}{z}} \qquad \text{Eqn. [33]}$$

The solution of the problem, when temperature varies in time, is obtained by substituting Eqn. [33] into Eqn. [29] or [30]. This is followed by the separation of the variables and by integration on both sides of the equations, $t = 0$, $Y = 1$ as the initial condition.

The result for $p = 1$ from Eqn. [29] is:

$$\lg Y = -\frac{1}{D_r} \int_0^t 10^{\frac{T(t')-T_r}{z}} dt' \qquad \text{Eqn. [34]}$$

and for $p \neq 1$ from Eqn. [30]:

$$Y^{1-p} = 1 - \frac{1}{D_r} \int_0^t 10^{\frac{T(t')-T_r}{z}} dt' \qquad \text{Eqn. [35]}$$

It is obvious when comparing Eqn. [34] with Eqn. [14] and Eqn. [35] with Eqn. [32] that the definite integral in the above relations is equal to F.

In the system used for calculating property variations there are altogether six constants, A_i, A_p, z, p, T_0 and D_r, which have to be determined experimentally. T_r is generally selected by some kind of common agreement. The experiments are usually measurements of the property value as a function of time at different constant temperatures. Five constants: A_i, A_p, z, p, D_r can be found by the least squares method. T_0 is the lower limit temperature. At this temperature property changes in time are so small, that they are negligible.

According to this method, instead of equivalent times the relative property values are calculated at the nodal points: The calculation of the tempera-

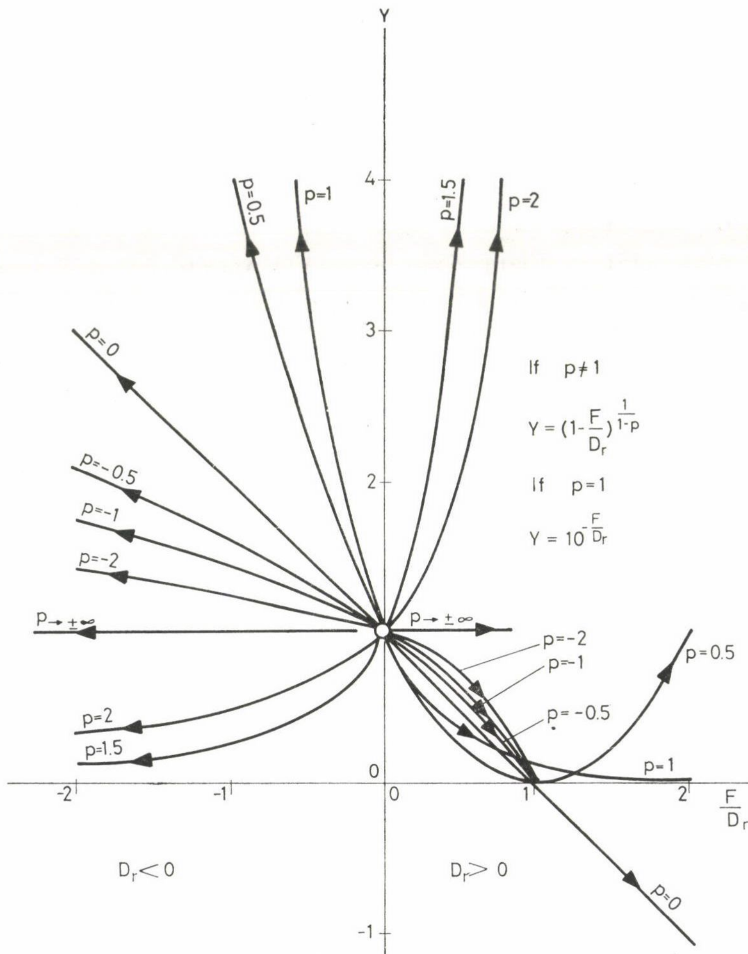

Fig. 8. Variation of the relative property value (Y) vs. F/D_r at different parametric values of the reaction order (p)

ture increment ($\Delta T'$) and the new temperature is followed by the calculation of the increments of the equivalents ($\Delta F'$). Next the relative property values are transformed by means of Eqn. [32] ($p \neq 1$) or Eqn. [14] ($p = 1$) into the corresponding equivalents and the increments of the equivalents are added to these values. The next step is the calculation of the new relative property values (Y') by inverse transformation.

However, instead of Eqn. [12] and Relation [13] a previously published method is used (KÖRMENDY, 1982, 1982a) for the calculation of $\Delta F'$. The method is based on the approximation that the temperature variation is linear within the time increment ($\Delta t'$).

Results are only displayed when time-temperature data are introduced into the calculation, as for the infinite cylinder. The results displayed are the following:

Serial numbers (l, n) for the nodal point of the maximum temperature
The value of the maximum temperature (T_{max})
Average temperature (T_m)
Serial numbers (l, n) for the nodal point of the minimum temperature
The value of the minimum temperature (T_{min})

For the 1st property:

Serial numbers (l, n) for the nodal point of the maximum relative property value
Maximum relative property value (Y_{max})
The average of the relative property values (Y_m)
Serial numbers (l, n) for the nodal point of the minimum relative property value
Minimum relative property value (Y_{min})

The same results are displayed for the 2nd and all further properties.

A special algorithm has been developed to find the maximum and minimum values, together with the associated serial numbers (l, n), and to calculate the average.

It is obvious that the concept of "slowest heating point" has been radically replaced by the concept "locations of the extreme values". The locations of the extreme values may move as the heat treatment time advances. For instance, if the heat transfer coefficients at the end plates differ ($\alpha_a \neq \alpha_b$), then the location of the minimum temperature when heating the container (the "slowest heating point") will move along the axis of the cylinder.

The computer programme has been so constructed, that all temperatures and optionally the relative property values are printed out at the end of the calculations. It is therefore possible to continue the calculations at a later time, beginning with the end-state of the former calculations.

The various steps in the programme are illustrated in Fig. 9.

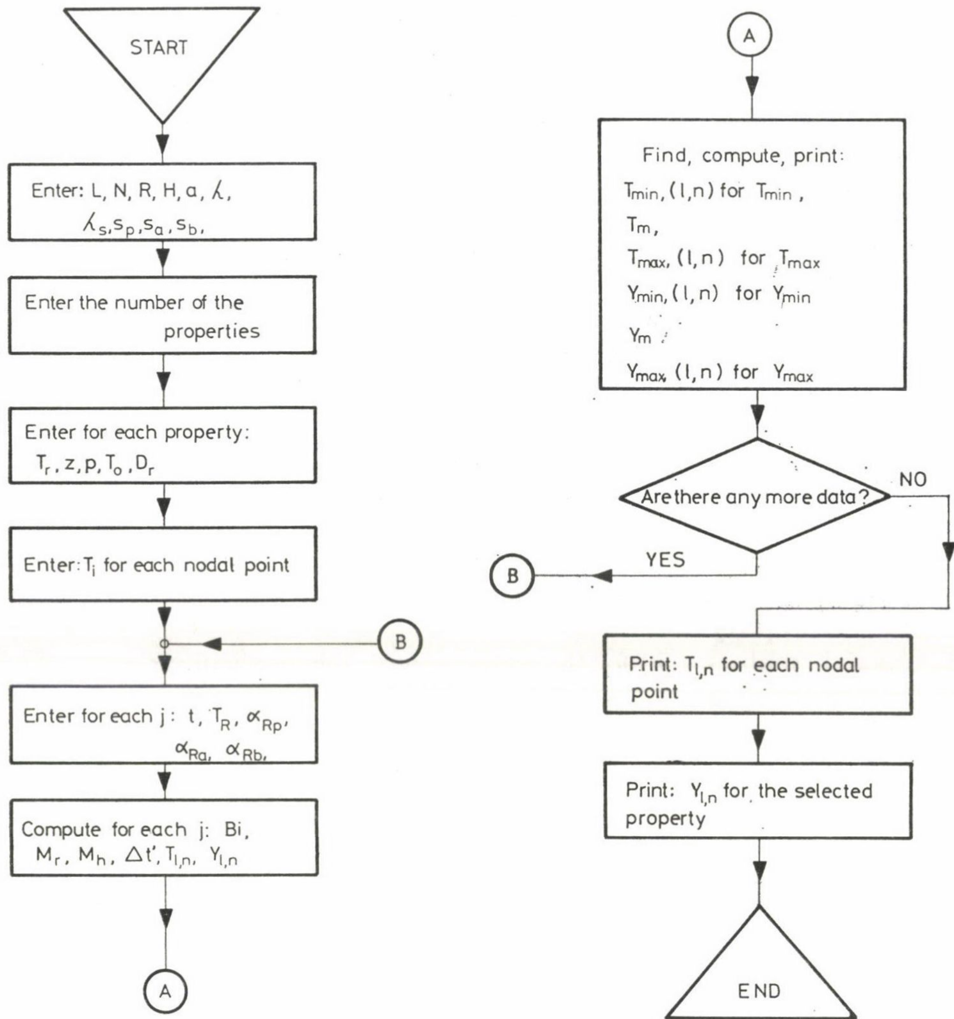

Fig. 9. Flow chart of the calculation programme for the "finite cylinder"

3. Strong natural convection in cylindrical containers

Under "strong natural convection" we mean that, except for a negligibly thin layer at the boundary surface, the temperature within the container is almost the same everywhere at a given time. Thus, a single temperature, the average, characterizes the whole content of the container.

Besides geometric data such as the radius of the inner surface (R), the volume of the foodstuff (V) and the thickness of the container wall (s), the following physical properties are needed for the calculations: the thermal

conductivity (λ), specific heat (c), density (ϱ), coefficient of volumetric expansion (α_V) and dynamic viscosity of the liquid foodstuff (η), thermal conductivity of the container wall (λ_s). Suitable average values have to be substituted for all physical properties, except for dynamic viscosity, where the temperature dependence of viscosity has to be taken into account. Seven paired data for viscosity vs. temperature between 20 and 140 °C must be introduced for the calculations. The data are stored and interpolation is based on the relation:

$$\log_{10}\eta = A_1 + A_2 / (T + 273.15) \,, \qquad \text{Eqn. [36]}$$

where A_1 and A_2 are constants and their values depend on the end values of the interpolation range.

The convective heat transfer coefficients for the outside of the container (α_R) or the relevant Biot numbers (Bi) are also needed. The calculations give the average temperature within the container (T_m) as a function of heat treatment time, if the initial temperature within the container (T_i) and the ambient temperature (T_R) as the function of heat treatment time are known. The Biot number is assumed to be constant within each time interval, as in cases of heat conduction in cylindrical bodies (see Fig. 4).

The calculation is based on the following differential equation:

$$kS(T_R - T_m) - \varrho c V \frac{dT_m}{dt} = 0 \,, \qquad \text{Eqn. [37]}$$

which is the result of a simple heat balance for the food material. The same simplification regarding the container have been adopted here as for heat conduction in Para. 2.1. Therefore, Eqn. [1] and [2] are assumed to be valid. The Biot number is calculated by substituting $E = R$. The overall heat transfer coefficient is then calculated by the relation:

$$k = \left(\frac{1}{\alpha} + \frac{1}{\alpha_m}\right)^{-1} = \left[\left(\frac{1}{Bi} + \frac{1}{Nu}\right)\frac{R}{\lambda}\right]^{-1} , \qquad \text{Eqn. [38]}$$

where

$$Nu = \frac{\alpha_m R}{\lambda} \qquad \text{Eqn. [39]}$$

The calculation method is based on the solution of Eqn. [37] under the following conditions: The variation of the ambient temperature and the overall heat transfer coefficient within the time interval (Δt_j) are approximated by a linear function. The average temperature in the container (T_{mj}) at the begin-

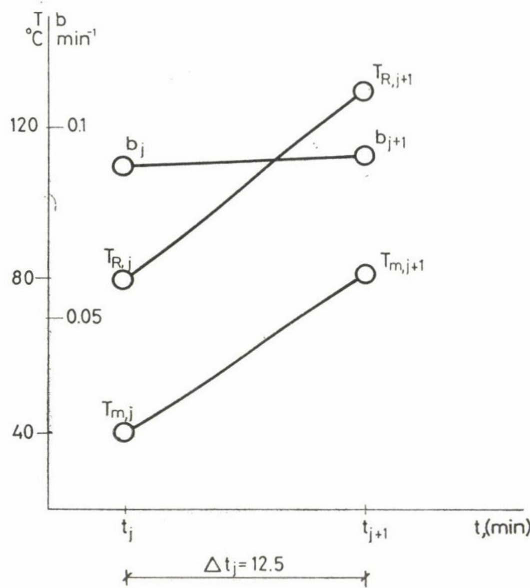

Fig. 10. An example of strong natural convection within the container. Linear variation of the retort temperature vs. heat treatment time within given time interval. The computed variation for the average food temperature and the velocity coefficient

ning of the time interval (t_j) is known. The solution is

$$T_{m,j+1} = \left[\frac{T_{R,j+1} - T_{R,j}}{b_{m,j}\,\Delta t_j} - (T_{R,j} - T_{m,j})\right] e^{-b_{m,j}\Delta t_j} + $$

$$+ T_{R,j+1} - \frac{T_{R,j+1} - T_{R,j}}{b_{m,j}\,\Delta t_j} \qquad \text{Eqn. [40]}$$

and

$$b_{m,j} = \frac{1}{2}(b_j + b_{j+1}) \qquad \text{Eqn. [41]}$$

The velocity coefficient of heat exchange b is simply proportional to k:

$$b = \frac{S}{\varrho c V} k \qquad \text{Eqn. [42]}$$

which is naturally valid for the averages (b_m, k_m), too.

Figure 10 illustrates an example for the variation of T_R, T_m and b within a time interval. The Nusselt number is calculated using the following formula (HIDDINK et al., 1976):

$$Nu = K(Gr \cdot Pr)^m = K(B \cdot |T_s - T_m|)^m , \qquad \text{Eqn. [43]}$$

where

$$B = \frac{gR^3\alpha_V\varrho^2 c}{\eta\lambda} \qquad \text{Eqn. [44]}$$

Thus, R has been chosen as the characteristic length in the Grashof number. The value of η is always that associated with the temperature $\frac{1}{2}(T_s + T_m)$. The calculation is made after the following pattern:

Step 1: The calculation of T_s, if T_R, T_m and α are known. The method is based on the relation:

$$\alpha(T_R - T_s) = \alpha_m(T_s - T_m) \qquad \text{Eqn. [45]}$$

which is valid since the heat capacity of the container has been neglected. After substituting α and α_m from Eqn. [1] and [39] and Nu from Eqn. [43] followed by rearrangement and reduction, Eqn. [45] is transformed into the following formula:

$$T_R - T_s = \frac{KB^m}{Bi}\left|T_s - T_m\right|^m (T_s - T_m) \qquad \text{Eqn. [46]}$$

T_s is the only unknown value here, because the dynamic viscosity in B depends only on T_s, as T_m is known. The solution of the equation is obtained in an iterative manner as illustrated in Fig. 11. The figure illustrates the version $T_R > T_m$. The difference $T_R - T_m$ is divided into 10 intervals and the iteration begins with $T_s^x = T_m$, increasing the value of T_s^x by $10^{-1}(T_R - T_m)$ in each step. The current value of T_s^x is substituted into both sides of Eqn. [46]. If the right side of the equation is greater than the left side, then one step backwards is made and the procedure is repeated, this time increasing the value of T_s^x by $10^{-2}(T_R - T_m)$. The procedure is continued using smaller and smaller intervals until the interval is less than a prescribed value, say 10^{-3} °C. The same procedure is used when $T_R < T_m$, but here negative steps are taken from $T_s^x = T_m$.

Step 2: The calculation of b, when T_R, T_m, α and T_s are known. First Nu is calculated from Eqn. [39], then k from Eqn. [38] and b from Eqn. [42].

Step 3: The calculation of $b_{m,j}$ and $T_{m,j+1}$, if Δt_j, $T_{R,j}$, $T_{R,j+1}$, $T_{m,j}$ and α_j (or Bi_j) are known. The procedure starts with the calculation of $T_{s,j}$ and b_j through Step 1 and Step 2. The substitution of $b_{m,j} = b_j$ into Eqn. [40] gives the first approximation of $T_{m,j+1}$. Then follows the determination of the first approximation of $T_{s,j+1}$ and b_{j+1} by repeating Step 1 and Step 2. The first approximation of $b_{m,j}$ is obtained from Eqn. [41] and when this is substituted into Eqn. [40] the result is the second approximation of $T_{m,j+1}$ and consequently of b_{j+1}. The procedure is repeated until the relative difference between two consecutive average velocity coefficients ($b'_{m,j}$ and $b''_{m,j}$) is less than a

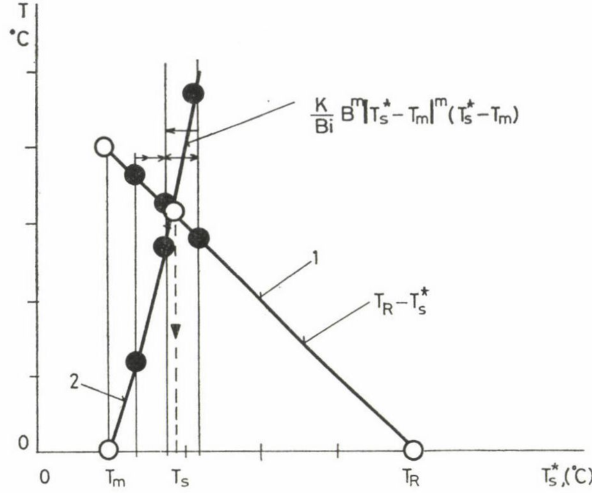

Fig. 11. Illustration of the iteration for finding T_s in Eqn. [46]. 1: the left side of Eqn. [46] vs. T_s^x; 2: the right side of Eqn. [46] vs. T_s^x

prescribed value according to

$$\left| \frac{b''_{m,j} - b'_{m,j}}{b'_{m,j}} \right| < 0.001 \qquad [47]$$

The consecutive average velocity constants within a single time interval are always calculated using the same value of b_j in Eqn. [41]. Fig. 10 illustrates the results of this calculational procedure.

Nomenclature

a	Thermal diffusivity (m^2 min^{-1})
A	Property value
A_i	Initial value of a property
A_p	Property constant (see Eqn. [31])
A_1, A_2	Constants in Eqn. [36]
b	Velocity coefficient (see Eqn. [42]) (min^{-1})
b_m, b'_m, b''_m	Average of the velocity coefficient (see Eqns [41] and [40]) (min^{-1})
B	Notation for the right hand side of Eqn. [44] ($°C^{-1}$)
Bi	Biot number (see Eqn. [1])
Bi_h	Biot number at one of the end plates of the cylinder. The $h = a$ indicates the lower and $h = b$ the upper end plate
c	Specific heat of the food material (J kg^{-1} $°C^{-1}$)

C	Number of elementary time steps within a time interval (see Eqns. [9], [10] and [11])
CL	Abbreviation for "container limits" in Fig. 1
D	Time constant ($p \neq 1$) or decimal reduction time ($p = 1$). See Eqns. [29], [30] and [33] (min)
D_r	The same as D at the reference temperature (min)
E	Characteristic length in dimensionless parameters (m)
F	Equivalent heat treatment time (min)
F_o	Fourier number
g	Acceleration of gravity (m s^{-2})
G_r	Grashof number
H	Length of the finite cylinder (m)
k	Overall heat transfer coefficient referred to the surface of the food material (W m^{-2} °C^{-1}, J min^{-1} m^{-2} °C^{-1}) in Eqns. [37] and [42]
k_m	The average of k
K	Dimensionless constant in Eqns [43] and [46]
l	Serial number of the nodal points in the radial direction ($1 \leq l \leq L + 1$)
L	Number of divisions in the radial direction for the allocation of the nodal points
m	Dimensionless exponent in Eqns. [43] and [46]
M_h	Axial module according to Eqn. [26]
M_r	Radial module according to Eqn. [5]
M'_r	Approximate value of M_r
n	Serial number of the nodal points in the axial direction ($1 \leq n \leq N + 1$)
N	Number of divisions in the axial direction for the allocation of the nodal points
Nu	Nusselt number (see Eqn. [39])
p	The constant for the reaction order (see Eqn. [30])
Pr	Prandtl number
PL	Abbreviation for "property limits"
r_l	Radius of a nodal point with the serial number l (m)
R	Radius of the cylinder (m)
s	Thickness of the container wall (m)
S	Surface area of the finite cylinder (m^2)
t	Time (min)
t'	Time as an integration variable in Eqns. [34] and [35] (min)
T	Temperature (°C)
T_i	Initial temperature (°C)
T_0	Lower temperature limit for the calculation of equivalent time or relative property value (°C)

T_r Reference temperature (°C)
T_R Ambient temperature (retort temperature, °C)
T_s^x Surface temperature as an iterational variable (see Eqn. [46], °C)
T' Temperature at a nodal point at the end of an elementary time step $\Delta t'$ (°C)
V Volume of the cylinder (m^3)
Y Relative property value (see Eqn. [31])
Y' Relative property value at the end of an elementary time step $\Delta t'$
z Constant in Eqn. [33], positive temperature difference for the decimal reduction of D (°C)
α Heat transfer coefficient according to Eqn. [2] (W m^{-2} °C^{-1})
α_R Heat transfer coefficient at the outside surface of the container referred to the surface of the food material (W m^{-2} °C^{-1})
α_V Coefficient of volumetric expansion for the food material (°C^{-1})
$\Delta F'$ Increment of the equivalent heat treatment time in time step $\Delta t'$ (see e.g. Eqn. [12], min)
Δh Distance of the nodal points in the axial direction (m)
Δr Distance of the nodal points in the radial direction (m)
Δt Time interval, i.e. difference between two consecutive heat treatment times introduced into the calculations (min)
$\Delta t'$ Elementary time step (see Eqn. [10], min)
$\Delta T'$ Temperature increment in the time step $\Delta t'$ (°C)
$\Delta T'_h$ Temperature increment at a nodal point, if heat is propagated only in the axial direction (see Eqns [18] and [19], °C)
$\Delta T'_r$ Temperature increment at a nodal point, if heat is propagated only in the radial direction (see Eqns [3], [4] and [17], °C)
$\Delta T'_s$ Temperature increment at the cylindrical surface of the infinite cylinder (see Eqn. [6], °C)
η Dynamic viscosity of the liquid food material (Ns m^{-2})
λ Thermal conductivity of the food material (W m^{-1} °C^{-1})
λ_s Thermal conductivity of the container wall (W m^{-1} °C^{-1})
ϱ Density of the food material (kg m^{-3})

Subscripts

a Refers to the lower end plate of the cylinder (Bi, s, α, α_R)
b Refers to the upper end plate of the cylinder (Bi, s, α, α_R)
c Refers to the axis of the cylinder (F, T)
d $d = 2$ or N in Eqn. [18]
j Serial number referring to heat treatment times introduced into the calculations and to other related quantities, either introduced or calculated (b, b_m, Bi, C, t, T_m, T_R, T_s, Δt, $\Delta t'$, α)
l Refers to the serial number of the nodal points in the radial direction, increasing towards the axis ($1 \leq l \leq L + 1$)

m	Refers to an average (b, F, k, T, Y)
min	Refers to a minimum quantity (T, Y)
max	Refers to a maximum quantity (T, Y)
n	Refers to the serial number of the nodal points in the axial direction, increasing towards the upper end plate ($1 \leq n \leq N + 1$)
p	Refers to the cylindrical surface of the finite cylinder (Bi, s, α, α_R)
s	Refers to the cylindrical surface of the infinite cylinder (T, F), or the surface of the food material (T)

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Acta Alimentaria, Vol. 16 (1) pp. 29—43 (1987)

INFLUENCE OF SUBSTRATE ON THE F-2 AND T-2 PRODUCTION OF FUSARIUM SPECIES

1. EFFECT OF CORN AND RICE SUBSTRATES

A. Badawey, A. Halász, J. Sawinsky and E. Kozma

Central Food Research Institute, H-1022 Budapest,
Herman Ottó út 15. Hungary
(Received: 2 July 1985; revision received: 19 February 1986;
accepted: 21 February 1986)

Twenty-one identified strains of Fusarium sp were examined for their ability to produce F-2 and T-2 toxin on sterilized, moistened rice and corn grains. The following strains were used: *F. culmorum* (234 B/5, 234 B/7, 13, 15, 25), *F. graminearum* (977/12, 11, 8, 15, 39/3), *F. moniliforme* (187 B/19, 228 B/9), *F. tricinctum* (20, 21, 17, 204/10) *F. roseum* (908 B/1, 8, 908 B/6, *F. semitectum* (14) and *F. sporotrichioides* (16).

The F-2 and T-2 toxin production was confirmed by GLC and estimated by TLC. The strains of individual species produced the following amounts F-2 toxin per kg of corn: 4 strains of *F. culmorum* (0.77 to 205.10 mg), 3 of *F. graminearum* (8.07 to 50.70 mg), 2 of *F. moniliforme* (5, 7 to 16.00 mg) 2 of *F. roseum* (2.82 to 8.56 mg), *F. semitectum* 27.5 mg and *F. sporotrichioides* 7.01 mg and one strain of *F. tricinctum* produced 1.44 mg T-2 toxin. On rice we found the following amounts of F-2 toxin per kg: 2 strains of *F. culmorum* (53.43 to 1166.13 mg), *F. moniliforme* (5.39 mg), 3 strains of *F. graminearum* (15.54 to 453.11 mg), one from each of *F. semitectum* and *F. sporotrichioides* (0.12 and 0.37 mg, respectively) while *F. roseum* did not produce any toxin, but in the case of *F. tricinctum* T-2 toxin production was 119 mg per kg.

Keywords: F-2 toxin, T-2 toxin, Fusarium species, substrate dependence

Species of Fusarium, such as *Fusarium roseum, Fusarium tricinctum,* and *Fusarium oxysporum* produce mycotoxins known as zearalenone (Mirocha et al., 1971) and trichothecenes (Bamburg & Strong, 1971). *Fusarium culmorum, Fusarium graminearum* (Christensen et al., 1965) and *Fusarium moniliforme* (Mirocha et al., 1969) were reported as zearalenone producers. Urry and co-workers (1966) confirmed the toxic compound to be an enantiomorph of 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcyclic acid lactone and named it zearalenone (Fig. 1).

It is the most frequently encountered mycotoxin in cereal grain. Bata and co-workers (1983) reported that in some countries of Eastern Europe, Fusarium plays an important role in fungal deterioration of feed quality and in animal diseases caused by mycotoxins. Feed may often be infected by several fungi, moreover the same fungal species often produces several fusariotoxins.

On the other hand, T-2 toxin (Fig. 2) is one of a family of closely related compounds produced by several Fusarium species. Bamburg and Strong (1971) reported also that T-2 toxin and related compounds have been impli-

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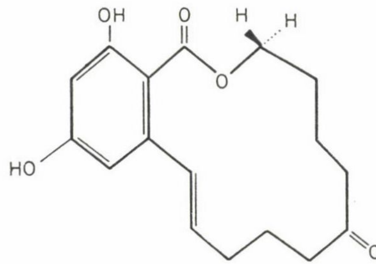

Fig. 1. Chemical structure of zearalenone 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid lactone

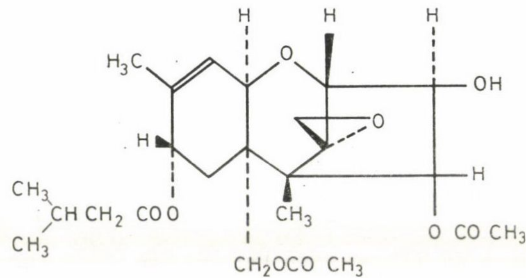

Fig. 2. Chemical structure of T-2 toxin (3-hydroxy 4, 15 diacetoxy-8-[3- methylbutyryl-oxy]-12,13-epoxy-trichothec-9-ene)

cated with a disease, known as the moldy corn toxicosis of swine, symptoms of which include refusal to eat, loss of weight, digestive disorders and diarrhoea, ultimately leading to death.

Aim of the present investigation was to study the growth and mycotoxin production of several identified Fusarium strains isolated from various grains such as corn and rice. Detection and determination of mycotoxin production were performed by thin-layer chromatography and confirmation was done by gas liquid chromatography. We studied the influence of different substrates and the effect of different strains on mycotoxin production.

1. Materials and methods

1.1. Investigated Fusarium strains

Species	Strains
Fusarium culmorum	234 B/5, 234 B/7, 13, 15, 25
Fusarium graminearum	977/12, 11, 18, 15, 39/3
Fusarium moniliforme	187 B/19, 228 B/9

Fusarium tricinctum	20, 21, 17, 204/10
Fusarium roseum	908 B/1, 8, 908 B/6
Fusarium semitectum	14
Fusarium sporotrichioides	16

Cultures used in the present investigation were obtained from Mycotoxin Laboratory of the Central Food Research Institute, Budapest, Hungary. Strains were identified according to BOOTH (1971), BÁNHEGYI and co-workers (1985).

Prior to use all the strains were subcultured on potato-dextrose agar for 14 days at 25 °C and stored at 4 °C according to ISHII and co-workers (1974).

1.2. Investigated substrates

1.2.1. Preparation of corn substrate. Corn was cooked for 30 min at boiling temperature in excess of water. Three hundred g of the cooked grain were put into a 1000 cm^3 Erlenmeyer flask and sterilized at 120 °C for 15 min.

1.2.2. Preparation of rice substrate. One hundred g of polished rice were soaked in tap water for 1 h, transferred to a 500 cm^3 Erlenmeyer flask and sterilized at 120 °C for 15 min.

1.2.3. Inoculation of the substrate. The sterilized corn and rice samples, resp., were inoculated with 2 cm^3 cell suspension (10^6 cell cm^{-3}) of identified Fusarium strains subcultured on potato-dextrose agar for 14 days at 25 °C. Samples were prepared in duplicates.

We prepared corn cultures with 15 and rice cultures with 12 different Fusarium strains (see Table 1).

1.2.4. Incubation of the samples. The inoculated samples were incubated at 20 °C for 7 days followed by 14 days at 8–12 °C and again at 20 °C for the last 7 days. At the end of the incubation period the samples were heavily overgrown by mycelium. Cell propagation and toxin production was stopped by drying the samples overnight in a pan at 60 °C.

1.2.5. Extraction of F-2 from corn and rice cultures. We used the partly modified method of MIROCHA and co-workers (1974). Twenty five g dry, ground sample was moistened with 7.5 cm^3 water and mixed thoroughly. The wet sample was extracted with ethyl acetate in a Soxhlet equipment for 7 h and the extract was evaporated (rotavapor) to near dryness. The residue was redissolved in 25 cm^3 $CHCl_3$ and transferred into a 250 cm^3 separating funnel and 10 cm^3 1 N NaOH was carefully added. To avoid the formation of an emulsion we rotated the funnel gently. When the layers separated the bottom layer ($CHCl_3$) was drained into a second 250 cm^3 separating funnel and the treatment was repeated with 10 cm^3 1 N NaOH. The second $CHCl_3$ layer was discarded. The combined aqueous layers were extracted with 2×5 cm^3 $CHCl_3$ in a separating funnel. The $CHCl_3$ layers were discarded.

Table 1

Identified Fusarium species investigated for mycotoxin production on moist autoclaved rice and corn

Fusarium species	No. of strains[a]	Substrate Rice	Corn
F. culmorum	13		+
	15		+
	25	+	
	234 B/5	+	+
	234 B/7		+
F. graminearum	11		+
	15	+	
	18		+
	39/3	+	
	977/12	+	+
F. moniliforme	187 B/9		+
	228 B/9	+	+
F. roseum	8		+
	908 B/1	+	
	908 B/6		+
F. semitectum	14	+	+
F. sporotrichioides	16	+	+
F. tricinctum	17	+	+
	20	+	
	21	+	
	204/10		+

[a] The strains were collected by J. SAWINSKY, Mycotoxin Laboratory of the Central Food Research Institute, Budapest, Hungary

The pH of the aqueous layer was adjusted to 9.5 by adding 2 *N* H_3PO_4 and the sample was extracted with 15 cm³ $CHCl_3$. The separated $CHCl_3$ layer was drained through a column filled with anhydrous Na_2SO_4 (5 g). The partition procedure was repeated twice and afterwards the aqueous layer was discarded.

The $CHCl_3$ layer was evaporated to dryness, and the residue transferred into a screw cap vial with 1 cm³ acetone and dried with nitrogen.

1.2.6. Separation of F-2 by thin-layer chromatography (TLC). For the TLC separation of F-2 silica gel plates of 20×20 cm size and 0.25 mm thickness were used (Polygram Sil G) after activation at 110 °C for 30 min.

The crude extract from para. 1.2.5. was dissolved in 200 μl acetone, from this samples of 10, 20 μl volumes were applied to the start line of the plate. Zeara-

lenone standard solution (50 ppm) was applied to identify the F-2 on the TLC plate. Beside the control F-2 spot (10 μl) from the standard toxin solution further 10 μl were as internal standard superimposed on one of the samples. Plates were developed with the following solvent systems:

— toluene–ethylacetat–chloroform 95:55:50 (v/v)
— chloroform–acetone 97:3 (v/v).

After development plates were heated for 5 min at 120 °C.

1.2.7. Detection of F-2 on TLC plates

1.2.7.1. Detection of F-2 by UV light — Plates were observed at $\lambda = 366$ and 254 nm. At 366 nm zearalenone shows faint blue spot with an R_f value of 0.5. At 254 nm the toxin shows a bright blue colour (Stoloff et al., 1971, Eppley, 1968).

1.2.7.2. Detection of F-2 toxin by colour-reagent — We sprayed the plates with 1% 4-methoxy-benzene-diazonium-fluoroborate (Sarudi, 1974) and heated for 10 min at 110 °C. Spots of zearalenone turned into red-brown.

1.2.8. Identification and quantitation of F-2 and T-2 by gas-liquid chromatography (GLC). Mycotoxin extracts were separated on TLC plates and detected under UV light (paras. 1.2.6., 1.2.7. and 1.2.9., 1.2.10., respectively). The detected spots were scraped off and extracted with acetone. The extract was evaporated to dryness and the residue redissolved in 200 μl acetone.

For GLC Carlo Erba Fractovap type 2300 equipment with flame ionization detector was used under the following conditions: 2000 mm, glass column length, 2.3 mm i.d., packed with 3% OV-17 on 80–100 mesh Gaschrom Q (Appl. Scientific Lab. Inc.). Retention time of F-2: 21′40″ at 274 °C; of T-2: 20′35″ at 269 °C. Nitrogen flow rate: 30 cm^3 min^{-1}. Injector temperature: 250 °C, chromatographic separation was started at 180 °C and heated to 280 °C, heating rate: 4 °C min^{-1}. Sensitivity: 0.05 μg per μl for standard F-2 and standard T–2 as well. Peaks were evaluated with the help of a calibration curve.

For the calibration curve standard solutions of 1 μg per μl were prepared of both toxins in acetone. Different aliquots were taken from the stock solutions, and evaporated to dryness under nitrogen stream. Twenty μl TRI-SIL/BT reagent was added to the residue, and after 20 min at 60 °C 1 μl of it was injected into GLC; then 0.2, 0.4, 0.6, 0.8, and 1 μl of F-2 standard and 0.1, 0.2, 0.3, 0.4, 0.5 μg of T-2 standard, respectively were injected onto GLC. The procedure above was repeated three times for each concentration.

The height of peaks were measured in mm and the regression equation was calculated.

The equation of calibration curve for F-2 toxin:

$$Y = 0.016\,x$$
$$r = 0.99$$

and in the case of T-2 toxin:

$$Y = 0.0023\,x + 0.112$$
$$r = 0.99$$

Y = injected standard sample (μg)
x = the height of peaks (mm).

The recovery of both kinds of toxin was 70% when 150 μg per kg of F-2 and T-2 toxin, respectively, were added.

1.2.9. Extraction of T-2 toxin from corn and rice substrate. Ten g ground sample was extracted with 200 cm^3 ethylacetate for 2 h at room temperature. To improve efficiency the mixture was shaken repeatedly (3×30 min). The solvent phase was separated by filtration on filter paper Whatman No. 1. The residues were extracted with 200 cm^3 methanol for 2 h at room temperature and separated. The two extracts were combined and evaporated to oily liquid residue and redissolved in 100 cm^3 33% aqueous methanol. After further defatting with petroleum ether (3×50 cm^3) the aqueous phase was evaporated to dryness in rotavapor heated by a glycerol bath.

1.2.10. Separation of T-2 toxin by TLC. The T-2 extract prepared according to para 1.2.9. was dissolved in 200 cm^3 acetone. From the crude extracts and standard T-2 solution (50 ppm) 10–10 μl were applied to the start line of silica gel plate 10×20 cm in size and 0.25 mm thickness (silica gel 60, Merck). Plates were developed in toluene–ethyl acetate–90% formic acid 6:3:1 (KIRCHNER, 1978).

1.2.11. Detection of T-2 on TLC plates. T-2 spots were detected by spraying the plates with 20% H_2SO_4 in 96% ethanol and heating for 10 min at 110 °C. In UV light $\lambda = 366$ nm T-2 shows blue green fluorescent spots (KAMIMURA et al. 1981).

2. Results

2.1. Recovery of F-2 toxin from corn samples

To determine recovery (%) of F-2 toxin from the grains, a corn sample was infected with F-2 standard methanol solution (50 ppm), from this stock 218.7 μl were used for 25 g sample to obtain a concentration of 350 μg per kg. Extraction was done according to para. 1.2.5. and determination by TLC. The recovery as mean value of 5 parallels was 73% (Table 2). The lowest concentration detectable by this method was 150–180 μg per kg.

2.2. Recovery of F-2 toxin from rice samples

Rice samples infected with pure zearalenone at a concentration of 350 μg per kg were extracted as given in para. 1.2.5. F-2 was separated from the crude

Table 2

Recovery and lowest TLC-detectable concentration of F-2 and T-2 toxins from corn and rice samples

Mycotoxin	Added (μg per kg)	Recovery (%) corn	Recovery (%) rice	Lowest detectable concentration (μg per kg) corn	Lowest detectable concentration (μg per kg) rice
Zearalenone	350	73 ± 2.9	71 ± 2.8	150—180	160—190
T-2 toxin	500	70 ± 2.7	70 ± 2.3	100—250	200—260

Recovery % based on 5 determinations ± standard deviation of measurements

extract and determined by TLC (see paras. 1.2.6. and 1.2.7., respectively). The recovery as mean value of 5 parallels was 71%, the lowest detectable concentration 160–190 μg per kg (Table 2).

2.3. Recovery of T-2 toxin from corn samples

Corn sample was infected with toxin T-2 at a concentration of 500 μg per kg. After extraction as given in para. 1.2.9. the crude extract was separated (para. 1.2.10.) and the toxin determined by TLC. As a mean value of 5 parallels recovery of the added toxin was 70%, the lowest detectable concentration 200–250 μg per kg (Table 2).

2.4. Recovery of T-2 toxin from rice samples

Rice samples infected at a concentration of 500 μg per kg were separated and T-2 determined by TLC. As given in Table 2 recovery was found to be 70%, the lowest detectable concentration of T–2 was 200–260 μg per kg.

2.5. Sensitivity of TLC method for F-2 detection

Sensitivity as the smallest amount (μg) of toxin per spot was determined in the case of pure zearalenone as well as in the case of crystalline F-2 added to grain samples (350 μg per kg) and extracted from corn and rice, respectively.

The F-2 was detected under UV light and confirmed by Sarudi reagent. As shown in Table 3, from pure F-2 solution 0.03 μg per spot was the smallest detectable amount.

In the case of F-2 added to a grain sample the sensitivity is poorer, as the lowest detectable amount is 0.04 μg per spot. The decrease of sensitivity is due to the pigment content of the grain extract which causes a faint colour on the plate.

2.6. *Sensitivity of TLC method for T-2 detection*

The lowest amount (μg) of T-2 toxin spot was 0.05 μg in the case of pure toxin solution and 0.07 μg T-2 in the case of solutions prepared from infected grain samples (Table 3). The decrease of sensitivity is the same as in the case of F-2 toxin and is due to the pigment content of the grain extract which lowers the detectability of the toxin.

2.7. *F-2 production of Fusarium sp. on corn*

The amounts of F-2 produced were quantitated by GLC and are shown in Table 4.

Table 3

Sensitivity of the TLC method for F-2 and T-2 determination

Mycotoxin	Detection	Pure toxin solution 50 ppm (μg per spot)	Standard toxin added to grain extract
Zearalenone	UV/254 nm, confirmed by Sarudi reaction	0.03	0.04
T–2 toxin	UV/233 nm, confirmed by 20% H_2SO_4	0.05	0.07

Table 4

Zearalenone production of Fusarium species on wet autoclaved corn and rice

Fusarium species	Strains No.	Detected concentration (mg per kg)[a] corn	rice
F. culmorum	234 B/5	121.2	53.4
	234 B/7	205.0	NI
	13	0.8	NI
	15	57.3	NI
	25	NI	1166.1
F. semitectum	14	27.5	0.1
F. sporotrichioides	16	7.0	0.37
F. roseum	908 B/6	2.8	NI
	908 B/1	NI	ND
	8	8.5	NI
F. graminearum	977/12	50.7	15.5
	11	8.07	NI
	18	4.0	NI
	39/3	NI	137.18
F. moniliforme	228 B/9	5.7	5.39
	187 B/19	16.0	NI
F. tricinctum	204/10	2.6	ND
	17	150.0	ND

[a] Determinations were made by GC in two parallels (duplicate)
NI: Not investigated
ND: Not detectable

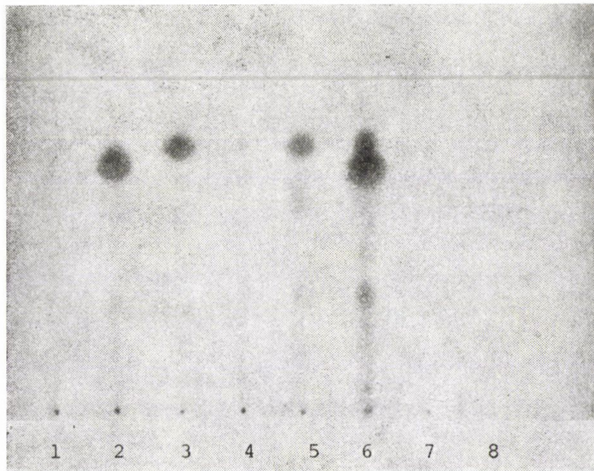

Fig. 3. Separation and detection of F-2 toxin from corn and rice samples. Developing system: $CHCl_3$-ethanol (97:3). Detection: sprayed with Sarudi reagent. Application: to spots 1, 2, 3, 4 corn samples strain No. 8, 234 B/7 and 15–20 μl from each; to spots 5, 6 rice samples strain No. 234 B/5 and 25–20 μl from each; to spot 7 corn samples strain No. 4 + 10 μl F-2 standard; to spot 8 F-2 standard (50 ppm) 10 μl, $R_f = 0.5$

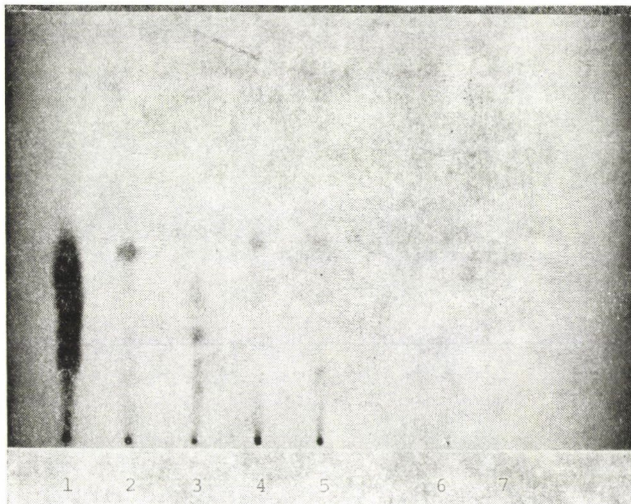

Fig. 4. Separation and detection of F—2 toxin from corn and rice samples. Developing system: toluene-ethylacetate-$CHCl_3$ (95:55:50). Treatment: sprayed with Sarudi reagent. Application: to spots 1, 3 rice samples strain No. 228 B/9 and 21–20 μl from each; to spots 2, 4, 5 corn samples strain No. 908 B/6, 228 B/9 and 18–20 μl from each; to spot 6 5 μl from extracted corn strain No. 18 + 10 μl F-2 standard; to spot 7 10 μl F-2 standard (50 ppm) $R_f = 0.49$

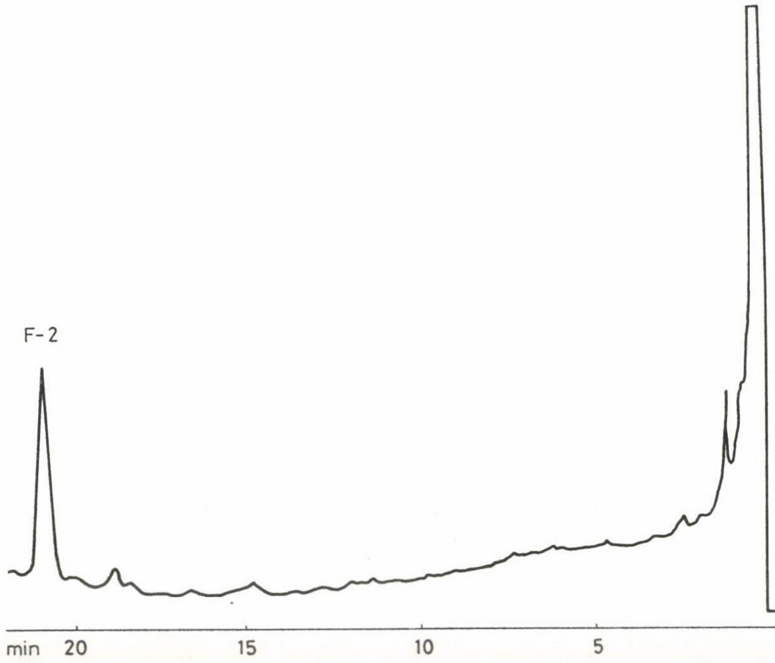

Fig. 5. GLC chromatogram of extracts of moldy corn culture No. 3, showing the peak of F-2 toxin

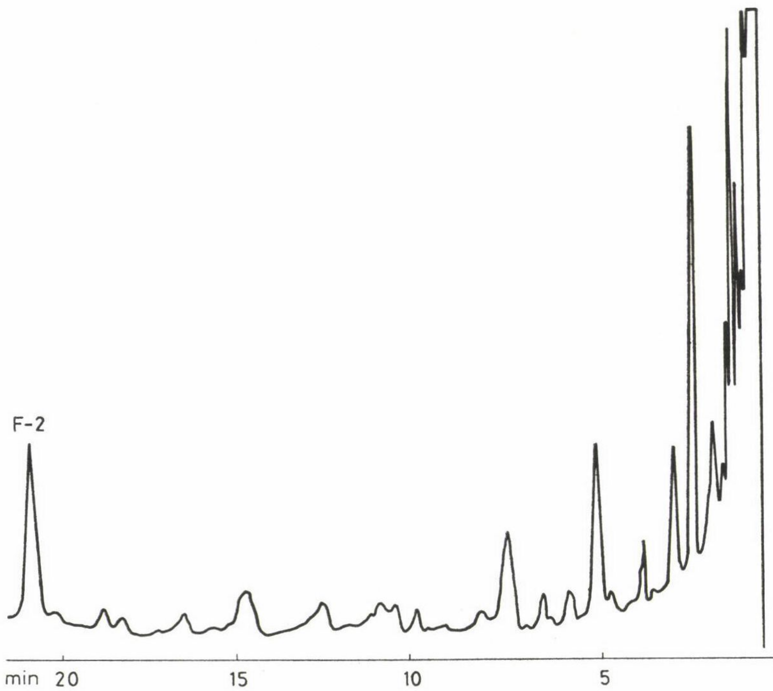

Fig. 6. GLC chromatogram of extracts of moldy corn culture No. 6, showing the peak of F-2 toxin

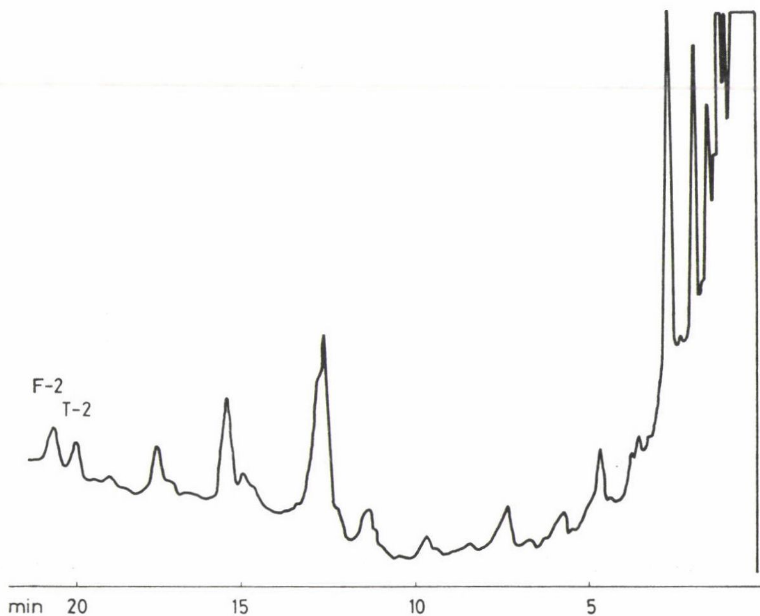

Fig. 7. GLC chromatogram of extracts of moldy corn culture No. 7, showing the peaks of F-2 and T-2 toxin

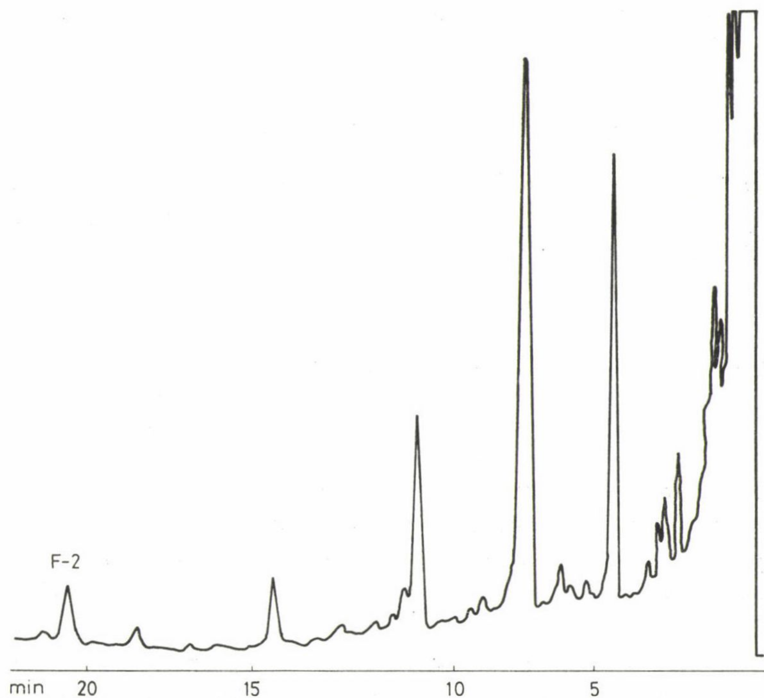

Fig. 8. GLC chromatogram of extracts of moldy rice culture No. 5 showing the peak of F-2 toxin

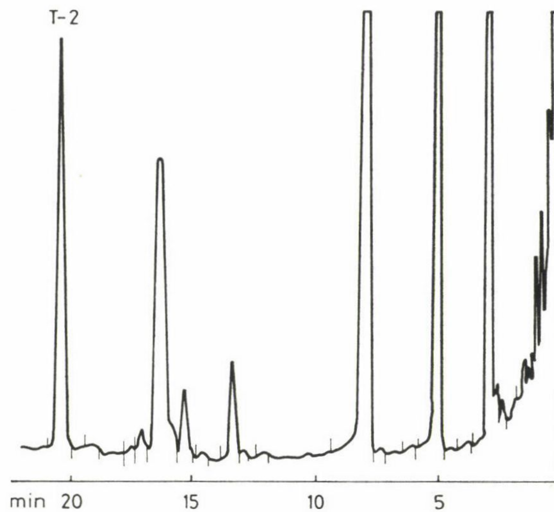

Fig. 9. GLC chromatogram of extracts of moldy rice culture No. 3, showing the peak of T-2 toxin

2.8. F-2 production of Fusarium sp. on rice

The F-2 toxin yields obtained are shown in Table 4, quantitation was made by GLC.

2.9. T-2 production of Fusarium tricinctum *strains on corn and rice*

The T-2 toxin production of *F. tricinctum* strains is shown in Table 5. The GLC chromatograms of the investigated corn and rice samples are shown in Figs. 3 to 9.

3. Discussion

3.1. Influence of the substrate on F-2 production

Results summarized in Table 4 show that depending on the substrate the same strain produces highly different amounts of zearalenone under identical conditions. All investigated strains except *F. culmorum 25* yielded higher toxin quantities on corn. According to BOTTALICO and co-workers (1983) corn appears to be a more appropriate substrate than rice for mycotoxin production. However, the highest zearalenone content was found on rice substrate inoculated with *F. culmorum 25* (1166 mg per kg).

This result agrees with MARASAS and co-workers (1979) who reported that zearalenone could be found in 4 out of 5 *F. culmorum* strains, ranging

from 320 to 1400 mg per kg. CALDWELL and co-workers (1970) reported F-2 production of three *F. tricinctum* strains in the range of 0.2–6.0 mg per kg, much lower than our findings of 2.59 to 150.00 mg per kg. As in both cases corn was used as substrate this great difference shows a high variability even within the same strain.

3.2. Influence of the strain on F-2 production on corn and rice substrates

Table 4 shows F-2 concentrations measured on different corn and rice substrates. We took into consideration only those species from which more than one strain was investigated. *F. graminearum* produced mycotoxin on corn in the range of 4.0–50.7 and on rice between 15.5–453.0 mg per kg. These results are better than those reported by THIEL and co-workers (1982) (8.9–13.7 mg per kg on corn) but lower than the maximal yields of SHERWOOD and PEBERDY (1974), which were 500–2000 mg per kg.

We found in agreement with data in the literature, that even closely related strains show very different F-2 toxin producing capacity. For instance we found one *F. roseum* strain which did not produce zearalenone at all on rice. Standard deviation of mycotoxin production determined in 5 parallel samples inoculated with 5 different strains is, in the average, about 25% of the mean value. Thus differences in the measured F-2 values (Table 4) seem to be significant. However, it is noticeable that mycotoxin production even of the same strain under identical conditions and on the same type of substrate varies with time. In a later experiment the detected F-2 values differ considerably from those of the first experiment. Possible explanations for this phenomenon could be a different pretreatment of the grain or the change in the F-2 producing capacity of the strains during the year. This needs further investigations.

3.3. T-2 production of F. tricinctum *strains*

In the literature *F. tricinctum* has been reported to produce a variety of mycotoxins including several trichothecenes (BAMBURG & STRONG, 1971; LANDSEN et al., 1978; ILUS et al., 1977; MIROCHA & CHRISTENSEN, 1974). BOTTALICO and co-workers (1983) reported F-2 and T-2 toxin production in *F. culmorum, F. graminearum* and *F. tricinctum* strains isolated from cereals in Italy.

Our experiments agree with data in the literature. Among the investigated *F. tricinctum* strains we found F-2 production on corn ranging from 2.59–150 mg per kg and T-2 production in the range of 1.4–119 mg per kg on both corn and rice.

Table 5

Production of T-2 toxin by Fusarium tricinctum *strains on moist autoclaved corn and rice*

Fusarium species	Strains No.	Substrate	T-2 toxin (mg per kg)[a]
F. tricinctum	204/10	corn	1.44
F. tricinctum	17	corn	ND
F. tricinctum	21	rice	119.00
F. tricinctum	20	rice	ND
F. tricinctum	17	rice	ND

[a] Determinations were made by GC in duplicate
ND: Not detectable

F. tricinctum 17 does not produce T-2 either on corn or on rice. Strain No. 21 has a good productivity on corn, while No. 204/10 produces small amounts of F-2 (2.69) and T-2 (1.4 mg per kg).

The other species used in our experiments have not been examined for T–2 production until now.

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Acta Alimentaria, Vol. 16 (1), pp. 45—52 (1987)

ZEARALENOL PRODUCTION OF FUSARIUM SPECIES IN RICE AND CORN CULTURES

A. Badawey, J. Sawinsky and A. Halász

Central Food Research Institute, H-1022 Budapest,
Herman Ottó út 15. Hungary
(Received: 2 July 1985; revision received: 19 February 1986;
accepted: 20 February 1986)

Three species of Fusarium were tested for their ability to produce zearalenol on wet autoclaved corn and rice. They belonged to the following strains:
F. culmorum 234 B/15, 234 B/7, 15, 25, *F. graminearum* 977/12, 11, 39/3 and *F. roseum* 8, 908/11. These strains of individual species produced the following amounts of zearalenol on corn: 3 strains of *F. culmorum* 480 to 1680 μg per kg, 2 of *F. graminearum* 213 to 500 μg per kg and *F. roseum* 580 μg per kg. On the other hand the following amounts of the toxin were produced on rice: 2 strains of *F. culmorum* 400 to 8800 μg per kg, 2 of *F. graminearum* 1200 to 1920 μg per kg and *F. roseum* 350 μg per kg.

Keywords: Fusarium species, zearalenol, rice, corn

Zearalenol (6-(6,10 dihydroxyundecyl)-β-resorcyclic acid-μ-lactone) is one of the naturally occurring derivatives of zearalenone isolated from Fusarium (Hagler et al., 1979). The structure of zearalenol is shown in Fig. 1.

Mirocha and co-workers (1979) found zearalenol in addition to zearalenone in samples of problem feed, and as products of metabolism in animals (Mirocha et al., 1981). The oestrogenic effect of zearalenol is 4–5 times higher than that of zearalenone (Mirocha et al., 1978).

Stipanovic and Schroeder (1975) reported the isolation of zearalenol from cultures to *F. roseum "Gibbosum"* and *F. roseum semitectum* grown on moist autoclaved sorghum or cracked corn. The aim of our investigation was to determine

- the zearalenol synthesizing ability of three F-2 producing Fusarium species and
- the substrate effect on zearalenol production.

1. Materials and methods

1.1. Organism and culture preparation

The strains selected for the investigation were F-2 producer and were obtained from the Mycotoxin Laboratory of the Central Food Research Institute, Budapest, Hungary. The strains belong to *F. culmorum, F. graminearum* and *F. roseum* species as summarized in Table 1.

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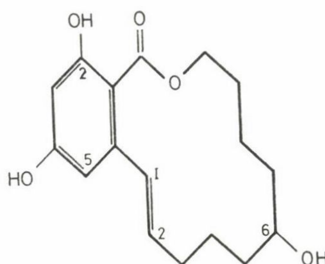

Fig. 1. Chemical structure of zearalenol

Table 1

Fusarium species investigated for zearalenol production on moist autoclaved corn and rice grain

Fusarium species	Strains No.	Substrate	
		corn	rice
F. culmorum	234 B/5	+	+
F. culmorum	234 B/7	+	—
F. culmorum	25	—	+
F. culmorum	15	+	—
F. graminearum	977/12	+	+
F. graminearum	11	+	—
F. graminearum	39/3	—	+
F. roseum	8	+	—
F. roseum	908 B/1	—	+

The strains were identified according to BOOTH (1971).

Prior to use, all the strains of Fusarium species were subcultured on potato–dextrose agar for 14 days at 25 °C followed by storage at 4 °C according to ISHII and co-workers (1974).

1.2. Preparation of corn and rice substrates

Corn was cooked for 30 min at boiling temperature in excess of water. Three hundred g of the cooked grain was put into an Erlenmeyer flask and sterilized at 120 °C for 15 min. One hundred g of polished rice was soaked in tap water for 1 h then transferred into an Erlenmeyer flask and sterilized at 120 °C for 15 min.

The moisture content after autoclaving was between 50 and 55%.

1.3. Conditions of substrate inoculation and culture incubation

The sterilized corn and rice samples were inoculated with 2 cm^3 cell suspension of identified Fusarium strains subcultured on potato–dextrose agar for 14 days at 25 °C. Samples were prepared for *F. graminearum* 977/12 and *F. culmorum* 234 B/5 on rice and corn, resp, (Table 1).

The inoculated samples were incubated at 20 °C for 7 days followed by 14 days at 8–12 °C and again at 20 °C for the last 7 days. At the end of the incubation period the samples were heavily overgrown by mycelia.

Cell propagation and toxin production was stopped by drying the samples overnight at 60 °C.

1.4. Extraction of zearalenol from corn and rice cultures

The F-2 toxin extraction method of MIROCHA and co-workers (1974) was modified for zearalenol extraction. Twenty five g dry ground sample was moistened with 7.5 cm³ water, extracted with ethyl acetate in a Soxhlet equipment for 7 h and the extract was evaporated (rotavapor) near to dryness. The residue was redissolved in 25 cm³ $CHCl_3$, transferred into a 250 cm³ separating funnel and 10 cm³ 1 *N* NaOH was carefully admixed. To avoid emulsification the funnel was gently rotated. When the layers separated the bottom layer ($CHCl_3$) was drained into a second 250 cm³ separating funnel and the treatment was repeated with 10 cm³ 1 *N* NaOH. The second $CHCl_3$ layer was discarded, the combined aqueous layers were repeatedly extracted with 2×5 cm³ $CHCl_3$ in a separating funnel. The $CHCl_3$ layers were discarded. The pH of the aqueous layer was adjusted to 9.5 by adding 2 *N* H_3PO_4 and again extracted with 15 cm³ $CHCl_3$. The separated $CHCl_3$ layer was drained through a column filled with anhydrous Na_2SO_4 (5 g).

The partition procedure was repeated twice and afterwards the aqueous layer discarded while the $CHCl_3$ layer was evaporated to dryness. The residue was transferred into a vial with 1 cm³ acetone and dried with nitrogen.

1.5. Separation of zearalenol by TLC

For TLC separation of zearalenol silica gel plates of 20×20 cm size and 0.25 mm thickness were used (Polygram Sil). Layers were activated by heating at 110 °C for 30 min.

The crude zearalenol extracts from the previous step were dissolved in 200 μl acetone. From these samples 10, 20 μl volumes were applied to the start line of the plate. Zearalenol standard solution (50 ppm) was also used to identify zearalenol. 10, 12.5 and 15 μl zearalenol standards were run. Plates were developed in $CHCl_3$–ethanol (97:3). After development plates were heated for 5 min at 120 °C.

1.6. Detection of zearalenol by UV light

Plates were observed at $\lambda = 366$ and 254 nm. Zearalenol shows faint blue colour with $R_f = 0.4$. At 254 nm the toxin shows bright blue colour.

1.7. Detection of zearalenol by colour reagent

The plates were sprayed with 1% 4-methoxy-benzene-diazonium-fluoroborate in water (SARUDI, 1974) and heated for 10 min at 110 °C. Spots of zearalenol turned into red-brown.

1.8. Quantitation of zearalenol by overpressure layer chromatography (OPLC)

For the determination of zearalenol by OPLC Chrompres 10 overpressure layer chromatograph (Labor MIM, Budapest, Type OE-306) was used. Samples were run on Kieselgel 60 F_{254} layer with impress sealed edges in chloroform–acetone (9:1). Thickness: 0.25 mm, cushion pressure: 10 bar, temperature: 25 °C.

Extracts of corn and rice samples prepared as given in 1.4 were dissolved in 200 μl acetone. Aliquots of 10, 20 μl were applied to the start line of the plate. Ten, 12.5 and 15 μl of zearalenol standard solution (50 ppm) was also run for identification and quantitative determination of the toxin by OPLC.

After developing the plates were sprayed with 1% 4-methoxy-benzene-diazonium-fluoroborate (SARUDI, 1974) and heated for 10 min at 110 °C. Spots of zearalenol turned into red-brown.

2. Results

2.1. Recovery of zearalenol added to corn and rice samples

To corn and rice samples prepared according to para. 1.2 pure zearalenol was added at a concentration of 350 μg per kg. The toxin was extracted and separated from the crude extract as given in paras. 1.4 and 1.5 and determined by OPLC. As shown in Table 2 the recovery was approx. 67% in both cases.

2.2. Separation and detection of zearalenol by TLC

For the estimation of zearalenol by thin-layer chromatography, culture extracts were applied to the chromatographic plates together with various amounts (10, 12.5 and 15 μl) of zearalenol standard solution (50 ppm).

Table 2

Recovery of zearalenol added to corn and rice

Mycotoxin	Added (μg per kg)	Recovery (%)	
		corn	rice
Zearalenol	350	68 ± 2.1	67 ± 2.5

Recovery based on 5 determinations; ± standard deviation of measurements

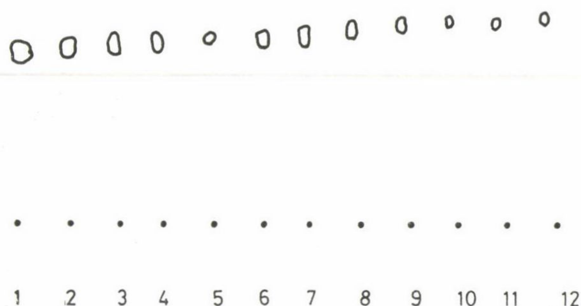

Fig. 2. Separation and detection of zearalenol from corn and rice samples. Developing system: chloroform–ethanol (97:3). Treatment: spraying with Sarudi reagent. Applied amounts: No. 2, 3, 9 rice samples 3 μl, 10 μl, 20 μl; No. 4, 6, 7, 8, 10 corn samples 20 μl, each; No. 1, 5, 11, 12 zearalenol standards (50 ppm) 20 μl, 12 μl, 7.5 μl, 10 μl. R_f value: 0.4

Figure 2 shows the separation and detection of zearalenol from corn and rice sample extracts.

2.3. Determination of zearalenol by OPLC

Figure 3 shows the separation of the toxin by OPLC after development with Sarudi solution (para. 1.8). The R_f value of zearalenol was 0.24. The amounts of toxin in the extract dilutions were determined by comparing colour intensity of sample spots on the plate with those of the standards. Table 3 shows the zearalenol production of Fusarium species on moist corn and rice cultures.

2.4. Sensitivity of TLC and OPLC methods for zearalenol

To determine sensitivity of TLC method we investigated the lowest detectable zearalenol amount per spot. Pure 50 ppm toxin solution was applied to the plate in the range of 1–10 μl. After development the toxin was detected in UV light and by Sarudi reagent. The results on Table 4 show that the sensitivity of the method was 0.03–0.04 μg.

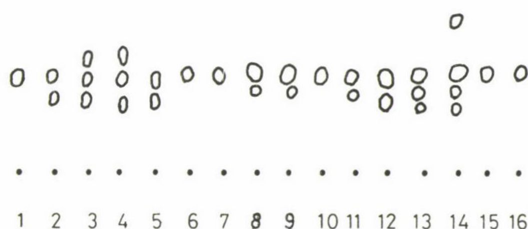

Fig. 3. Separation and quantitation of zearalenol by OPLC. Developing system: chloroform–acetone (9:1). Applied amounts: No. 1, 6, 10, 15, 16, zearalenol standards (50 ppm) 7.5 μl, 7.5 μl, 10 μl, 12 μl, 12 μl; No. 11, 12, 13, 14, 2 rice samples 20 μl; No. 3, 4, 5, 7, 8, 9 corn samples 20 μl. R_f value: 0.24

Table 3

Zearalenol production of Fusarium species in moist corn and rice cultures

Fusarium species	Strains No.	Zearalenol[a] (μg per kg)	
		corn	rice
F. culmorum	234 B/5	1680	400
F. culmorum	234 B/7	1140	NI
F. culmorum	25	NI	8800
F. culmorum	15	480	NI
F. graminearum	977/12	500	1920
F. graminearum	11	213	NI
F. graminearum	39/3	NI	1200
F. roseum	8	580	NI
F. roseum	908/B1	NI	350

[a] Determinations were made by OPLC, mean value of duplicate determinations is given.
NI: Not investigated

Table 4

Sensitivity of the TLC method for zearalenol determination

Detection	Standard zearalenol (μg)	Standard toxin added to 20 μl grain sample
254 nm UV	0.04	0.05
Sarudi reaction	0.03	0.04

Table 5

Sensitivity of the OPLC method for zearalenol determination

Detection	Standard zearalenol (μg)	Standard toxin added to 20 μl grain sample
254 nm UV	0.04	0.05
Sarudi reaction	0.02	0.03

The effect of extractable compounds on the sensitivity of the TLC method was measured with zearalenol samples dissolved at a concentration of 50 ppm in grain extract prepared according to para. 1.4. Volumes of 1–10 μl were applied to the plate. After development the toxin was detected as given in para. 1.7. In the case of grain extract the sensitivity of the method is decreased to 0.05–0.04 μg as a result of separation.

The sensitivity of OPLC method was determined in a similar way as in the case of TLC. The lowest detectable zearalenol quantities were found to be 0.04 μg in UV light and 0.02 μg with Sarudi reagent. The effect of grain extract causes a decrease in the sensitivity of OPLC determination (Table 5).

2.5. Zearalenol production on corn substrate

As shown in Table 4, all the investigated strains produced zearalenol on corn. The highest amounts were synthesized by two *F. culmorum* strains, 1680 and 1440 μg per kg, respectively. The third *F. culmorum* strain, the *F. graminearum* strains and the investigated *F. roseum* were much less effective in zearalenol production. The lowest amount — only 213 μg per kg — of the toxin was found in *F. graminearum No. 11* culture.

2.6. Zearalenol production of Fusarium species on rice substrate

Each of the investigated Fusarium strains produced zearalenol on rice substrate (Table 5). Highest concentration was found with *F. culmorum No 25* (8800 μg per kg) and *F. graminearum 977/12* (1920 μg per kg), respectively.

3. Discussion

3.1. The suitability of TLC and OPLC methods for zearalenol determination

The developed extraction method gives an acceptable recovery for zearalenol (67%) on both grains. After an appropriate separation of the crude extract on silicagel TLC plate, the toxin sample is ready for quantitative determination.

Sensitivity of the TLC method is 0.05 μg per spot, using $CHCl_3$–ethanol (97 : 3) as developing system. In the case of OPLC $CHCl_3$–acetone (9 : 1) is a suitable solvent.

The lowest detectable amount is 0.02 μg per spot of zearalenol. The developed method enabled us to determine zearalenol from rice or corn at concentrations of at least 140 μg per kg.

3.2. Effect of Fusarium species on zearalenol production

F. culmorum strains produced the highest concentrations of toxin on both substrates (1680, 8800 μg per kg). The investigated *F. roseum* strains were weak producers (580 and 350 μg per kg). Among the three *F. graminearum* strains two (977/12 and 39/3) synthesized high amounts of the toxin (1920 and 1200 μg per kg) on rice and two strains (11 and 977/12) produced low quantities (213 and 500 μg per kg) on corn, respectively.

These findings agree with the examination of F-2 and T-2 production of Fusarium strains (BADAWEY et al., 1987). Among the same species strains can show a great variation in toxin production efficiency.

3.3. Effect of substrate on zearalenol production of Fusarium species

F. culmorum 234 B/5 produced a zearalenol concentration of 400 μg per kg on rice, but 1680 μg per kg on corn.

F. graminearum, however, was more active on rice (1920 μg per kg) than on corn (500 μg per kg). These findings agree with those of MIROCHA and coworkers (1979) on zearalenol production on oats and corn (4.0 μg per g and 0.15 μg per g, respectively) and with our observations on F-2 production of Fusarium strains (BADAWEY et al., 1987). It is interesting but not surprising that *F. culmorum* 234 B/5 produced also in the case of F-2 higher toxin amounts on corn (121.25 μg per kg) than on rice (53.43 μg per kg).

However, *F. graminearum* 977/12 did not prefer rice to corn for F-2 production but was also more active on corn. So it seems to be no correlation between F-2 production and zearalenol production of Fusarium strains in respect to substrate preference.

As zearalenol is several times more active oestrogenic compound than F-2 (MIROCHA et al., 1979) the fact that both corn and rice are possible substrates for zearalenol producing Fusarium strains is of great practical importance. The OPLC method gives an easy way for routine estimation.

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Acta Alimentaria, Vol. 16 (1), pp. 53–59 (1987)

THERMAL ANALYSIS AND INFRARED SPECTROSCOPY STUDY OF CHANGES IN HEAT-DENATURED RAPESEED PROTEINS

M. KLEPACKA

Agricultural University of Warsaw, Faculty of Food Technology,
Department of Fermentation and Fruit and Vegetable Technology,
ul. Rakowiecka 26/30, 02-526, Warsaw. Poland

(Received: 6 August 1985; accepted: 28 January 1986)

Proteins from rapeseed (three varieties), extracted with water or salt solutions (5% K_2SO_4 and 10% NaCl) were heated at 80 °C and 100 °C. The precipitates were examined, using thermal analysis and infrared spectroscopy. Analysis of the thermoanalytical curves of the precipitates showed that the losses in mass (TG curve) in the range of 400–800 °C temperature were irreversibly dependent in case of the precipitates obtained (80 °C and 100 °C), on proteins extracted with 10% NaCl, as compared with the precipitates obtained from proteins extracted with water or 5% K_2SO_4. Similarly different dependences were observed in protein precipitates from salt extracts with NaCl during the analysis of relationship between absorptions Amide I and II and the band at 1090 cm^{-1}. The stated differences depended mainly on the protein fractions and not on the variety of the rapeseed.

Keywords: thermal analysis, infrared spectroscopy, heat-denatured proteins

In the previous work, the different thermal properties of rapeseed proteins, extracted with water or salts, were described. Differences were found in the thermal stability of the proteins and in the mineral composition of thermally denatured protein precipitates (KLEPACKA et al., 1983).

The analysis of protein precipitates presents difficulties as only a small group of analytical techniques is suitable for their examination. Of these methods, the use of thermal analysis seemed to be promising to study protein denaturation (KARMAS & DIMARCO, 1970; MISHIN & GARBUZOV, 1951; STEIM, 1965).

In the present work, the results of studies on the comparison of certain properties of the protein in three varieties of rapeseed are given, namely: classical rapeseed, rapeseed of low and doubly low erucic acid content. To study thermally denatured proteins in form of precipitates, thermal analysis and infrared spectroscopy were used.

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1. Materials and methods

1.1. Materials

The studies were conducted with three varieties of rapeseed: classical variety (Górczanski), variety of low erucic acid content (Quinta) and variety of double low — low glucosinolate, low erucic acid content (Start).

1.2. Methods

The investigations were carried out on the precipitates formed by heat treatment (at 80 °C or 100 °C for 30 min) of water soluble proteins (WSP) and salt soluble proteins: 10% NaCl (SSP–NaCl) or 5% K_2SO_4 (SSP–K_2SO_4). The techniques applied were identical with those in the previous work (KLEPACKA et al., 1983).

The precipitates were exposed to thermal analysis, using Paulik–Paulik–Erdey derivatograph. The experimental conditions were as follows: weight of the samples from 33 mg to 94 mg, DTA 1/3, DTG 1/5 at a heating rate of 9 °C per min. The samples were heated to 800 °C. Determination was conducted under nitrogen atmosphere.

The IR measurements were performed with Zeiss spectrophotometer, model IR–75. About 1 mg of the precipitates was pressed into tablets with KBr (250 mg), then desiccated above P_2O_5. The conformation structure of denatured proteins, band of half-width Amide I and beta form content, were determined according to CHIRGADZE and co-workers (1973) and CHIRGADZE and BRAZHNIKOV (1974) and MIYAZAWA and co-workers (1962).

2. Results

The results of thermal analysis of the precipitates are presented in Fig. 1. We analysed four curves recorded on the derivatograph: curve of differential thermal analysis (DTA), curve of change in temperature (T), curve of thermogravimetry (TG) and curve of derivative thermogravimetry (DTG).

It was established that the differences between the thermoanalytical curves of the precipitates from the studied rapeseed varieties were not significant. Hence, in Fig. 1, the results obtained for the precipitate of only the variety of doubly low erucic acid content, are shown. The data showing the differences in weight losses of the samples are presented in Table 1. The loss of weight in the temperature range of 120–140 °C was dependent on the loss of water. In the particular samples, the loss in weight was found to be in the ranges from 4.1% to 7.7% and from 4.9% to 12.1%, respectively, for the precipitates obtained at 80 °C and 100 °C, respectively. Similar effects in the temperature

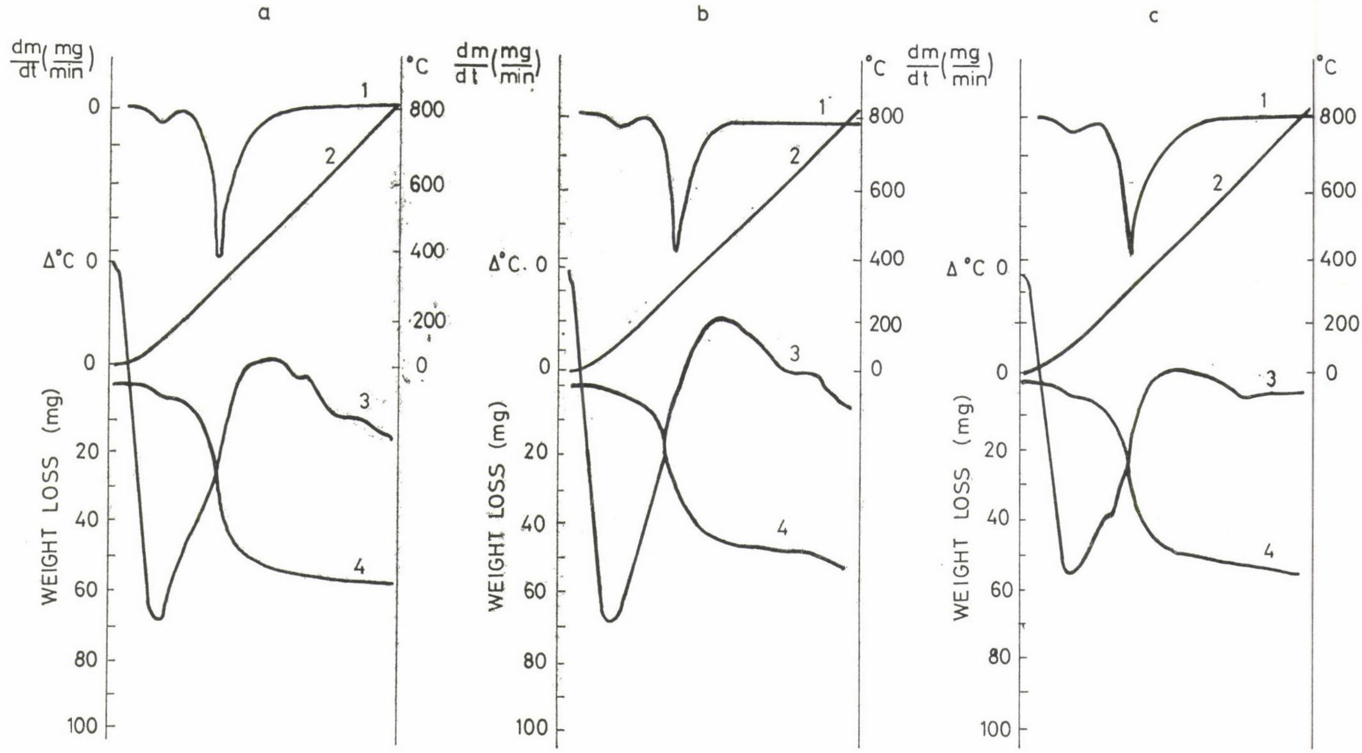

Fig. 1. Thermoanalytical curves of precipitates formed by heat treatment (100 °C, 30 min) from protein extracted with: (a) water, (b) 10% NaCl, (c) 5% K_2SO_4, from rapeseed of doubly low erucic acid content

Table 1

Loss in substance weight (%) of the protein precipitates

Temperature range of analysis (°C)	Temperature of protein denaturation (°C)	Samples of precipitates from								
		water soluble proteins			salt soluble proteins (10% NaCl)			salt soluble proteins (5% K_2SO_4)		
		C	LEAR	DL	C	LEAR	DL	C	LEAR	DL
120–140	80	4.1	5.9	7.3	7.1	6.4	7.7	5.7	5.7	5.0
	100	5.8	7.8	7.1	12.1	7.2	5.7	5.3	4.9	6.5
220–400	80	57.5	52.4	52.9	55.9	54.4	45.0	50.7	54.6	50.0
	100	47.8	45.3	52.4	43.9	52.0	50.0	57.5	53.1	52.6
400–800	80	11.0	13.1	11.7	16.4	12.5	16.7	15.9	11.3	13.3
	100	13.1	14.1	13.1	10.6	8.0	14.3	19.7	13.6	13.1

Varieties of rapeseed: C: classical; LEAR: of low erucic acid content; DL: of doubly low erucic acid content

range of 110–140 °C were observed by Mishin and Garbuzov (1951) during the examination of proteins.

In the temperature range of 220–400 °C considerable weight losses amounting to 57% were observed.

When analysing the differences in weight losses as obtained in the temperature range of 400–800 °C (Table 1), greater differences appeared in the precipitates obtained by heating the extracts WSP and SSP-K_2SO_4 at 100 °C, than by heating them at 80 °C (excepting the doubly low variety). Reverse dependence was observed in the samples obtained by heating the NaCl extracts.

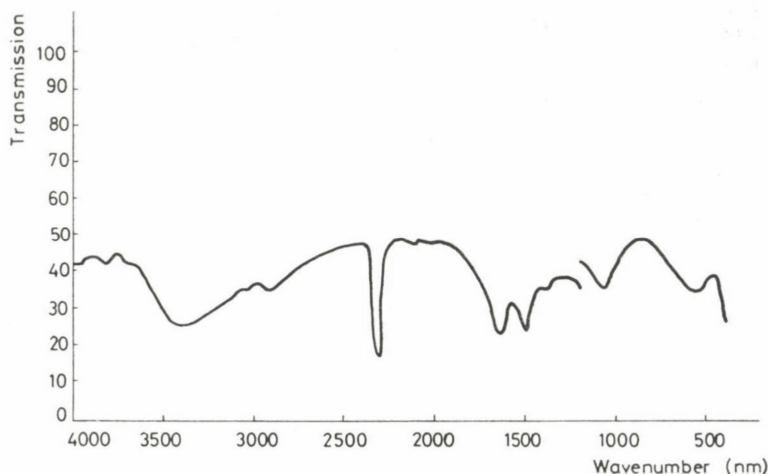

Fig. 2. IR spectrum of the precipitate formed by heat treatment (100 °C, 30 min) from protein extracted with 5% K_2SO_4 from classical rapeseed. The precipitate was pressed into a pellet with KBr

Table 2

Spectral parameters of some amide bands of the protein precipitates

Index	Temperature of protein denaturation (°C)	Samples of precipitates from								
		water soluble proteins			salt soluble proteins (10% NaCl)			salt soluble proteins (5% K_2SO_4)		
		C	LEAR	DL	C	LEAR	DL	C	LEAR	DL
Band half-width	80	50	50	50	43	43	36	43	36	50
Amide I$\Delta_{1/2}$ cm^{-1}	100	50	50	50	50	50	43	50	50	50
In content	80	18	19	18	19	19	19	18	19	18
Beta form (%)	100	18	18	18	19	18	18	18	19	19
$A_{Amide\ I}/A_{1090}$ cm^{-1}	80	1.15	1.13	1.12	1.10	1.11	1.12	1.11	1.06	1.04
	100	1.09	1.11	1.07	1.15	1.17	1.15	1.05	1.04	0.96
$A_{Amide\ II}/A_{1090}$ cm^{-1}	80	1.13	1.12	1.13	1.07	1.08	1.08	1.10	1.04	1.03
	100	1.08	1.08	1.07	1.15	1.15	1.13	1.02	1.01	0.94

Varieties of rapeseed: C: classical; LEAR: of low erucic acid content; DL: of doubly low erucic acid content; A: absorption

The weight losses in the precipitates obtained at 80 °C were higher than in the precipitates obtained at 100 °C.

The thermal effect, weakly marked on the shoulder of the DTA curve in the temperature range of 230–250 °C, was visible in the analysed samples, with the exception of the proteins precipitated at 100 °C from the NaCl extracts.

Infrared spectroscopy revealed in all the samples, that the frequencies of vibrations appear at the same wavenumbers. For example, a spectrum is given in Fig. 2. In the spectra, the characteristic frequencies of amide vibrations at 1635 cm^{-1} (Amide I) and at 1500 cm^{-1} (Amide II) were observed and this is in accordance with the data of CHIRGADZE and co-workers (1973).

The half-width of Amide I band in the range of 36–50 cm^{-1} probably indicates the contribution of polypeptide segments in beta and random forms. They are in conformity with the results of KOSTYRA and co-workers (1984). The range of Amide V band, at 650 cm^{-1} confirms also the presence of the random form (MIYAZAWA et al., 1962).

The relationship between the absorption of Amides (I and II) and the band at 1090 cm^{-1} depended mainly on the extraction medium of proteins (Table 2). The precipitates obtained in extracts WSP and SSP–K_2SO_4 showed higher values of the measured ratio in the samples denatured at 80 °C than at 100 °C. The precipitates obtained in the SSP–NaCl extract gave the reverse relationship.

3. Discussion

Data of thermal analysis show that about 50% of the examined precipitates were decomposed at 220–400 °C. This is a consequence of the degradation of amino acids and proteins. KARMAS and DIMARCO (1970) showed when

studying thermoprofiles of amino acids and proteins, that in the temperature range of 230–300 °C, amino acids become carbonized, sublimated and decomposed. Also, LIEN and NAWAR (1974) in their studies on the thermal decomposition of certain amino acids in the temperature range of 180–270 °C found that their degradation results in formation of such compounds as ammonia, propane, carbon dioxide, acetone, piridine and acetonitrile. Certain amino acids are capable of sublimation in the temperature range of 205–280 °C (KARMAS & DIMARCO, 1974). This phase change of amino acids is probably responsible for the observed endothermal effects in DTA curve in the temperature range of 230–250 °C.

In the temperature range of 400–800 °C the tendency of increasing or decreasing of weight losses of the precipitates (at 80 °C and 100 °C) are dependent on the solvent used for protein extraction. It is assumed that weight losses in the discussed temperature range are related to the mineral composition of the protein precipitates and they confirm the results of our previous work (KLEPACKA et al., 1983). That study showed that the particular protein fractions isolated with water or salts are capable of forming combinations with phosphorus and other mineral compounds, during heating at 80 °C or 100 °C. Further confirmation of different properties of SSP–NaCl, as compared with WSP and SSP–K_2SO_4 was absence of endothermal effect in the temperature range of 230–250 °C in DTA curves from the precipitate at 100 °C and differential relationship in the measured absorption ratio of Amide I and II and the band at 1090 cm^{-1}. Supposedly the band at 1090 cm^{-1} derives from the group of phosphoric esters (from phytates) associated with proteins. Decrease in phosphorus content in precipitates obtained by denaturation of proteins extracted with NaCl (at 100 °C) (KLEPACKA et al., 1983), probably caused the increase in dependence between Amides I and II and the band at 1090 cm^{-1}.

4. Conclusion

On the basis of the conducted studies on denatured proteins at 80 °C or 100 °C it was established that the observed differences were dependent mainly on the extracting agents, i.e. on the protein fractions. Two values

— weight losses in the TG curve in the temperature range of 400–800 °C,

— ratio between Amides I and II and the band at 1090 cm^{-1}

revealed the reverse dependence of denatured SSP–NaCl at 80 °C and 100 °C, as compared to those of WSP and SSP–K_2SO_4. On this basis it may be assumed that the SSP–NaCl forms different network structure during denaturation (at 80 °C or 100 °C), as compared with WSP and SSP–K_2SO_4.

The differences between the studied rapeseed varieties were insignificant, they did not appear in the thermal analysis and in spectral analysis, only

dependences of Amides I and II and the band at 1090 cm^{-1}, were observed. The variety of doubly low erucic acid content revealed lower values of the measured ratio than the other two varieties.

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Acta Alimentaria, Vol. 16 (1), pp. 61—65 (1987)

STUDIES ON EUCALYPTUS SEED PROTEIN FRACTIONATION

(PRELIMINARY REPORT)

B. MANDAL,[a] S. GHOSH MAJUMDAR[b] and C. R. MAITY[c]

[a] Department of Chemistry, B. N. Mahavidyalaya, Itachuna-712 147, Hooghly West Bengal. India

[b] Department of Chemistry, University of Burdwan, Burdwan-713 104, West Bengal. India

[c] Department of Biochemistry, Burdwan Medical College, Burdwan-713 104, West Bengal. India

(Received: 15 August 1985; accepted: 12 December 1985)

Three protein fractions and one glycoprotein fraction with molecular weights of 295 000, 240 000, 170 000 and 65 000, respectively, were separated from *Eucalyptus hybrida* seed protein by SDS-polyacrylamide gel electrophoresis. Amino acid composition of these protein fractions including glycoprotein and carbohydrate composition of the glycoprotein fraction were determined. Three protein fractions contained 17 amino acids including 10 essential amino acids while glycoprotein fraction contained 8 amino acids and 35% carbohydrates.

Keywords: *Eucalyptus hybrida,* seed protein fractionation, polyacrylamide gel electrophoresis

Recently, eucalyptus *(Eucalyptus hybrida)* is being widely planted in different forests of India for its rapid growth and valuable pulpwood. Therefore, seeds are available in large quantities in different local forests which are up till now unexplored. The fatty acid composition and nutritional potentiality of the seed oil has been reported earlier (MANDAL et al., 1984a, 1985) from our laboratory. The protein content of the defatted eucalyptus seed cake varied from 13.5 to 14.2% and its balanced amino acid composition suggests that it might be a valuable supplement in animal feed (MANDAL et al., 1980, 1982, 1984b, 1984c, 1984d). On this basis, the eucalyptus seed protein may be of commercial importance. Therefore, further informations on the seed protein fractions, their molecular weight and amino acid composition are highly needed. In this communication, we report the results of such a study.

1. Materials and methods

Eucalyptus hybrida (Myrtaceae) seeds were collected from the local forests of Burdwan (W. B.), India and completely defatted by repeated solvent (hexane) extraction and dried in air at room temperature.

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1.1. Isolation and fractionation of seed protein

Seed protein was extracted with 0.5 mol NaCl in 0.025 mol TRIS-glycine buffer (pH 8.3) by shaking in centrifuge tube for 1 h using seed meal to solvent ratio of 1 : 20 (w/v). Extract was clarified by centrifugation at 10 000 g for 15 min and then dialysed against 0.025 mol TRIS-glycine buffer (pH 8.3) for 15 h. The extract was pooled and made up to a known volume and used for electrophoresis. The nitrogen content of the extract was determined by the micro-Kjeldahl method (AOAC, 1970) and protein content was calculated using a factor of 6.25. Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis was performed according to the procedure of WEBER and OSBORN (1969) with minor modification. In the procedure used, protein extract was treated with 0.1% SDS in 0.025 mol TRIS-glycine buffer (pH 8.3) for 2 min at 100 °C. An aliquot containing 200 μg protein was applied on each of the gels Electrophoresis was carried out using 7.5% acrylamide gels in 0.025 mol TRIS-glycine buffer (pH 8.3) for 8 h at 5 mA per tube. Marker proteins used were 11 S protein fraction (300 000) of sunflower seed protein, pyruvate kinase (230 000), aldolase (160 000) and human serum albumin (68 000). Gels were stained in 0.25% Comassie Brilliant Blue-R 250 in MeOH–H_2O–AcOH (5–4–1, v/v). Destaining was accomplished with the same solvent in ratio of 5–88–7 (v/v). Glycoprotein fraction was localized by periodic acid-shiff (PAS) test following the method of SMITH (1968), which gave a magenta colour indicating the presence of glycoprotein.

1.2. Amino acid analysis

The gel bands were extracted with 0.1 mol NaOH and N content in the aliquot was determined by micro-Kjeldahl method (AOAC, 1970). A suitable quantity of the aliquot (1.5 mg N) was taken and neutralized by the addition of 0.1 mol HCl. Concentrated HCl was then added to bring the final concentration to 6 mol HCl and the contents were hydrolysed by refluxing for 24 h in evacuated tubes. The hydrolysate was then filtered and excess acid was removed by repeated evaporation under reduced pressure. For the determination of sulphur containing amino acids, the samples were first oxidized with performic acid for 18 h according to the method of LEWIS (1966) before acid hydrolysis. Residues obtained upon acid hydrolysis and evaporation were dissolved in citrate buffer (pH 2.2). The amino acids were analysed in a Beckman amino acid analyser using M–72 type resins.

1.3. Carbohydrate analysis

Glycoprotein fraction recovered from gel band was hydrolysed for 10 h at 100 °C with 2 mol H_2SO_4 in evacuated tube. The excess acid was neutralized by adding solid $BaCO_3$ and filtered. The filtrate was concentrated and applied

to a column (0.5×5 cm) of dowex-50×2(H^+) and washed with water. The washings were concentrated, subjected to paper chromatography (n-butanol–pyridine–water, in ratio of 2 : 2 : 1) according to the method of SMITH and SEAKINS (1976) and sugars were identified from their R_f values referring to those of standard samples. Saturated aqueous solution of aniline hydrogen oxalate was used as staining reagent. Quantitative estimations of sugars were done by PhOH–$-H_2SO_4$ method (DUBOIS et al., 1956).

2. Results

Electrophoretic patterns of eucalyptus seed protein and those of standard proteins are shown in Fig. 1. It is evident from the electrophoretic pattern that eucalyptus seed protein consists of three protein fractions, I, II and III of molecular weights of 295 000, 240 000 and 170 000, respectively, and one glycoprotein fraction (IV) of 65 000. The nitrogen percentage of these fractions, I, II, III and IV were 14.2, 13.6, 14.0 and 10.3, respectively. The distribution of protein fractions as percentage of total recovered protein was: fraction I (295 000), 39%; fraction II (240 000), 13%; fraction III (170 000), 35% and fraction IV (65 000), 13%. Amino acid composition of the protein fractions including glycoprotein are given in Table 1. The results revealed that protein fractions, I, II and III contain 17 amino acids including 10 essential amino acids while glycoprotein fraction (IV) consists of 8 amino acids with higher levels of threonine (26.4 g per 16 g N) and serine (16.4 g per 16 g N). The carbohydrate content of the glycoprotein fraction (IV) was 35% and it gave glucose (28.3%), galactose (18.1%), glucosamine (5.4%), mannose (31.6%), mannitol (12.4%) and xylose (4.2%) on acid hydrolysis.

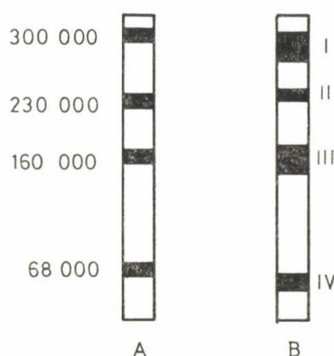

Fig. 1. Electrophoretic patterns of reference proteins and eucalyptus seed protein A. Reference proteins (from top to bottom): 11 S fraction (300 000) of sunflower seed protein, pyruvate kinase (230 000), aldolase (160 000) and human serum albumin (68 000); B. Eucalyptus seed protein (from top to bottom): fraction I (295 000), fraction II (240 000), fraction III (170 000) and fraction IV (65 000)

Table 1

Amino acid composition (g per 16 g N)[a] *of eucalyptus seed protein fractions*

Amino acids	Fractions			
	I	II	III	IV
Glycine	2.0	3.6	2.3	6.8
Alanine	2.0	3.0	3.1	5.6
Threonine	0.6	0.3	1.2	26.4
Serine	0.7	0.9	2.0	16.4
Valine	1.0	0.8	3.0	9.2
Leucine	6.6	5.2	4.3	—
Isoleucine	2.1	2.3	1.1	—
Proline	1.0	1.1	2.2	5.4
Phenylalanine	2.1	2.1	3.1	—
Methionine	0.8	0.6	0.1	—
Cystine	0.5	0.6	0.3	—
Histidine	2.8	2.7	2.6	—
Tyrosine	2.8	1.6	2.4	8.8
Arginine	10.1	10.9	11.4	—
Lysine	5.3	5.1	4.4	—
Aspartic acid	7.0	8.7	9.4	9.3
Glutamic acid	28.3	26.3	29.4	—

[a] Values not corrected for any destruction due to acid hydrolysis
— : not present

3. Conclusions

Eucalyptus seed protein fractionation by SDS-polyacrylamide gel electrophoresis showed three protein fractions and one glycoprotein fraction present therein. Studies on some plant seed proteins such as sunflower (BAUDET & MOSSE, 1977), soybean (WOLF, 1977), mustard *(Brassica juncea)* (GURURAJ RAO, 1980) and guar *(Cyamopsis tetragonoloba)* (NATH, 1980) shows that they generally consist of four to six fractions. It is not surprising, therefore, that our study has shown that eucalyptus seed protein consists of four fractions including one glycoprotein fraction.

The amino acid composition of the protein fractions varied from one another and also from the seed protein itself (MANDAL et al., 1982). It was found that protein fractions (I, II and III) contain same number of amino acids but amino acid contents differ from fraction to fraction. The amino acid composition of the glycoprotein fraction (IV) was completely different from those of other protein fractions. The amino acids threonine and serine were the major components along with glycine, alanine, valine, tyrosine and aspartic acid as the minor ones. It is probable that in glycoprotein fraction (IV), threonine and serine are mainly involved in amino acid-sugar linkages. Thus higher levels of amino acids, threonine and serine in fraction IV indicate the presence of large numbers of 0-glycosidic linkages between sugars and amino acids, threonine and serine. It is in this context that the sum of amino acid contents

(g per 16 g N) of the protein fractions including glycoprotein fraction, varied in the range of 75.98 to 88.04. This lower value is probably due to destruction of some amino acids during acid hydrolysis.

It could be of interest to determine the structure of carbohydrate moieties, the nature of the bonds linking them to the peptide and the probable distribution of these units in glycoprotein fraction of eucalyptus seed protein. Such studies are in progress and the observations made will be reported in a later communication.

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The authors are grateful to Dr. P. BANERJEE, Chemistry Department, North Bengal University for his technical help and to the Director of Central Food Technological Research Institute, Mysore for some analyses. This work was supported in part by a research grant from the Council of Scientific and Industrial Research (CSIR) of India.

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Acta Alimentaria, Vol. 16 (1), pp. 67–77 (1987)

EFFECT OF WATER FROM DIFFERENT SOURCES IN SAUDI ARABIA ON THE RHEOLOGICAL PROPERTIES OF WHEAT FLOUR AND BREAD QUALITY

A. S. MESALLAM, M. I. EL-SHAARAWY and A. I. MUSTAFA

Department of Food and Dairy Technology, King Faisal University
Al-Ahsa, Saudi Arabia
(Received: 22 August 1985; accepted: 21 January 1986)

Ten water samples were collected from different locations of the Eastern Province in Saudi Arabia. They represent tap water, springs and wells water, potable treated water and sea water. The pH, total salinity, contents of phosphate, chloride, calcium, magnesium, sodium, potassium, iron, zinc, and copper were determined in all samples. Effect of water source on rheological properties of two levels of extraction of hard Saudi wheat flour was studied using the Farinograph and the amylograph. Quality of breads prepared thereupon was evaluated by measuring the specific loaf volume, crust and crumb colours, symmetry, grain, break and shred, and texture. As expected, it was found that sea water contained the highest salinity and mineral content, followed by tap water, whereas the treated water showed the lowest salinity and mineral content. It was shown that the higher the salinity of water, the higher the stability of doughs and peak viscosity of water–flour suspensions. The best bread quality was obtained with waters having total salinity between 1000–1500 mg per kg; quality suffering a decrease towards the higher margin.

Keywords: wheat flour, water analysis, bread quality

Water is the major ingredient in breadmaking, forming more than 50% of the dough (MALEKI et al., 1980) and 35% of bread (WATT-BERNICE & MERRILL-ANNABEL, 1975). It is necessary for gluten formation (BLOKSMA, 1971), starch gelatinization (SANDSTEDT, 1961) and to provide medium for the multitude of interactions occurring throughout the bread-making process (PONTE, 1971). In the final product it plays a significant role in bread staling (ASEMAN, 1976) and in keeping qualities. Kind and concentration of the dissolved salts affect water absorption by the flour (GALAL et al., 1978), dough fermentation by baker's yeast (SNELL & FORTMANN, 1962), dough machinability, and texture of the finished product (MATZ, 1972).

Owing to the well designed policy of the Saudi Government, the Kingdom has been declared self-sufficient in wheat and flour (ANON., 1985a). A Certificate of Honour was granted to the Saudi Minister of Agriculture and Water by the FAO in November 1984 (ANON., 1985b). Evaluation of water sources in Saudi Arabia and their influence on Saudi flour doughs and bread making is therefore important.

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The main water source in Saudi Arabia is the ground water, formed essentially from rainfall (AL-BESHR, 1983). Rainfall is very low and unpredictable. Runoff is therefore irregular and storage of surface water is almost negligible. Two thirds of the Kingdom is believed to be underlain by sedimentary formations, of which sandstone and limestone are the main sources of ground water (EL-KHATIB, 1980). The ground water is available in form of natural springs, shallow wells or otherwise artesian wells.

Municipal water is drawn from ground water, and chlorinated for household use. Its high salt content, however renders it unpotable. Drinking water depends on commercially treated water, locally or otherwise imported bottled water or mineral water.

Another water source of increasing significance for domestic and industrial purposes in coastal cities, is the desalinized water. Except for bottled water, breadmaking in the Kingdom utilizes water from all of the above-mentioned sources depending on location and size of the oven or bakery.

The present work investigates effect of water sources on breadmaking to find out how far each source would suit this process. Since salt is added during dough preparation, and due to world water scarcity and particularly in Saudi Arabia, sea water is likewise tried. This would also help comparison with water of low salt content.

1. Materials

1.1. Water samples

Ten water samples representing the above-mentioned sources were collected from the Eastern Province in Saudi Arabia, as shown in Table 1. Samples were kept in deep freezer until utilized. Deionized water was used as control.

Table 1

Source of water samples investigated

Sample No.	Source	Location
Control	Deionized water	College Lab.
1	Municipality water	Al-Khobar
2	Municipality water	Al-Dammam
3	Municipality water	Al-Ahsa (Hofuf)
4	Municipality water	Al-Ahsa (Mobarraz)
5	Natural spring	Al-Ahsa (Ein-ul-Harra)
6	Artesian well	Al-Ahsa (Qatar Road)
7	Artesian well	Al-Ahsa (Eastern Villages)
8	Treated water	Al-Khobar
9	Treated water	Al-Hofuf
10	Arabian gulf water	Al-Khobar

Table 2

Recipe of loaf bread prepared

Ingredients	Amount (g)
Flour	100
Water	as required[a]
Sugar	3
Salt	2
Yeast	2
Glyceride monostearate	0.5
Ascorbic acid	0.005

[a] The quantity determined by the farinograph

1.2. *Flour samples*

Two samples representing the commercially available Saudi flour of 85% and 75% extraction rates were obtained (Saudi Mills Co., Dammam).

1.3. *Other bread ingredients*

Sugar, salt, yeast, glyceride monostearate and ascorbic acid (Table 2) were added as indicated under "bread making".

2. Methods

2.1. *Water analysis*

Three determinations were carried out on each sample.

2.1.1. Total salinity. Salinity is measured as total salts (in mg per kg) by an Electric Conductivity Meter, Model MC–1 (Electrical Instruments Ltd., Surrey, England).

2.1.2. Metal content. Content of four major and four trace metals is determined by atomic absorption spectrophotometry (EL-SHAARAWY, 1979) in a Perkin Elmer AAS, Model 603 using air/acetylene flame. Lanthanum oxide is added to overcome interference with calcium determination (EL-SHAARAWY, 1971).

2.1.3. Sulphate. Sulphate is determined according to the AOAC (1980) gravimetric method.

2.1.4. Chloride. Chloride is determined volumetrically by titration with silver nitrate (USDA, 1954).

2.1.5. pH. pH is determined using a pH meter.

2.2. *Rheological properties of the dough*

Water absorption and mixing behaviour of the doughs prepared with different water samples were measured in a Brabander farinograph. Brabander amylograph is utilized to measure pasting and viscosity properties of the flour. Methods of the AACC (1976), were followed. Two determinations were carried out on each sample.

2.3. *Bread making*

Panned bread was prepared by the method of TSEN and TANG (1971) involving the one step mixing of all ingredients (salt is not included in the dough prepared from sea water). Water was added as determined by the farinograph for optimum consistency. After mixing, the dough was cut, manually punched, and kept in fermentor (National MFG Co., Lincoln, Nebr.) at 32 °C for 15 min. It was then molded in aluminium molds and kept back to proof in the fermentor for further 45 min; after which time it is baked at 218 °C. Three loaves were produced from every sample, and each used separately for weight and volume determinations.

2.4. *Assessment of bread quality*

Loaf weight was determined to nearest 1 g. Volume was measured by the seed displacement method (AACC, 1976). Sensory attributes of the bread were determined organoleptically by ten panelists selected from the college staff, following the Multiple Comparison Method (KRAMER & TWIGG, 1979) and a centrigrade scoring scale.

3. Results and discussion

3.1. *Water analysis*

Results of water analysis are presented in Tables 3 and 4. Maximum mineral content allowed by the SAUDI ARABIAN STANDARDS ORGANIZATION (1984) in drinking water is also cited for comparison.

Table 3 shows that the pH values of all water samples fall within a narrow range of 7.7–8.2, thus within that defined by the Saudi Standard. No correlation between water salinity, anionic or cationic content of water and its pH value is noted.

As expected, sea water shows very high content of salinity, sulphate, chloride, calcium, magnesium, sodium and potassium. Among other samples, Al-Khobar municipal water, followed by the Dammam sample show highest

Table 3

Acidity and salinity of water samples

Sample No.	Source and location		pH value	Total salinity (mg per kg)	Sulphates (mg per kg)	Chlorides (mg per kg)
Control	Deionized water,	Lab.	5.85	2.5		
1	Municipality,	Khobar	7.72	2 464	778	1 101
2	Municipality,	Dammam	8.12	2 143	593	972
3	Municipality,	Hofuf	8.19	1 248	411	439
4	Municipality,	Mobarraz	7.94	1 116	390	327
5	Springs,	Mobarraz	7.98	1 436	543	559
6	Well,	Qatar Rd	8.12	1 152	395	394
7	Well,	Villages	7.94	1 140	375	346
8	Treated,	Khobar	8.13	438	127	115
9	Treated,	Hofuf	7.70	282	49	82
10	Gulf water,	Khobar	7.89	33 920	3804	20 751
Saudi standard for drinking water		Max. range	6.5–9.2	1 472	400	600
		Optimum	7–8.5		200	200

Table 4

Metallic content of water samples
(mg per kg)

Sample No.	Source and location		Ca	Mg	Na	K	Zn	Fe	Co	Mn
1	Municipal,	Khobar	93	53	375	23	0.07	—	—	—
2	Municipal,	Dammam	88	45	325	19	0.53	—	—	—
3	Municipal,	Hofuf	73	40	200	14	0.74	—	—	—
4	Municipal,	Mobarraz	53	28	150	12	0.10	—	—	—
5	Spring,	Mobarraz	70	33	200	14	0.04	—	—	0.1
6	Wells,	Qatar Rd.	60	40	175	12	0.26	—	—	0.1
7	Wells,	Villages	58	33	175	12	0.02	—	—	—
8	Treated,	Khobar	4	2	3	3	0.06	—	—	—
9	Treated,	Hofuf	2	1	1	2	0.04	—	—	—
10	Gulf,	Khobar	4600	1800	10 095	568	0.06	0.3	0.1	0.1
Saudi standard for drinking water		Max.	200	30	not given		15	1	1.5	5
		Optimum	75	or 150			5	0.1	0.05	0.05

—: Nil

levels of salinity, sulphate, chloride, calcium and magnesium, evidently exceeding those set for drinking, and particularly for bottled water. Two maxima for magnesium concentration are set in the Saudi project, depending on sulphate level. Thus up to 30 mg per kg water is allowed at a sulphate concentration of 250 mg per kg, whereas 150 mg per kg are tolerated at lower sulphate concentrations. Only the former level is cited in Table 4, since sulphate concentrations of all the samples (except that of the treated) are much higher than 250 mg

per kg. Magnesium content of the two treated water samples, concurrently of low sulphate level, is however, extremely low. Likewise are concentrations of total salinity and of other minerals determined. This is conceivable from the ion-exchange treatment applied. It is to be noted that no level for sodium nor for potassium is set in the pertinent Saudi Standard.

As for concentration of the trace metals zinc and manganese, all samples, including sea water, lie within the allowed limits. Except for sea water, all other samples proved practically void of detectable levels of iron or copper.

It may be concluded that municipal water of either Khobar or Dammam do not meet Saudi specifications for drinking water, whereas all water samples of Al-Ahsa, including those from wells, are considered acceptable or at least marginal from the salts point of view. It is worth mentioning however, that both Khobar and Dammam enjoy in the moment drinking water from the desalination project in Azizeyya, which started pumping after completion of this investigation.

3.2. Dough mixing characteristics

Table 5 shows that water absorption capacity is higher for the higher extraction flour, possibly due to its larger content of highly imbibing pentosans (El-Wakeel et al., 1975).

Examination of Tables 3, 4 and 5 indicates no correlation between water absorption and its pH value. However, water absorption is found to decrease slightly with increase of total salinity and content of individual ions, particularly potassium, in water. This is most clear from comparison of the two extremes, i.e. the sea water and the deionized water (having salt content of only 25 mg per kg). This agrees well with earlier findings. Water absorption was

Table 5

Effect of water from different sources on the mixing properties of Saudi wheat flour (determined by farinograph)

Sample No.	75% extraction flour					85% extraction flour				
	Water absorption (%)	Mixing time (min)	Stability time (min)	Break-down time (min)	Mixing tolerance index (B.U.)	Water absorption (%)	Mixing time (min)	Stability time (min)	Break-down time (min)	Mixing tolerance index (B.U.)
Control	68.9	8	16.0	22 25	30	75	5.25	9.5	18.00	50
1	67.5	10.0	19.75	25.0	20	73.8	6.75	12.50	20.50	50
2	67.3	9.50	19.00	24.0	25	74.0	6.50	12.00	19.75	50
3	68.2	9.25	17.75	23.5	30	74.1	6.25	11.75	19.25	50
4	68.8	8.75	17.75	23.0	30	74.5	6.00	10.75	18.75	50
5	68.1	9.25	17.75	23.0	30	74.4	6.25	11.50	19.00	50
6	68.8	8.75	17.25	23.0	30	74.8	6.00	11.25	18.75	50
7	68.8	8.75	17.00	23.0	30	74.6	6.00	10.75	18.50	50
8	68.8	8.25	16.50	22.7	30	74.9	5.50	9.75	18.00	50
9	68.8	8.25	16.50	22.7	30	74.9	5.50	9.75	18.00	50
10	65.8	14.00	30.00	40.0		70.0	9.00	13.00	30.00	20

reported to decrease with content of sodium chloride alone (GALAL et al., 1978; VETTER, 1981; SALOVAARA, 1982), or when replaced at 20% and 40% levels with chlorides of potassium, calcium or magnesium, or sulphates of sodium or magnesium (SALOVAARA, 1982). The latter author found insignificant variations in water absorption among samples of different salts. However, MORAD and co-workers (1981) reported increased absorption with ground water of higher hardness than tap water.

Dough mixing time (indicated as development or peak time), stability, and break-down time, all show general increase with water salinity, anionic and cationic content (Table 5). Moreover, they are higher for the lower extraction flour. Table 3 and 5 indicate no clear effect of water pH on rheological properties of the dough. However, TSEN (1966) reported a decrease in dough resistance between pH 4.2. and 7.3. Differences in resistance among our doughs may therefore be attributable solely to variations in mineral content of water. In fact, pH values of doughs were almost constant at 5.5., indicating some buffering action. Ground water, compared to the softer tap water of some Egyptian cities was reported to increase mixing and stability times of the dough (MORAD et al., 1981). Different salts were found to result in different effects. Thus sodium chloride strengthened the dough (GALAL et al., 1978) as did when partially replaced by magnesium or sodium sulphate (SALOVAARA, 1982). Replacement of sodium chloride by potassium chloride or magnesium acetate showed no significant effect, whereas replacement by magnesium or calcium chlorides weakened the dough (SALOVAARA, 1982).

Effect of electrolytes on dough rheology may be explained on basis of gluten aggregation (SALOVAARA, 1982). Ions may enhance either protein association or dissociation to become dominant (BENNETT & EWART, 1965).

Mixing tolerance index (MTI) shows an elevated value with the higher extraction rate (Table 5). This may be explained by the higher protein content of the 85% extraction flour.

Except for sea water, other water samples do not affect the MTI of the 85% extraction flour. The same holds true with the 75% extraction flour only with water samples having salinity of up to 2000 mg per kg. At higher salt concentrations, the MTI decreases significantly. This depressing effect is most profound with sea water, where MTI shows more than 50% depression with the high extraction flour and becomes unmeasurable with the low extraction type.

3.3. *Viscosity of flour suspensions*

Comparison of flour suspensions prepared with deionized water and sea water (Table 6) shows significant increase with salinity in pasting time and temperature and in peak viscosity of suspensions of both degrees of flour ex-

Table 6

Effect of water from different sources on the viscosity of flour water suspensions

Sample No.	75% Extraction					85% Extraction				
	Pasting		Peak viscosity			Pasting		Peak viscosity		
	Temperature (°C)	Time (min)	B.U.	Temperature (°C)	Time (min)	Temperature °C)	Time (min)	B.U.	Temperature (°C)	Time (min)
Control	55.5	17	460	83	35.5	57.0	18	260	75	30
1	57.5	18	620	86	37.5	61.0	21	300	81	34
2	57.5	18	610	85	37.0	60.0	20	290	79	33
3	57.0	17	590	84	36.5	59.0	19	275	78	32
4	57.0	17	580	84	36.0	58.5	19	270	77	32
5	57.0	17	580	84	36.0	58.5	19	280	77	32
6	56.5	17	550	84	36.0	58.5	19	280	77	31
7	56.5	17	540	84	36.0	58.0	19	275	77	30
8	55.5	17	480	83	35.5	57.5	18	260	75	30
9	55.5	17	470	83	35.5	57.5	18	260	75	30
10	66	24	870	93	42.0	69.0	26	555	94	42

Table 7

Effect of water from different sources on bread volume

Sample No.	Weight (g)	Volume (cm^3)	Specific volume (cm^3g^{-1})
Control	236	490	2.08
1	221	510	2.31
2	236	545	2.31
3	233	642	2.76
4	226	612	2.71
5	220	557	2.53
6	240	605	2.52
7	221	557	2.52
8	227	550	2.42
9	223	550	2.47
10	228	280	1.23

Table 8

Scoring of panned bread as affected

Character	Total grade	Scores given for bread pre-							
		Control		1		2		3	
		$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Loaf volume	20	14	1.05	14	0.67	15	0.94	17	1.15
Crust colour	10	8	0.82	8	0.94	9	0.94	9	0.94
Symmetry	10	7	1.15	6	1.15	7	0.94	8	1.05
Bread shred	10	7	1.05	6	1.05	7	1.05	8	0.94
Grain	20	12	1.15	12	1.33	12	1.15	15	1.05
Texture	20	12	1.15	10	1.15	12	1.7	16	1.15
Crumb colour	10	8	1.15	8	0.82	8	1.35	8	1.15
Total scores	100	68	3.83	64	2.21	70	2.31	81	2.98

n = 10 panelists

traction. The other suspensions show slight though steady increase in these values with water salinity.

However, pasting time of the 75% extraction flour is practically constant at salinity values below 2000 mg per kg. It is known that increase in viscosity of flour suspensions is directly related to quantity and swelling properties of flour starch and gluten (BROOKE, 1939).

3.4. *Bread quality*

As expected, use of sea water significantly decreased loaf volume, weight, and specific volume (Table 7). Deionized water, on the other hand, is found to yield small bread, hence, too, of low specific volume, and undesirably high compactness.

Absence of dough tightening salts would lead to sticky doughs (BROWN, 1939). Evidently, mineral contents of lower than 500 mg per kg or higher than 2000 mg per kg water yield bread of low volume and/or high weight, hence of low specific volume. Water samples having 1000–1500 mg per kg salts proved to produce enhanced specific volumes. Which of the studied electrolytes is more responsible for better results needs further investigation. Calcium, sulphate and bicarbonate had been identified as potential factors affecting dough fermentation by yeasts (SNELL & FORTMANN, 1962). Sodium chloride and other salts present normally in water were shown to have significant effect on dough machinability and texture of the finished product (MATZ, 1972). Sodium bicarbonate or magnesium oxide were reported to affect fermentation and decrease loaf volume only when added at concentrations exceeding 1000 mg per kg or 500 mg per kg for the two salts, respectively (BLOKSMA, 1971).

Results surmised from the specific volume determinations are confirmed by the sensory panel (Table 8), as indicated from scores given to loaf volume, grain and texture.

by water from different sources

pared with different water samples

4		5		6		7		8		9		10	
$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
17	1.05	16	0.94	17	1.15	16	0.94	15	1.15	15	1.15	8	0.82
9	0.67	9	1.25	9	0.82	8	1.05	8	0.67	8	0.67	6	0.94
9	0.82	8	1.25	8	1.05	9	1.05	7	0.94	7	1.05	5	1.14
9	0.94	7	0.67	8	1.15	9	0.82	8	0.94	8	0.94	2	1.15
16	1.15	15	1.15	18	1.25	18	1.15	14	1.05	14	1.15	4	0.94
18	1.05	15	1.76	18	1.15	18	1.15	12	1.49	13	1.33	4	1.05
8	1.15	8	1.15	8	1.33	8	1.05	8	1.05	8	1.15	6	0.92
86	3.33	78	3.06	85	3.06	86	3.5	72	3.09	73	2.75	34.1	2.04

Moreover, bread prepared with water of 1000 to 1500 mg per kg salt content is shown to have the best symmetry, break and shred, and overall acceptability. Except for bread prepared with sea water, crust and crumb colours are apparently not affected by changes in mineral content.

In conclusion, water having 1000–1500 ppm salts would be most suitable for bread making. Neither municipality water of Khobar or Dammam, nor treated water used for drinking in Saudi Arabia falls in this category. Further investigation is recommended for testing partially desalinized sea water.

*

The kind assistance of Dr. MUSTAFA ABU RADY of the Soil & Water Department, King Faisal University in carrying out metal determination in water is gratefully acknowledged.

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Acta Alimentaria, Vol. 16 (1), pp. 79–93 (1987)

ON-LINE MONITORING AND CONTROL OF PHOSPHATE CONCENTRATION IN DIFFERENT FERMENTATION TECHNOLOGIES

M. Pécs[a], L. Szigeti[a], A. Boros[a], L. Nyeste[a], J. Holló[a], J. Báthory[b], I. Pataky[b] and M. Éry[b]

[a] Department of Agricultural Chemical Technology, Technical University, Budapest, H-1111 Budapest, Gellért tér 4. Hungary
[b] Phylaxia Veterinary Biologicals Ltd., H-1483 Budapest 10, P.O. Box 23. Hungary
(Received: 4 September 1985; accepted: 2 January 1986)

A computer-coupled autoanalyzer-fermentor system was developed for on-line studying of microbial metabolism. Phosphate consumption, and other parameters were investigated in three fermentation processes. Phosphate consumption in the primary metabolism was monitored in *Methylomonas sp.* and *Saccharomyces cerevisiae* fermentations, and metabolic changes were studied in bacitracin — secondary metabolite — fermentation. The computer-coupled system was capable to realize the minimal variance control of phosphate level during the microbial process. It has been demonstrated that the system is suitable for on-line data processing and control, and for the analysis of synthetic and industrial fermentation media. It provides useful information for further development of the fermentation of primary and secondary metabolites. It can be flexibly applied to various analytical purposes and to the study of different quality fermentation broths.

Keywords: on-line monitoring, on-line control, phosphate concentration, fermentation, microbial metabolism

The automation of fermentation systems has been lagging at least ten years behind the general development of chemical industry. This lag is mainly due to the immense complexity of microbiological systems in comparison to the mechanism of chemical conversion. The number of parameters to be measured and controlled is much higher. Controlling the pressure, temperature and rate of supply is not sufficient, measurement of at least part of the reacting molecules and products formed is also required. Current methods seem to be suitable for solving hardware and software problems of fermentation process control, the main challenge facing experts today is the development of appropriate chemical sensors. A new pathway is the application of computer-coupled discrete sensors (gas chromatography, mass spectrometry, HPLC, cytofluorography, autoanalyzer, etc.). In this paper we wish to present our results with an on-line system of computer-coupled autoanalyzer-fermentor developed in our laboratories.

There are relatively few publications available on the analysis of fermentation broths by means of an autoanalyzer. Some of these (Roy & Buccafuri, 1980; Hőgye & Nagy, 1981; Mor et al., 1974) present only off-line analysis

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

and even with on-line systems, application to process control is rather rare (DEAN & STEWART, 1979).

An explanation for this may be the prolonged dead-time of autoanalyzers: 3 to 30 min between sampling and peak evaluation. Since, however, fermentation is a prolonged process, such dead-time may be considered permissible.

The fundamental problem is taking samples from the fermentation broth, as a pure liquid sample has to be obtained from a three-phase disperse system. For separation of the solid fraction as well as the cells and bubbles various methods have been developed: continuous dialysis (RÖHR et al., 1976; ZABRISKIE & HUMPHREY, 1978; VOGELMANN et al., 1974; CHOTANI & CONSTANTINIDES, 1982; VALENTINI & RAGAZZANO, 1982); sedimentation (LEISOLA & KAUPPINEN, 1978; OJAMO & LEISOLA, 1979; LEISOLA et al., 1979); continuous filtration (MOR & FIECHTER, 1974; LEISOLA & KAUPPINEN, 1978; LEISOLA et al., 1980; KUHLMANN et al., 1982; HILL & THOMMEL, 1979; IMMING et al., 1982; KUHLMANN et al., 1984; SCHMIDT et al., 1984, 1985; SCHEPEL et al., 1984). In our system mainly continuous dialysis, and in some cases also continuous filtration have been applied (PÉCS et al., 1985).

In developing our system our aim was to attain flexibility. As a result, the system developed can be flexibly changed i.e., by exchanging the reaction module it can be used for analyses of different components, on the other hand, by modification of the sampler unit, it is adaptable for the analysis of different broths. In the course of our investigations, we tested the system by application of a reactor module for the measurement of the inorganic phosphate and studied three different fermentation processes. The measurement of phosphate concentration was justified by the simplicity of the method and by its role in controlling primary and secondary metabolism.

1. Materials and methods

1.1. The measuring system

The measuring system was composed of the following units:

- Fermentor: 3 dm^3 Biotec fermentor body with measuring and control systems developed in our laboratory (VERES et al., 1981).
- Computer: TRS-80 microcomputer with laboratory-made interface and valve control (SZIGETI et al. 1986).
- Autoanalyzer: Contiflo analyzer (Labor MIM, Hungary) with a sampling unit equipped with magnetic valves (Fig. 1).

For the analysis of different broths two samplers working on identical principles have been elaborated (Fig. 2). In experiments carried out on synthetic media the sample recycled from an external circulation loop is passed through the alternative magnetic valves into the continuous dialyzer. (Valves:

Reichelt Chemie Technik 95115, see Fig. 2/a.) The bacitracin broth contains solid particles (soy cake, corn starch), therefore the recycling unit was equipped with a continuous filter and bubble separator (Fig. 2/b).

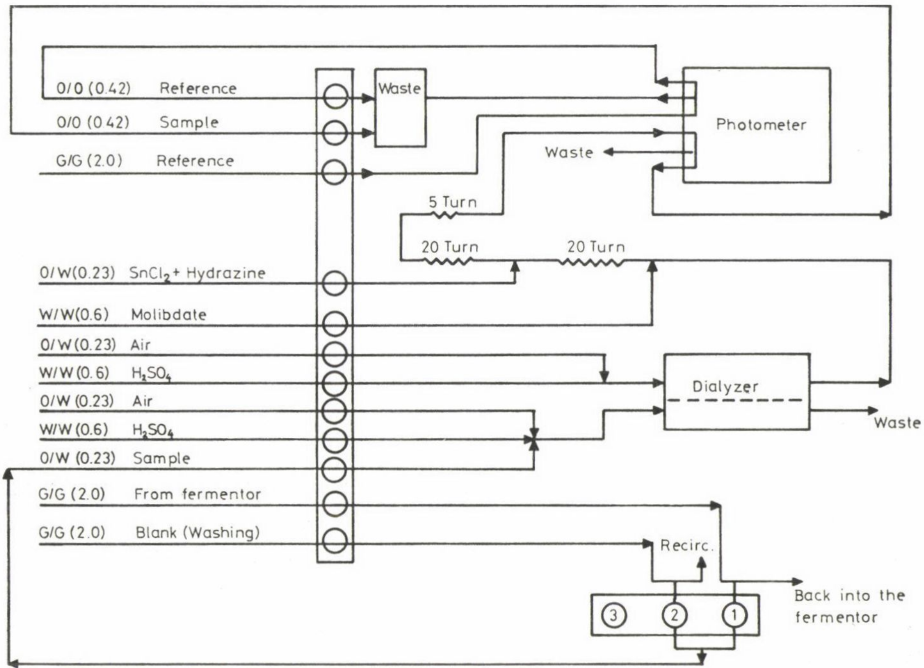

Fig. 1. Flowchart of the autoanalyzer system. ①, ②, ③: magnetic valves

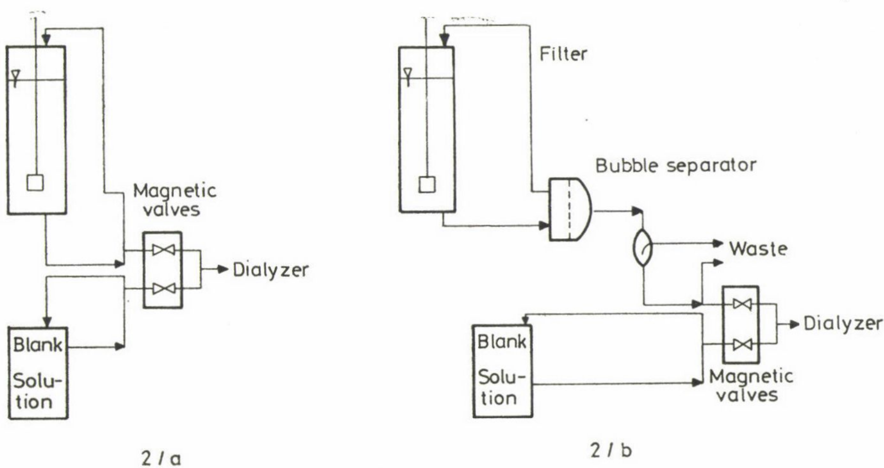

Fig. 2. Sampler units. a: for synthetic media; b: for industrial media

(Valves: Radelkis titrimeter valves, OF-510. Filtermembrane: Sartorius cellulose acetate membrane-filter, pore diameter: 0.8–1.2 μm.) Detailed description of the structure, operation and software of the system had been presented in earlier publications (PÉCS et al., 1985; SZIGETI et al., 1986).

1.2. Test fermentations

1.2.1. Production of single cell protein (SCP) on methanol base with a *Methylomonas sp.* strain. Temperature: 33 °C; pH: 6.8; mixing: 700–1000 rpm, aeration: 1 vvm. Fermentation media: methanol 10 cm^3 per dm^3, $(NH_4)_2SO_4$ 1.6 g per dm^3; $MgSO_4 \cdot 7\,H_2O$ 0.36 g per dm^3; $FeSO_4 \cdot 7\,H_2O$ 0.04 g per dm^3; $CaCl_2 \cdot 2\,H_2O$ 0.05 g per dm^3; NaCl 0.1 g per dm^3; H_3PO_4 and the KOH variant. pH control was carried out with the use of a 12.5% NH_3 solution.

1.2.2. Production of baker's yeast with a *Saccharomyces cerevisiae* strain. Temperature: 30 °C, pH: 4.5, mixing: 800 rpm, aeration: 1 vvm. Composition of media: glucose 30–100 g per dm^3, $(NH_4)_2SO_4$ 5 g per dm^3, $MgSO_4$ 0.1 g per dm^3, $CaCl_2$ 0.1 g per dm^3, NaCl 0.1 g per dm^3, biotin 25 μg per dm^3, phosphate level control by addition of 2 g per dm^3 KH_2PO_4 solution.

1.2.3. Bacitracin fermentation with *Bacillus licheniformis* mutant strain (Phylaxia Co.). Temperature: 35 °C; without pH control; mixing: 600–800 rpm; aeration: 1 vvm. These experiments were carried out in a Biofer type laboratory fermentor (8 dm^3 useful volume). The media contained approximately 6% solid granular substances (soy cake and corn starch), ammonium sulphate and other mineral salts. They contained, however, no phosphate salts, the phosphate measurable in the broth was derived from natural resources, mainly from soy cake. In the course of experiments, the following parameters were obtained by off-line methods: total carbohydrate concentration (glucose measurement after hydrolysis), free glucose concentration (without hydrolysis), phosphate concentration (off-line control measurement), cell concentration (protein concentration after digestion by lysozyme), bacitracin concentration (by biological titration), phage-titer (for two different types, by plack method).

1.3. Control algorythm

Principles and mode of operation of the minimal variance (MV) adaptive algorythm applied for controlling phosphate concentration were presented in a previous paper (PÉCS et al., 1985). In the course of the fermentation process the decrease in phosphate concentration was controlled through the algorythm by the supply of a calculated amount of concentrated phosphate solution. This algorythm takes into consideration the changing dynamic characteristics of the process.

2. Results and discussion

2.1. Monitoring phosphate consumption in SCP fermentation on methanol base

Our system developed for on-line measurement of phosphate concentration was first adopted for the measurement of substrate consumption in primary metabolism. For this purpose we applied *Methylomonas sp.* production on methanol carbon source, a process used in a technology currently under development in our laboratory for the production of single cell protein. We wished to monitor phosphate incorporation into the cells and, on the basis of this, to set up a material balance for batch and continuous fermentation processes. In addition to the determination of phosphate concentration, pH and dissolved oxygen measurements were also carried out by the computer system. Optical density and intermittent measurements of the dry matter content were performed by the off-line method.

In the course of batch fermentation experiments, phosphate incorporation was studied in balanced growth phases free of limitation (Fig. 3).

Studying the biomass formed as a function of phosphate consumption, we obtained straight lines in the batch fermentations investigated (Fig. 4), with the slope showing the yield constant.

The straight lines were found to be nearly parallel and calculated slopes were nearly identical (Table 1).

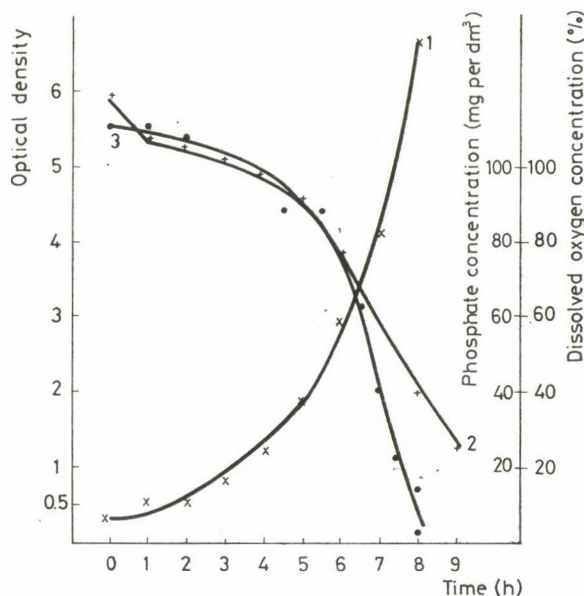

Fig. 3. Cultivation of *Methylomonas sp.* on methanol. 1: optical density, 2: dissolved oxygen concentration, 3: phosphate concentration (mg per dm^3)

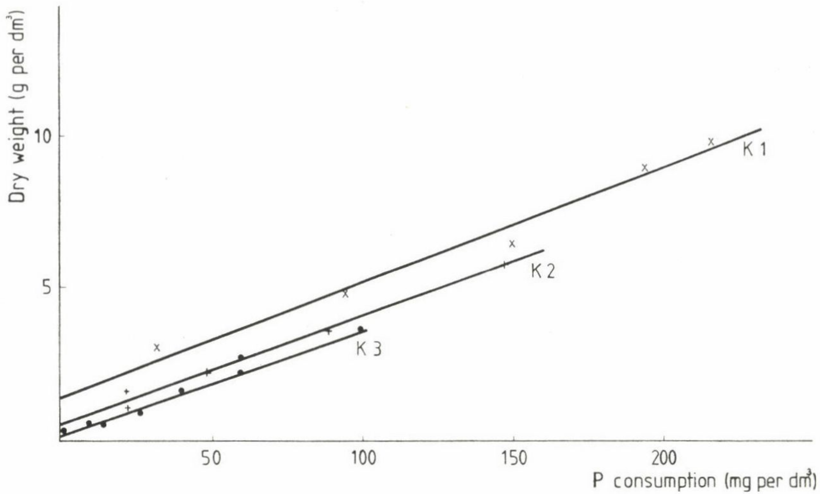

Fig. 4. Yield curves of three batch *Methylomonas sp.* cultures

Interceptions of the lines differed owing to the different initial concentrations.

The extent of phosphate incorporation can also be calculated by the material balance related to the total fermentation time. Addition of the amounts of phosphate passed into the system with the inoculum or media or supplied in the course of the experiments, minus the amount measured in the cell-free liquid at the end of the process, will give phosphate concentration in the cells. With this method of calculation all errors of concentration and volume measurements will interfere with the results, therefore, total error will be large (Table 1). In our continuous fermentation experiments two steady states have been evaluated (the third was washed out). In both cases steady state phosphate concentration was hardly detectable, owing to the poor signal/noise ratio, we could only estimate that the value was around 0–1 mg P per dm^3. Presumably this concentration is in the limiting region, this assumption has not been verified, however, by further substrate measurements. From the data obtained the

Table 1

Yields of batch experiments with Methylomonas sp.

		Calculated yield (g cell per g P consumed)	
		from slope	from material balance
Batch experiments	K1	36.064	35.252
	K2	37.917	42.60
	K3	34.785	39.99

Table 2

Yields of continuous Methylomonas *fermentations*

	D1	D2
Dilution rate (h^{-1})	0.176	0.354
Inlet phosphate conc. (mg P per dm^3)	104	104
Dry matter content (g per dm^3)	3.81	4.15
Outlet phosphate conc.	0–1	0–1
Yield (g cell per g P)	36.99	40.29
Yield (g cell per g methanol)	0.481	0.524

phosphate balance of the steady state can be determined, and from this, the yields can be calculated (Table 2).

The values thus obtained are approximately in agreement with the results of batch fermentations. The yields related to methanol are unusually high under the given experimental conditions. In our assumption, methanol assimilation and dissimilation in the metabolism of the strain proceed separately (REUSS et al., 1974), leading to higher energy (ATP) yields than required for cell growth. This independent dissimilation may be slowed down by the low level of phosphate concentration, resulting in higher yields related to methanol.

The results obtained in a series of experiments were checked also by another method. From the reciprocal values of the yields, phosphate concentration of the cells can be calculated. Comparison of these values with those obtained by elementary analysis is shown in Table 3.

The results obtained by different methods proved to be nearly identical. As corroborated by experimental results, the system is suitable for monitoring the process of fermentation and for quantitative study of the metabolism.

Table 3

Calculated and measured phosphate content of Methylomonas sp. *biomass*

		Phosphate content (%)	
		from slope	from material balance
Batch experiments	K1	2.77	2.84
	K2	2.64	2.35
	K3	2.87	2.50
Continuous experiments	D1		2.70
	D2		2.45
By elementary analysis		2.53	

2.2. Control of phosphate concentration in the course of yeast production

In the following step the system developed has been further extended: in addition to the measurement of phosphate concentration, control has also been realised. With this in view, we first solved automatic phosphate supply by passing a sterile phosphate solution into the fermentor through computer controlled magnetic valves. Control was realised by inserting in the software an algorythm adapted to the dynamics of the process. Minimal variance (MV) controllers serve to minimize changes in the values of variables related to preset values. On the basis of previous results, in each cycle of measurement

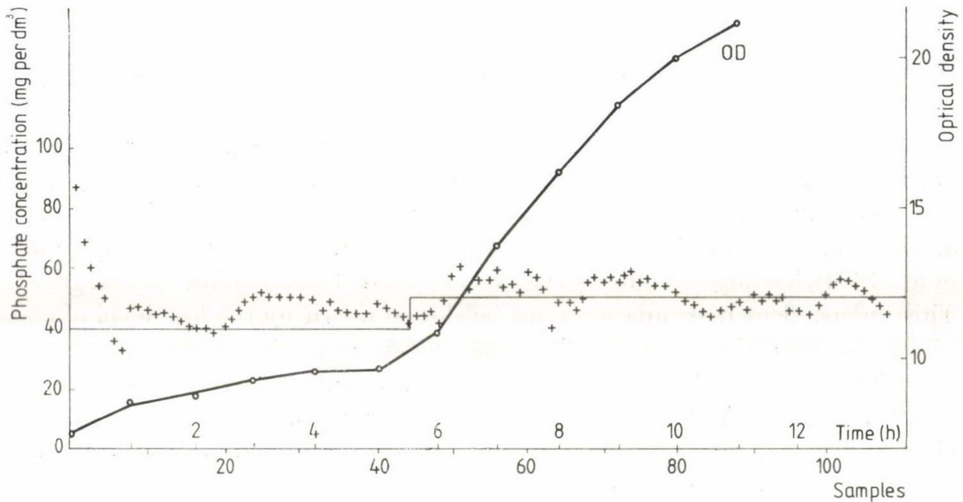

Fig. 5. Baker's yeast propagation with phosphate level control. Initial glucose concentration: 30 g per dm³. OD: Optical density. Preset levels of the phosphate: 40 and 50 mg P per dm³

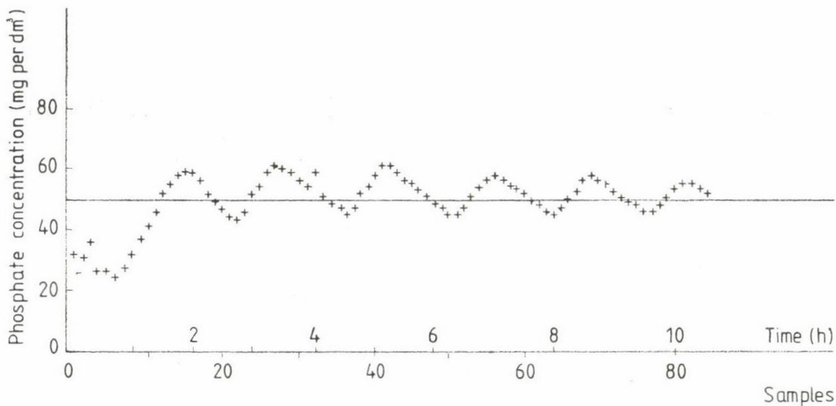

Fig. 6. Phosphate control during baker's yeast propagation. Initial glucose concentration: 100 g per dm³. Preset level of phosphate control: 50 mg P per dm³

(every 7.5 min), the computer renews the parameters of the control model and determines the extent of interruption, or periods of valve opening. The period of phosphate measurement from sampling to peak evaluation is altogether 15 min, the frequency of sampling is, however, twice as high, leading to a measurement cycle of 7.5 min.

Test experiments involved the growth of *Saccharomyces cerevisiae* (baker's yeast) under dynamic parameters of phosphate consumption were determined from the results of monitoring experiments as described above, and on the basis of this, operation of the hardware and software has been coordinated. Actual control experiments have been started after completion of this. The first experiment was set off with 100 mg per dm^3 phosphate concentration and 30 g per dm^3 glucose concentration, with 40 mg P per dm^3 as initial set point of the control. In the fourth hour of the process both cell growth and phosphate consumption ceased after the consumption of glucose. Upon the addition of 100 g per dm^3 glucose solution metabolism was restarted and control was in operation for further 8 h (Fig. 5), the set-point was adjusted to 50 mg P per dm^3.

The second experiment was set off with 100 g per dm^3 glucose concentration and 50 mg P per dm^3 as the set-point for both initial phosphate concentration and control. In the course of controlling, phosphate concentration showed periodical fluctuations around the set-point and control was satisfactory throughout the experiment (more than 10 h) (Fig. 6).

According to the theory of MV controllers, standard deviation of the controlled variable around a preset value is in agreement with the deviations in variable measurements. From calibration experiments, the standard deviation of measurements was determined at 50 mg P per dm^3 concentration, then from the results of control experiments the standard deviation of phosphate concentration related to the same set-point has been evaluated. In the second phase of the first experiment 66 data, and in the control phase of the second experiment 49 data were investigated. By comparing the standard deviations of measurement and control with the F-test, we obtain

$$F\ (65{,}22) = 2.621 \qquad F\ (0.95) = 1.89$$
$$F\ (48{,}22) = 1.116 \qquad F\ (0.95) = 1.91$$

i.e., in the first case standard deviations differ, thus, the conditions are not fulfilled by the control, whereas in the second case deviation was not significant, so the control was actually MV type. The reason of the difference may be that in the second case the degree of interruption was limited (20 sec. valve opening).

2.3. *Study of the metabolism in bacitracin fermentation*

Phosphate concentration has a fundamental role in controlling the fermentation of secondary metabolites. It has been stated for various secondary metabolites (antibiotics, alkaloids, etc.) that no synthesis can be observed in the

presence of phosphate (MARTIN, 1977). The system developed can be utilized considering only phosphate concentration, mainly in the production of secondary metabolites by fermentation. Our first task was to study the metabolism and metabolic changes in bacitracin fermentation. Owing to the industrial fermentation media applied, the system was equipped with a continuous membrane filter and bubble separator (Fig. 2). The computer system performed on-line data lodging of phosphate and dissolved oxygen concentration and the pH value, whereas the parameters listed in para. 1.2. were measured off-line.

According to earlier investigations, bacitracin fermentation has 3 phases (Figs. 7 and 8).

The first is the phase of increasing growth rate, characteristic of decreasing pH and dissolved oxygen (DO) values. The end of the phase is indicated by the consumption of free glucose (RCH), higher pH values and DO stagnation. The end of the second phase is marked by a minimal pH value and the onset of bacitracin synthesis. The third phase is characteristic of increasing pH

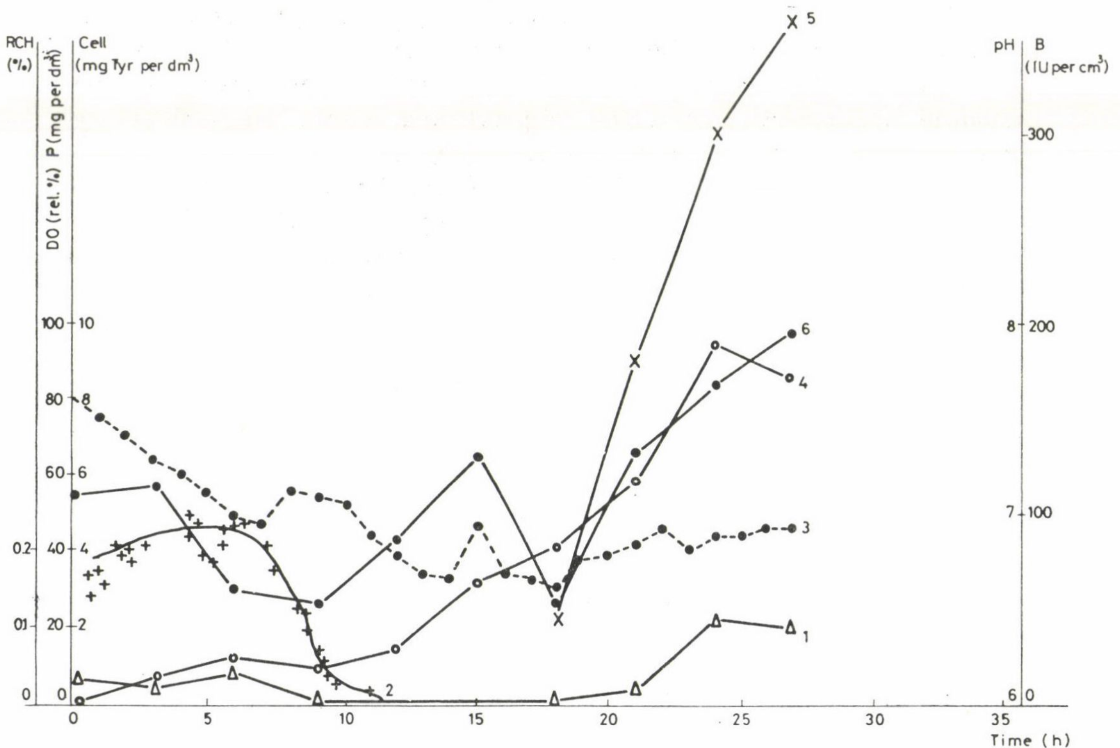

Fig. 7. Typical bacitracin fermentation without phosphate addition. 1: RCH (△-△-): reducing carbohydrates (%), 2: P (+-+-+): phosphate concentration (mgP per dm³), 3: DO (●---●---●) dissolved oxygen concentration (rel.%), 4: Cell (○—○): cell concentration (mg Tyr per dm³, see methods), 5: B (×—×): bacitracin concentration (IU per cm³), 6: pH —●—

values, intensive production of antibiotic and a decrease in cell concentration. This picture can be described in further detail by additional parameters obtained on the basis of phosphate concentration measurements (Figs. 7 and 8).

In the lag phase phosphate level seems to be stagnating or may show some slight increase, which may be attributed to the phosphate ions released from soy-cake by hydrolysis. In the phase of increasing growth rate, a marked decrease can be observed in the pH value as well as in phosphate and dissolved oxygen concentration. After the consumption of free glucose, phosphate consumption also slows down, total consumption can be observed, however, only about in the middle of the second phase.

2.3.1. Bacitracin fermentation by phosphate addition. In the following phase, we studied the effect of various methods of phosphate addition upon metabolism. First, the initial concentration has been raised, then in subsequent experiments small amounts of phosphate were added after phosphate consumption.

The addition of 100 mg P per dm³ phosphate in the form of a KH_2PO_4 solution before the inoculation resulted in a two-step "diauxial" slope of consumption (Fig. 9).

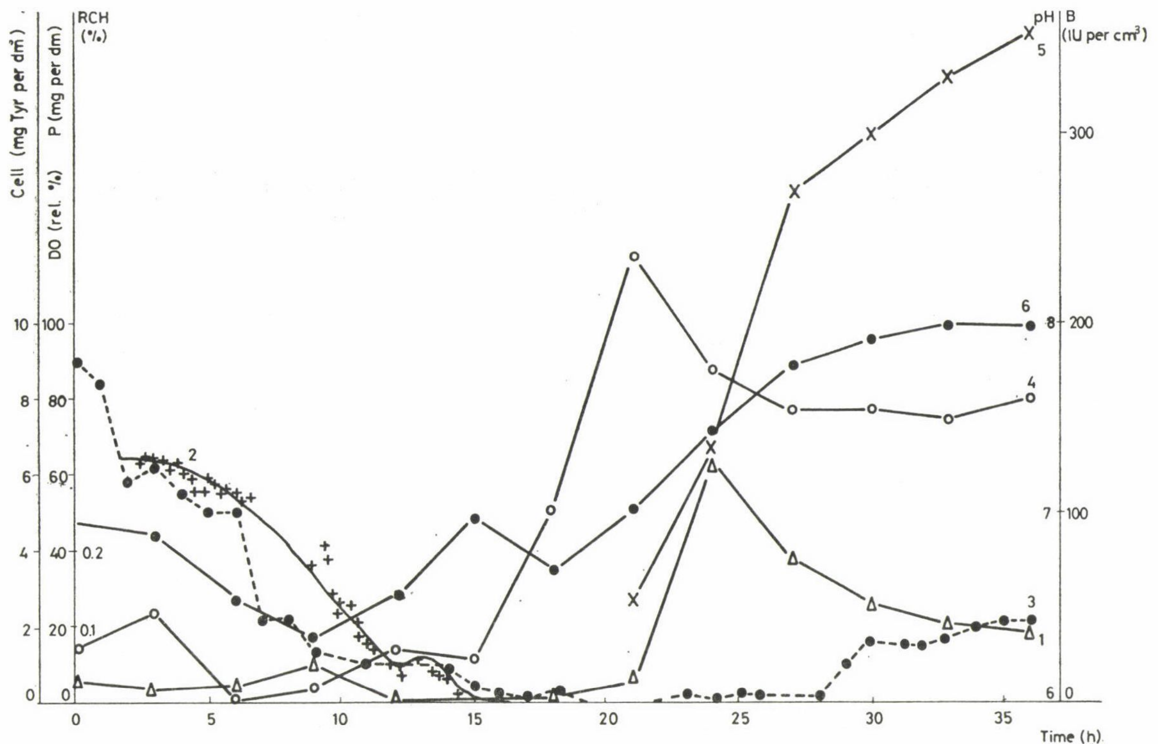

Fig. 8. Typical three-phase bacitracin fermentation with diauxic phosphate consumption. Symbols: see Fig 7

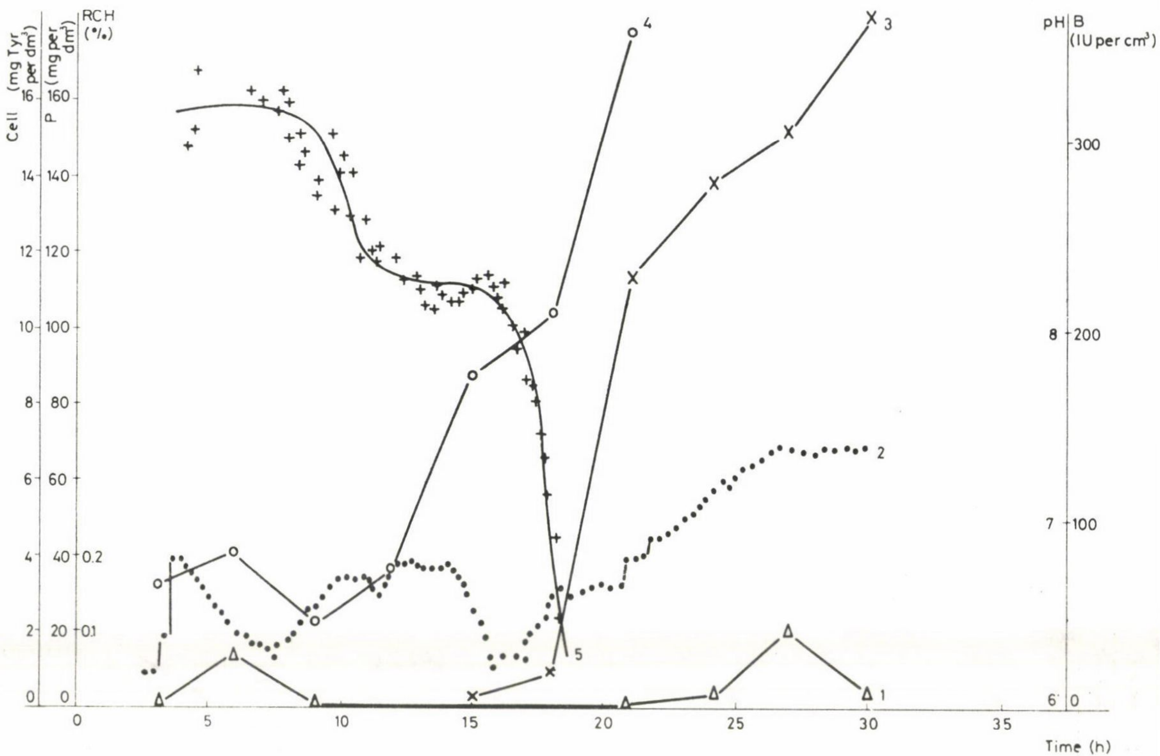

Fig. 9. Bacitracin fermentation with initial phosphate addition (100 mg P per dm³ in the form of KH_2PO_4). The phosphate consumption shows diauxic character. 1: RCH (△—△); 2: pH (. . . .); 3: B (×—×); 4: cell (○—○)

In accordance with the above, the first phase ends with glucose limitation, slowing down the whole process of consumption, which accelerates again after a period of adaptation. It may be remarkable that almost twice the amount of phosphate can be completely metabolized, resulting in particularly high cell concentration. The course of fermentation, later phases of the process as well as product formation are practically unaltered. The change in the slope of pH values may be interpreted by higher buffer capacity, which is due to higher phosphate concentration.

In other experiments, we started phosphate addition after consumption of the initial amount and endeavoured to achieve steady state and control at a low phosphate concentration (0–5 mg P per dm³). In this case, however, control was less accurate then described in the previous section partly because filtration and bubble separation extended the dead time, and partly because the supply of phosphate could be solved only with larger amounts (min. 5 cm³ of 3.52% KH_2PO_4 solution). In tracing the course of experiments (e.g. Fig. 10)

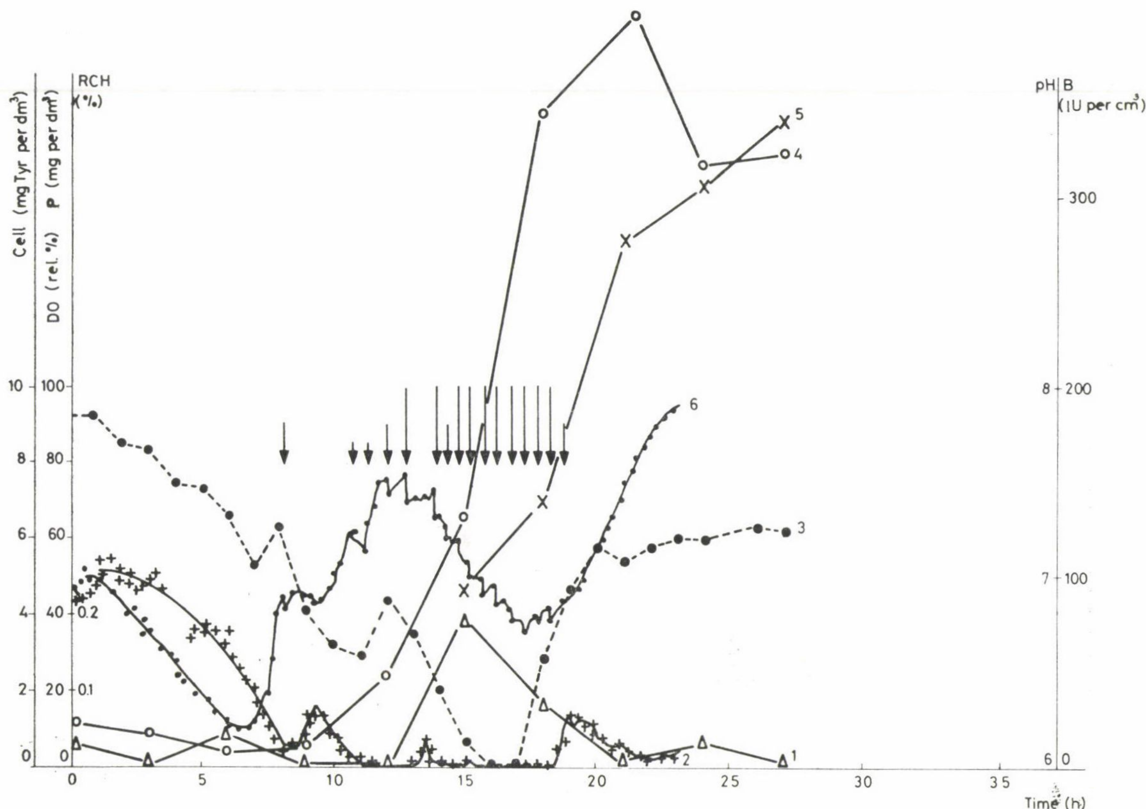

Fig. 10. Bacitracin fermentation with phosphate addition during the second phase. Symbols: see Fig. 7, the arrows demonstrate phosphate additions (5, 10 and 20 cm^3 of 3.52% KH_2PO_4 solution)

we may state that phosphate was utilized in much higher amounts than before, which resulted in intensive cell growth.

At a certain point, however, phosphate consumption suddenly ceased as shown in Fig. 10. In the 18th hour a sudden increase, followed by a slow decrease in phosphate concentration was observed. The sudden increase at nearly steady state phosphate incorporation may be due to a dramatic change in the metabolism, e.g. limitation of a non-identified component of the medium. Another effect of phosphate addition is the change in pH value, the decrease caused by phosphate addition is shown in Fig. 10.

The study of product formation leads to the conclusion — in accordance with literature data — that the presence of phosphate interferes with the production of antibiotics. In batch experiments the formation of bacitracin started only after total consumption of the phosphate. In the course of phosphate addi-

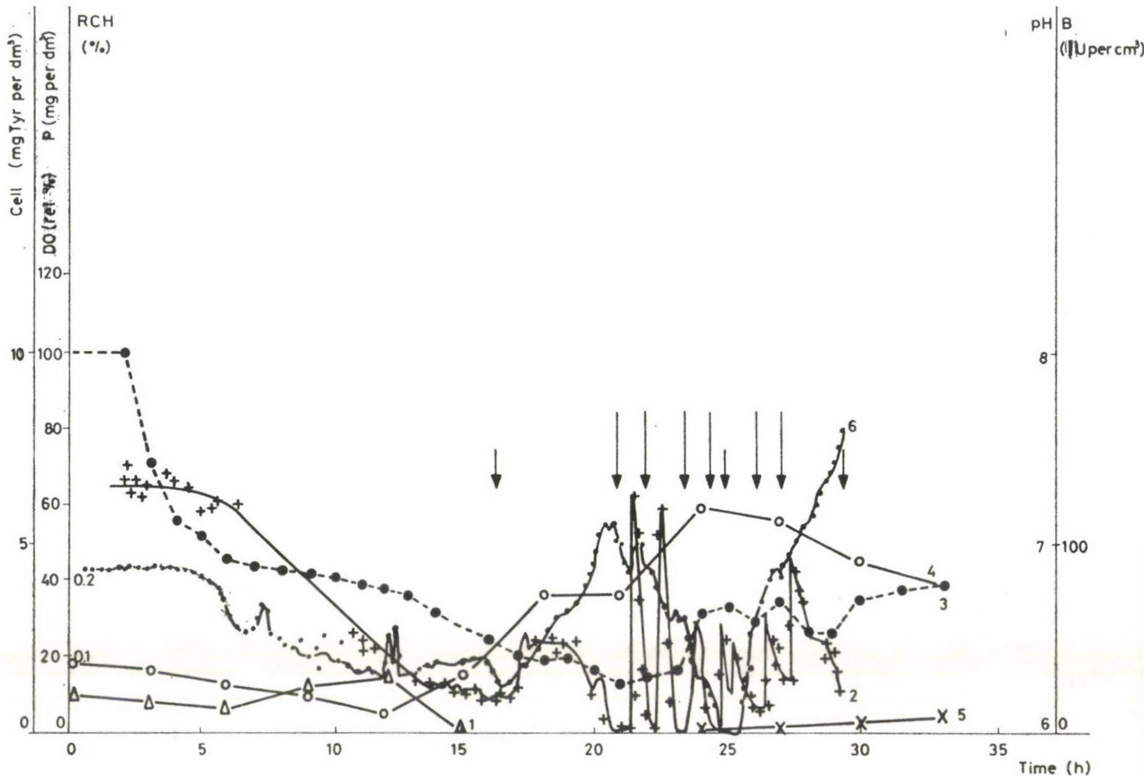

Fig. 11. Bacitracin fermentation with phosphate addition. Symbols: see Fig. 7. The phosphate level is high, the antibiotic formation is very weak

tion, product formation started if phosphate concentration was not higher than 5 mg P per dm³. At higher levels, the formation of products slowed down and completely ceased at a level of 20–30 mg P per dm³ (Fig. 11).

*

The authors wish to express grateful acknowledgement for the grant supplied by the Institute for Science Organization and Informatics.

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Acta Alimentaria, Vol. 16 (1), pp. 95–101 (1987)

Abstracts

of selected papers presented at the

YEAST CONFERENCE

ORGANIZED BY

THE HUNGARIAN SCIENTIFIC SOCIETY FOR FOOD INDUSTRY

Budapest, Hungary
29 May 1985

MANUFACTURE OF BAKERS' YEAST IN HUNGARY

L. Sági

Budapest Distilling Enterprise,
H-1089 Budapest, Diószeghy S. u. 8. Hungary

Two problems are prevailing at present in the manufacture of bakers' yeast: a. State and future of bakers' yeast manufacture in Hungary; b. Realization of dried yeast manufacture and the introduction of this new product to the home market.

The use of bakers' yeast has been steadily increasing during the last 15 years. In 1984 the demand for it was higher than the capacity of the bakers' yeast producing factory. Demand is not uniform during the year. During 1984 on six occasions were peaks observed outdoing 1.5 times the average demand. Thus, beyond the average demand the problem of peak demands had to be solved. Money to build a new factory was not available therefore, the existing factory was complemented by a plant producing dried yeast. The advantage of dried yeast over the compressed product is its substantially longer shelf-life.

NOVELTIES IN BAKERS' YEAST PRODUCTION

Gy. Folláth

Budafok Yeast and Distilling Factory,
H-1222 Budapest, Gyár u. 5—11. Hungary

In the course of introducing the production of dried yeast a new fermentor of 75 m^3 capacity was built in with tubular aeration and programmed nutrient addition. A new yeast strain has been applied with 15% higher leavening capacity. The yield of yeast increased, however, at increased costs, because the

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

new strain requires the addition of a higher amount of biotin. The capacity of the drying equipment is 150 kg per hour. Further possibilities of development are: a. introduction of more efficient sterilization in order to reduce the number of wild yeasts; b. development of a better refrigerating system to prevent compressed yeast from spoilage; c. development of a better yeast strain than the one used at present, suitable for the leavening of pastries of high and low sugar content; d. development of heat tolerant and alcohol tolerant strains by genetic means and thereby reducing the cooling water requirement.

CONTRADICTIONS IN TAXONOMY AND IDENTIFICATION DUE TO NEW RESULTS

T. Deák

Department of Microbiology, University of Horticulture, H-1118 Budapest, Villányi út 35—43. Hungary

The taxonomy of yeasts has been substantially changed by results achieved in recent years in biochemistry and molecular biology (composition of the cell wall, types of coenzyme Q, DNA base composition and homology, etc.). However, these methods cannot be used in routine tests. On the other hand, the results of traditional identification tests do not coincide always with the taxonomic classification based on new methods. The division of the genus Candida lags behind the possibilities of identification.

POSSIBILITIES OF THE INDUSTRIAL APPLICATION OF MODERN GENETICS

M. Sipiczki

Department of Genetics, Kossuth Lajos University, H-4010 Debrecen, Egyetem tér 1. Hungary

Yeasts can be advantageously used as model organisms in basic genetic research. Improved strains have already been used in the brewing industry in the 40's. However, genetically improved bakers' yeast was patented much later and the genetics of wine yeasts was studied even later. The methods of improving yeasts fall into two main groups: classical methods and new methods. In Hungary the latter methods are used only in basic research, yet it would pay to introduce them in practice.

SUGAR METABOLISM IN YEASTS

E. NOVÁK

National Institute of Public Health,
H-1097 Budapest, Gyáli u. 2. Hungary

On investigating the sugar metabolism in 44 yeast species it was found that beside invertase and alfa-glucosidase other enzymes also participate in the hydrolysis of sugars. The number of the possible combinations of the metabolic pathways of individual sugars among the known strains amounts to 125. In summing up the theoretical study of 200 species and the experimental investigation of 44 species it can be concluded that our knowledge of the sugar metabolism in yeast is still incomplete.

YEASTS AS QUALITY CHARACTERISTICS OF FOODS

V. TABAJDI-PINTÉR

Centre for Veterinary and Food Control,
H-1095 Budapest, Mester u. 82. Hungary

In certain foods spoilage is caused by yeasts, each food having its characteristic species. In recent years various procedures were worked out for each group of foods to qualify individual batches. The related activities were carried out in the following grouping: unification of test methods; storage stability tests; establishment of tolerable yeast level.

Based on the data obtained a clear picture was formed on the contamination by yeasts of various foodstuffs all over the country. The distribution of the population of yeast can be characterized by the mean and standard deviation; the knowledge of standard deviation is necessary to formulate a monitoring system and to allow the comparison of results.

STUDY OF BAKERS' YEAST AND BREWERS' YEAST BY POLYACRYLAMIDE GEL ELECTROPHORESIS

B. MÁTRAI and A. HALÁSZ

Central Food Research Institute,
H-1022 Budapest, Herman O. u. 15. Hungary

Methods of morphology, physiology and serology may be widely complemented by physico-chemical methods. The electrophoresis of proteins proved to be a satisfactory method for diagnostics and taxonomy. The aim of this study was to find the taxonomic range on which yeasts can be separated, on the basis of their enzyme tests of their protein patterns. Whether these tests are suitable for the detection of contaminating microbial flora or prove the purity of pitching yeast were investigated.

POSSIBILITIES AND RESULTS IN THE IMPROVEMENT OF BREWERS' YEASTS

A. Maráz

Central Food Research Institute[a],
H-1022 Budapest, Herman O. u. 15. Hungary

Laboratory methods used in improving brewers' yeasts: 1. Identification of genes coding enzymes which participate in the carbohydrate metabolism in the brewing and detecting their effect; 2. Application of recombinant DNA technique for the reduction of the level of vicinal diketones causing unpleasant taste in beer; 3. Analysis by chromosome transfer of the genetic material of brewers' yeast; 4. Elimination of the disadvantageous properties of hybrids produced by protoplast fusion.

DETECTION OF WILD YEASTS IN BREWERY SAMPLES

K. Szentpétery

Kőbánya Brewery,
H-1106 Budapest, Maglódi út 17. Hungary

Two media used to detect wild yeast were tested in the brewery practice (LIN-I and LIN-II-agar). The main components of LIN-I-agar are crystal violet and a fuchsine-sulphite mixture as inhibitors. This medium is suitable for the cultivation of Saccharomyces strains. The main component in LIN-II-agar is copper sulphate as inhibitor. This medium is suitable for the cultivation of non-Saccharomyces strains. On investigating industrial samples (above all at the pithing yeast) evaluation was not satisfactory hindered by the growth of cultured yeast, therefore, heat treatment was applied as a complementing method. Samples were first kept at 50 °C for 20 min, then cooled down and inoculated onto the two media. The growth period was 48 or 72 h at 30 °C. The plates showed colonies originating only from wild yeasts.

DETERMINATION OF VIABLE YEAST COUNT IN FERMENTING BREWAGE BY MEANS OF REDUCING CAPACITY

E. Farkas-Szűcs

Borsod Brewery,
H-3574 Bőcs, Rákóczi u. 81. Hungary

The TTC reduction method is suitable for determination of the viable yeast count. Reducing capacity depends not only on the number of viable cells but on the age of the culture, too. For each sample taken at various times a new

[a] Present address: Department of Microbiology, University of Horticulture, H-1118 Budapest, Villányi út 35–43. Hungary

calibration curve has to be constructed. The TTC reduction procedure should be considered as an indirect method because it gives the reducing capacity of the yeast culture and not the cell count. In the brewing process, however, the fermenting capacity is in closer relation to the quality of the product than the yeast count.

TRENDS IN REACTOR RESEARCH AND TECHNOLOGY

B. Sevella and L. Nyeste

Technical University, Budapest,
H-1111 Budapest, Gellért tér 4. Hungary

Main characteristics of newly developed bioreactors: greater volume, increase in the oxygen transfer rate, reduction of energy requirement. Comparison of the traditional and the new technologies: traditional technologies will be used in the future as well, beside more developed new ones, just the same as traditional reactors fitted with mixer will not be everywhere replaced by new type bioreactors.

GROWTH AND ETHANOL FERMENTING CAPACITY OF YEAST CELLS ENCLOSED IN GEL

L. Boross, B. Szajáni, Zs. Buzás, B. Polyák, K. Dallmann, P. Papp, V. Halmos, Gy. Copf and J. Zsolt

Department of Biochemistry, József Attila University,
H-6701 Szeged, Eötvös u. 6. Hungary

Of the methods for immobilizing *Saccharomyces cerevisiae* cells and to test their ethanol fermenting capacity enclosing in Ca-alginate gel was found the most suitable. Changes in the cell count of the enclosed yeast were investigated. The effect of various conditions on the growth of cells enclosed and in suspension with particular reference to aerobic and anaerobic conditions was compared.

The carbohydrate metabolising and ethanol producing capacity of the enclosed yeast cells was found unchanged. Suitable reactors were constructed which allow to take samples from different places. The gel-enclosed cells were successfully kept alive during several months. Reactors operating with gel-enclosed yeast cells were found suitable for the continuous industrial production of ethanol.

UP-TO-DATE METHODS OF ALCOHOL FERMENTATION

B. Kovács

Research Institute of the Distilling Industry,
H-1089 Budapest, Diószeghy S. u. 8. Hungary

Technologies used in the distilling industry to produce alcohol from cereals and molasses: a. combined method as used in the Budafok Factory for Alcohol and Yeast; b. continuous preparation of maize and subsequent batch fermentation as applied in the Szabadegyháza Combinate. Continuous fermentation technology with the recirculation of yeasts and stillage. Fermentation of worts of higher sugar concentration in order to save energy needed for distilling (Biostil procedure). A complete technology of sweet sorghum processing with alcohol as one of the products. Requirements on the yeast strains applied in various technologies.

GENERAL PROBLEMS RELATED TO THE USE OF WINE YEASTS

Á. Ásvány

National Institute of Wine Classification,
H-1027 Budapest, Bem József tér 2. Hungary

Selection of wine yeasts started as early as 1901 at the Institute of Viticulture and Enology. Eighty seven strains with excellent properties were collected from different wine growing districts. The strains were maintained, proliferated and distributed to wine growers from all over the country, who required them. The changes occurring in viticulture in the 70s made new demands on wine microbiologists. Beside liquid and lyophilized cultures the demand for active dry yeast is steadily increasing.

STUDY OF PSYCHROPHILIC YEASTS

E. Pölös

University of Horticulture, Institute of Viticulture and Enology,
H-6001 Kecskemét, Kisfái u. 10. Hungary

Mosts with high sugar content (25%) were fermented at 10 °C and 5 °C by psychrophilic yeasts. The sugar destruction and ethanol productivity was observed depending on strains after 6 weeks. Several strains were investigated and two strains were found best. The fatty acid and lipid composition of normal and psychrophilic yeasts were also studied. Thin-layer-chromatographic method was used for separating lipids and GC method for the determination of fatty acid composition. Based on the results of the experiments a correlation was established between membrane composition and fermentation ability at different temperatures.

POSSIBILITIES OF UTILIZING KILLER YEASTS IN WINE FERMENTATION

I. Wünsche

University of Horticulture, Institute of Viticulture and Enology, H-6001 Kecskemét, Kisfái u. 10. Hungary

The Killer toxins discovered in the 60s are macromolecular proteins. They act on the plasma membrane by changing permeability properties. They are destroyed by heat treatment and occur in different species. The effect of various factors on Killer yeasts was studied and the destroying effect was found to depend on the pH, too. At present 10 Killer toxins of different types are known. Under suitable conditions genetically improved Killer yeasts may be used for wine fermenting. This was proven experimentally.

THE ROLE OF YEASTS IN SPARKLING WINE MANUFACTURE

P. Sárkány

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

Selected yeast strains are used in pure or mixed cultures according to the different technologies of sparkling wine factories.

The optimum amount of yeast added to the ground wine and the optimum viable cell number were experimentally established. Best results are obtained with large amounts of yeasts with special character containing at least 70% of young, budding cells. In the case of 2–3% inoculum the optimal viable yeast count in the wine is 50–60 million cells per litre.

Five to six months after fermentation the majority of yeast cells in the sparkling wine is destroyed. After the sixth month the character of the sparkling wine begins to develop and it becomes ready in the 9th to 12th month.

If the ground wine is fermented by sparkling wine yeasts and it is stored in pore-free containers under inert gas the flavour and small characteristics of the sparkling wine may be developed already after 6 month. Significant changes were observed during storage in the nitrogen containing components, thus in the amino acid composition, too.

In Hungary, experiments were carried out to utilize dried active yeasts. Rehydration operations, the shelf-life of yeast and the uncertain fermenting capacity hinder the utilization of dried yeast. Young yeast cells are susceptible to the alcohol content of the ground wine (10–12 v/w). Traditional manufacturers of real Champagne hold themselves aloof of applying new methods, of cell and enzyme immobilization and genetic manipulation.

The character of the sparkling wine is more affected by the quality of the ground wine, the ripening time and other technological factors than by differences in the yeast strains.

Acta Alimentaria, Vol. 16 (1), pp. 103 (1987)

BOOK REVIEW

Engineering properties of foods

(Food Science and Technology Series, Volume 19)

M. A. Rao and S. S. H. Rizvi (Eds)

Marcel Dekker, Inc., New York, Basel, 1986, 416 pages

Engineering properties of foodstuffs are important in the design and operation of equipments used in food unit operations. The unit operations: fluid flow, heat transfer, mass transfer, concentration, and dehydration are among the most important operations in the processing of foods. In these operations, the properties which play an important role are rheological properties of fluid foods, thermal properties, colligative properties, mass transfer properties, and thermodynamic properties related to the dehydration of foods. In addition, knowledge of rheological properties of solid foods will be useful in their handling and dielectric properties will be useful in applications related to the use of microwaves such as thawing and sterilization.

Among physical properties of foods, rheological properties related to texture have been covered extensively along with their sensory properties. These properties are extremely useful in product development and evaluation, as well as in assessing consumer response to food. However, in the design and handling of process equipment, rheological properties in terms of stresses, strains or strain rates are needed.

The book has attempted to provide the definition and theoretical background of each engineering property, the methods employed in determining the property, and a reasonable compilation of the values of the property for different foodstuffs.

The seven parts of the book give a review of the significant properties of foods:

- Rheological properties of fluid foods,
- Thermal properties of foods,
- Mass transfer properties of foods,
- Thermodynamic properties of foods in dehydration,
- Rheological properties of solid foods,
- Physicochemical and engineering properties of foods in reverse osmosis and ultrafiltration,
- Electrical properties of foods.

The book completed with 63 tables, 122 figures, diagrams and contains more than 700 bibliographic references. The lists of symbols help to learn the correlations between phenomena and properties of foods.

This book will be useful for researchers, practicing engineers and in teaching both senior level undergraduate and graduate level courses.

I. Varsányi

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

ANNOUNCEMENT

13th INTERNATIONAL SYMPOSIUM OF THE IUMS—ICFMH & FECS—WPFC

(Under the auspices of the Ministry of Health, Welfare and Social Security of Greece)

5—9 October 1987

I. In the worldwide problem of foodborne infections and intoxications, toxins play an important role. New knowledge on the nature and role of bacterial toxins, conditions for toxin formation in foods and analytical methods for their identification and quantification will be presented and discussed at the Symposium. There will also be a short session devoted to mycotoxins.

II. As a contribution to the International Drinking Water Supply and Sanitation Decade (1981—1990) declared by the World Health Organization, a part of the Symposium will be devoted to the "Microbiology of Drinking Water". Bacterial indicators, methodology and problems relating to the bacteriology of non-carbonated bottled waters will be presented and discussed.

III. A half day Seminar on "Hygiene in Food Production" for members of the food industry will also be arranged.

Official language: English.

Statement of interest should be addressed: Prof. J. A. Papadakis, Omirou 24, 10672 Athens, GREECE.

The Organizing Committee

CARIES PREVENTION BY DOMESTIC SALT FLUORIDATION

By K. Tóth

In English. 249 pages, 15 figures. 14 × 21 cm.
Hardcover $18.00/DM 54,—/£14.50
ISBN 963 05 3476 2

In Hungary a series of clinical trials started in 1966 with the aim of testing the caries-preventive effect of fluoride added to domestic salt in different concentrations. Experiments were carried out paralell with three different salt-F concentrations and as a result caries fell by more than 50 per cent. Investigations confirmed furthermore that fluoridesupplemented salt has the same effect on deciduous teeth as fluoridated water.

In this book also the physiological aspects of salt fluoridation like daily optimal intake, tolerable and harmful amounts, drinking habits are dealt with. Finally the recent results reached in the field of caries prevention are assessed.

AKADÉMIAI KIADÓ
Publishing House of the Hungarian
Academy of Sciences
Budapest

Science and Technology Policies in Finland and Hungary– A comparative Study

Edited by

K. O. DONNER and L. PÁL

In English. 1985. 371 pages, 16×23 cm
Hardcover $29.00/DM79.–/£19.75
ISBN 963 05 3977 2

Written by thirty-one Finnish and Hungarian authors, this volume is a first attempt amongst international science policy ventures to apply a comparative approach in analyzing the scientific and technological policies of two countries with different social systems.

From among the several debatable issues of science policy and management dealt with here, let us mention but a few examples: the institutional system of science and technology; "official" and actual research and development priorities, the training of scientific personnel; the ways of financing research and development; the role of information system, of prognostication and of planning in the research and development process; the problems of how to evaluate the research results, and so on.

Structurally, most of the chapters are built up as follows: they start with a common introduction, on which the general background and setting are given. This is followed by the Finnish sub-chapter, then comes the Hungarian sub-chapter. Finally, the chapter is completed with a common conclusion, in which the authors make an attempt to point out both the differences and the similarities existing in the given field in the two countries.

Still available:

The Family and its Culture
An investigation in Seven East and West European Countries

Edited by
M. Biskup, V. Filias, I. Vitányi

In English. 1984. 496 pages, 11 figures, 126 tables, 3 maps. 17×25 cm
Hardcover $45.00/DM 115,–/£27.00
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NOTICE TO CONTRIBUTORS

General. Manuscripts in English or Hungarian should be typed double-spaced on one side of the sheet and should not exceed 20 pages.

Title. The title should be concise and informative. It must be followed by the authors' names and the address(es) of the institute(s) where the work was carried out.

Summary. The article should be preceded by a summary (not exceeding 150 words) giving a self-explanatory compendium of the essence of the paper. At the end of the summary some 4 keywords should be put in alphabetical order.

Text. The article should be divided into the following parts: *Introduction* beginning on a new page without a title containing a survey of related literature and the objectives of the work, followed by *Materials and methods; Results; Conclusions.*

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

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

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

Periodicals: Names and initials of all the authors; year of publication in parentheses; colon; title of the paper; title of the periodical; inclusive page numbers.

Books: Names and initials of all the authors; the year of publication in parentheses; colon; title of the book; publishing firm, place of publication; inclusive page numbers.

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ACTA ALIMENTARIA

An International Journal of Food Science

Editor

J. HOLLÓ

Volume 16 **June 1987** **Number 2**

Akadémiai Kiadó
Budapest

D. Reidel Publishing Company
Dordrecht & Boston

ISSN 0139—3006

CODEN ACALDI

ACTA ALIMENTARIA

An International Journal of Food Science

Sponsored by the Joint Complex Committee on Food Science of the Hungarian Academy of Sciences and Ministry of Agriculture and Food.

Editorial office:

Central Food Research Institute, H-1525 Budapest, Herman Ottó út 15, Hungary

Acta Alimentaria is a quarterly journal in English, publishing original papers on food science. The main subjects covered are: physics, physical chemistry, chemistry, analysis, biology, microbiology, enzymology, engineering, instrumentation, automation and economics of foods and food production.

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P.O. Box 149, H-1389 Budapest 62, Hungary

For all other countries:

D. REIDEL PUBLISHING COMPANY
P.O. Box 17, 3300 AA Dordrecht, Holland

Publication programme, 1987: Volume 16 (4 issues).
Subscription prices per volume: Dfl. 266,—/$ 111.00 including postage.

Second class postage paid at New York, N. Y. USPS No. 756-270. U. S. Mailing Agent: Expediters of the Printed Word Ltd., 515 Madison Avenue (Suite 917), New York, N. Y. 10022, U. S. A.

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Printed in Hungary

ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

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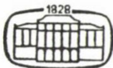

AKADÉMIAI KIADÓ
BUDAPEST

D. REIDEL PUBLISHING COMPANY
DORDRECHT & BOSTON

PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda, Budapest

Acta Alimentaria, Vol. 16 (2), pp. 105—110 (1987)

INFLUENCE OF SUBSTRATE ON MYCOTOXIN PRODUCTION OF FUSARIUM SPECIES

II. THE EFFECT OF SOY MEAL

A. BADAWEY, J. SAWINSKY and A. HALÁSZ

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

(Received: 2 July 1985; revision received: 19 February 1986; accepted: 20 March 1986)

Seven identified strains of Fusarium species were examined for the ability to produce zearalenone F-2 toxin on soy meal of two different moisture contents. F-2 toxin producers such as *F. graminearum*, *F. moniliforme*, *F. roseum*, *F. tricinctum* and *F. semitectum* were inoculated on soy meal substrates of 52% or 68% moisture content. The samples were incubated for 7 days at 20 °C, for 14 days at 8–12 °C, for 7 days at 20 °C. None of the above strains produced zearalenone on soy meal substrate.

Keywords: mycotoxin production, Fusarium strain, soy meal

Zearalenone (F-2 toxin) is an estrogenic metabolite produced by various species of Fusarium. URRY ad co-workers (1966) have identified the molecule as 6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcyclic acid lactone (Fig. 1). PALYUSIK (1972) and PALYUSIK and KOPLIK–KOVÁCS (1975) reported that most animal diseases caused in the last decades by molds were due to infection by Fusarium species.

Mycotoxicoses of animals attributable to Fusarium infected cereal grains include the estrogenic, emetic and feed refusal syndromes in swine are associated with corn and barley infected by *F. graminearum* (MIROCHA & CHRISTENSEN, 1974).

Zearalenone has been found in corn (both kernels and stalk), wheat, barley, oats, sorghum, sesame, potato, hay, silage, cassava and commercially

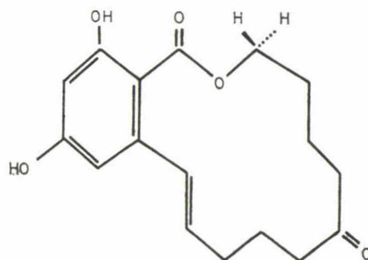

Fig. 1. Chemical structure of zearalenone
[6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcylic acid lactone]

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

formulated processed feed (MIROCHA et al., 1976; 1977). At present there is very little information available on the occurrence, relative incidence and toxigenic potential of Fusarium species on soy meal. Therefore identified toxigenic Fusarium strains were examined on autoclaved moistened soy meal to study their potential for mycotoxin production.

1. Materials and methods

Due to the lack of published data regarding the effect of Fusarium species on soy meal, a preliminary experiment was performed to select the optimal moisture content for maximal visible growth of Fusarium species.

For this purpose two identified Fusarium strains: *F. graminearum* 977/12 and *F. culmorum* 234 B/5 were used. Visible growth was observed every 24 h for 5 days at different percentages of moisture content. Table 1 shows that highest visible growth was obtained at 52 and 68% moisture contents. Percentage of moisture means water quantities added to the dry substrate before autoclaving.

Table 1

Visible growth at 25 °C temperature as affected by moisture in soy meal

	Time (day)	Moisture (%)				
		24	37	46	52	68
Fusarium graminearum	1	—	—	—	T	++
	2	—	—	—	++	+++
	3	—	—	T	+++	++++
	4	—	—	+	++++	++++
	5	—	—	++	++++	++++
Fusarium culmorum	1	—	—	—	+	++
	2	—	—	T	++	+++
	3	—	—	+	+++	+++
	4	—	—	++	++++	++++
	5	—	—	++	++++	++++

Symbols:
—: No visible growth
T: Trace of visible growth
+: Scattered colonies
++: Mycelial growth almost covered the surface
+++: Luxuriant growth covered the entire surface
++++: Heavy growth

1.1. Investigated Fusarium strains

Cultures used in the present investigation were obtained from Mycotoxin Laboratory, Central Food Research Institute (Budapest). Strains were identified according to BOOTH (1971) and BÁNHEGYI and co-workers (1985).

All the strains of Fusarium species were subcultured on potato dextrose agar for 14 days at 25 °C, followed by storage at 4 °C (ISHII et al., 1974).

1.2. Preparation of soy meal cultures

Dry powdered soy meal (100 g) was transferred into a 1 dm³ flask and wetted by adding tap water to obtain 52% moisture content and sterilized at 120 °C for 15 min. Seven samples were prepared and inoculated with identified Fusarium strains. Another seven samples were prepared with excess of tap water to obtain 68% moisture content and inoculated.

1.3. Inoculation and incubation period

Sterilized ground soy meal samples at two different moisture contents were inoculated with identified Fusarium strains (Table 2).

Table 2

Known Fusarium species inoculated on soy meal substrate

Fusarium species	Strain No[a]
F. moniliforme	228 B/9
F. culmorum	234 B/5
F. semitectum	14
F. roseum	908 B/1
F. graminearum	977 B/12
F. tricinctum	17
F. tricinctum	204/10

[a] The strains were collected by J. Sawinsky, Mycotoxin Laboratory of the Central Food Research Institute, Budapest, Hungary

The incubation time and temperature used in the present study were 7 days at 20 °C, 14 days at 8–12 °C, 7 days at 20 °C. At the end of the incubation period, the moldy soy meal heavily overgrown by mycelium, was dried overnight at 60 °C and ground.

1.4. Extraction and determination of zearalenone by thin-layer chromatography

1.4.1. Extraction and clean up. Extraction was carried out according to the method of FRISCHKORN and co-workers (1978). To 10 g of moldy soy meal 20 g anhydrous Na_2SO_4, 20 g seasand and 15 cm³ absolute ethanol were added. The whole mixture was transferred to a mortar and ground for 15 min. Fifty cm³ ethanol was added to the mixture and it was shaken for 20 min: this step was repeated 3 times. The extract was filtered (Whatman No 1) and evaporated to dryness using a rotavapor apparatus.

The residue was dissolved in 50 cm^3 methanol–water (50 : 45) mixture and defatted with three portions of 50 cm^3 petroleum ether by shaking in a separatory funnel. The methanol–water solvent was adjusted to pH 13 using 2 *N* NaOH. After the addition of 50 cm^3 chloroform, the pH of the methanol–water solvent was readjusted to 9.5 using 2 *N* H_2SO_4.

The toxin was extracted with four 25 cm^3 portions of chloroform. The combined chloroform layers were passed through a column of anhydrous Na_2SO_4 and evaporated to dryness.

1.4.2. Thin-layer chromatography. The residue as obtained in para 1.4.1. was dissolved in 200 μl acetone. Ten, 20μl of the samples were applied to TLC plates (20$\times$20 cm, POLYGRAM SIL G) precoated with 0.25 mm silica gel. Before use the plates were activated by heating for 30 min at 110 °C, then cooled to room temperature. Ten μl of 50 ppm F-2 toxin standard solution was also run for identification.

2. Results and discussion

The moldy cultures were examined for F-2 toxin production by TLC. In order to determine the recovery (%) of F-2 toxin soy meal samples were injected with pure F-2 toxin at the concentration of 100, 200 μg per kg. Extraction was done according to para 1.4.1. and determination by TLC. The recovery as mean value of 5 parallels for two concentrations were 83.2 and 84.6%, respectively (Table 3).

Table 3

Recovery of zearalenone added to soy meal

Mycotoxin	Added (μg per kg)	Recovery (%)
F-2 toxin	100	83.2 $\pm$ 3.2
F-2 toxin	200	84.6 $\pm$ 3.8

The mean value and the value of standard deviation based on 5 determinations

All strains gave negative results i.e. no production of F-2 could be obtained in soy meal cultures. A confirmation was done using Sarudi reagent (SAWINSKY-ACSÁDI, 1983). Despite the fact that the investigated Fusarium strains had given heavy growth of mycelia, F-2 toxin was not detected on soy meal cultures.

Recently we found that the previously mentioned Fusarium species had the capacity to grow on corn and rice and to produce varying amounts of zearalenone (BADAWEY et al., 1987).

Comparing the results obtained in the present investigation with ground soy meal and those in our earlier studies with corn and rice, it appears that soy meal is a poor substrate for F-2 formation as compared to corn and rice.

HESSELTINE and co-workers (1966) inoculated a number of toxin producing strains of *Aspergillus flavus* and *A. parasiticus* on autoclaved cereal and oil seeds to determine differences in their ability of toxin production. Their results with *A. parasiticus* have shown that soy meal was a poor substrate even under the best conditions of toxin production.

In a first survey of 866 soy meal samples SHOTWELL and co-workers (1977) reported that neither zearalenone nor aflatoxin or ochratoxin could be detected in any of the samples, although zearalenone was detected in various samples of wheat. Our results agree with the previous reports and support the well known fact that the nature of substrate is only one of the conditions that affect mycotoxin producing molds.

The results lead us to suggest that further studies are needed to detect whether all soy meal varieties show the same resistance to F-2 toxin formation. Incidentally, the present experiments have shown that since the growth of Fusarium was abundant on soy meal substrate, there is no causal relation between growth and toxin production.

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Acta Alimentaria, Vol. 16 (2), pp. 111—127 (1987)

EFFECT OF IONIZING RADIATION ON THE LIPIDS IN FROZEN POULTRY

I. FATTY ACIDS AND HYDROCARBONS

K. Gruiz and I. Kiss

Department of Agricultural Chemical Technology, Technical University, Budapest.
H-1111 Budapest, Gellért tér 4. Hungary
Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15.
Hungary

(Received: 3 September 1985; revision received: 30 April 1986; accepted: 5 May 1986)

Reduction of the microbial cell count in frozen packaged broiler by ionizing radiation is an advantageous method from the viewpoint of both the consumption and the storage. The effect of a microbiologically optimal radiation dose of 4 kGy on the lipids of broiler was studied by chromatography and spectrophotometry. The results were compared with both untreated and positive control (radiation dose: 50 kGy). Lipid content of the investigated broilers was 8.6%, deriving partly from the muscular tissue, partly from the adipose tissue of the skin.

The amount of free fatty acids — produced mainly by hydrolysis — increased directly upon irradiation. However a similar increase was measured in untreated samples during storage. Although fatty acids deriving from glycerides and esters did not show significant changes as the result of 4 kGy radiation, significant changes were measured on the effect of extremely high (50 kGy) radiation dose. Of the nonsaponifiable compounds the relative amount of the short carbon chain hydrocarbons increased substantially in the irradiated samples.

Lipid products formed by irradiation (URP) were not detected.

Keywords: irradiation, fatty acids, hydrocarbons, irradiation of frozen poultry

Foods are suitable for consumption only if they meet the quality requirements. One of the requirements is to meet the limit values of microbiology, particularly the elimination of pathogenic microorganisms. Contamination of poultry with salmonellae or other pathogens is causing more and more frequently diseases all over the world. According to a Canadian statistics about 17% of all salmonella infections is trasmitted by poultry. Storage stability is limited also by the high cell count in goods coming from the slaughterhouse.

Ionizing radiation enable desinfection of the packaged, frozen poultry without losing its sensory value. In this study the lipid-chemical analysis of the muscular and adipose tissue of frozen broilers treated with a 4 kGy dose of ^{60}Co was carried out as a function of storage time.

The aim of our investigations was to find out whether the radiation technology applied by the authors, caused, beside meeting the microbiological requirements, chemical changes in the lipids of the tissues of broilers.

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

Researchers try to elucidate changes in lipids caused by gamma radiation in model experiments carried out generally with triglycerides or fatty acids. They draw conclusions from the results of these experiments as to the reactions occurring in complex systems, such as poultry or other foodstuffs. In a complex system lipids are present together with proteins and other low and high molecular mass substances, often bound to them, partly in moisture containing media (meat), partly in fatty media (depot fat).

Products of radiolysis upon irradiation of lipids are mostly derivatives of triglycerides or fatty acids. Based on model experiments MERRITT and TAUB (1983) investigated the derivatives of triglycerides, while NAWAR and HANDEL (1977) those of phospholipids. A part of the radiolysis products get into the aqueous phase, the other part into the organic solvent phase during extraction, depending on the length of the carbon chain or on the polarity of the groups bound to the hydrocarbon chain. Radiolysis products may be ranked into the following three groups:

— Compounds of low molecular mass, mostly volatile substances, chiefly hydrocarbons which are formed by decarboxylation often accompanied by splitting of the chain. Acids, lactons, aldehydes, ketons and propane diol diesters may also be formed.

— Group of recombination products: aldehydes, ketons, esters and lactons of longer chain. Hydrocarbons with long chains may also be formed by decarboxylation from fatty acids or by linking of two molecules.

— The third type of products is formed by oxidative changes, e.g. peroxide compounds.

The hydrolytic cleavage of fatty acids may also be accounted for, particularly with higher radiation doses. This process leads to an increase in the amount of free fatty acids (MERRITT & TAUB, 1983; VAJDI et al., 1982).

Evaluating the results of the investigations the fundamental findings summarised by BASSON (1983) have to be kept in mind, too. He found that the quantity and quality of the products of radiation treatment depends on the lipid content, on the total fatty acid content and its composition as well as on the quality of triglycerides and their distribution in the animal studied. Differences depending on the litter or even on individuals must also be accounted for.

1. Materials and methods

1.1. Irradiation

Frozen broilers, packaged in polyethylene shrink-film were treated in the panoramic radiation source at the Institute of Isotopes of the Hungarian Academy of Sciences, (^{60}Co 3.7 PBq) with an average dose of 4 kGy at D_{max} per D_{min}=1.5. During treatment temperature was −18 to −12 °C. Broilers

were stored between —15 and —18 °C. As an effect of the radiation dose the number of mesophilic aerobic and psychrophilic microbes was reduced by 2–3 or 4 orders of magnitude, respectively. The number of Enterobacteriaceae decreased by 4 orders of magnitude. Salmonellae were not found in the irradiated samples.

Samples treated with gamma-radiation were compared to the untreated samples and those treated with the extra large dose of 50 kGy. The latter dose was used because most radiolytic products at low radiation doses are below the threshold value of detectability.

The concentration of radiolytic products is in the ppm range. These quantities are of the same order of magnitude as those formed during storage but lower than those formed upon heat treatment.

1.2. Lipid-chemical analyses

Changes in the lipids were investigated on four times during storage: on the 2nd, 30th, 105th and 150th day, respectively.

The course of the analyses is shown in Fig. 1. The meat (1) and the skin + adipose tissue (2) of the broilers were separated and each was extracted in three steps with a chloroform–methanol mixture. The combined extracts were separated into two phases.

The chloroform containing phase contains tri-, di-, and mono-glycerides, free fatty acids, sterols and their esters as well as phospholipids.

The upper aqueous phase contains beside the carbonyl compounds (aldehydes, ketones) acids of short chains, oxi-acids, alcohols and other water soluble compounds. These will be described in para. 2.1.

1.2.1. Preparation of samples. Frozen broilers, packaged in polyethylene shrink-film, irradiated and untreated were thawed at room temperature (20–22 °C). Five chickens of about 1000 g weight of each sample were halfed. Five halves of each kind were separated into meat and depot fat + skin. Both the flesh (1) and the fatty part (2) were homogenized with a meat grinder.

For lipid extraction 100 g flesh and 50 g adipose tissue were used. Extraction was carried out in three steps with a chloroform–methanol 1 : 1 mixture.

The extracts were combined and filtered, and separated in two phases. The lower phase, containing methanol, chloroform and a small quantity of water, contained the lipids.

The lipid containing phase was dried over Na_2SO_4, filtered and evaporated under N_2. The residue was weighed and used for further analyses (IR, GC, TLC). Acidity was measured prior to evaporation in aliquots filled up to equal volume.

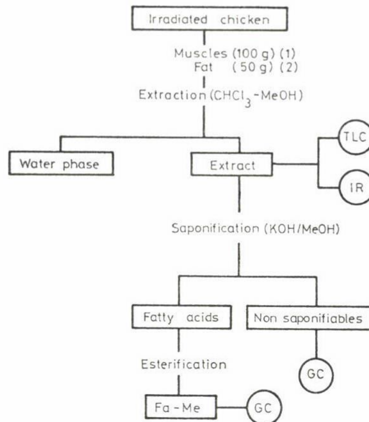

Fig. 1. Diagram of the processing of samples

Free fatty acids of the extracted lipids were measured by titration with alcoholic KOH solution, applying phenolphtalein indicator, in organic solvent according to the pertinent standard (HUNGARIAN STANDARD, 1981). Calculated from the amount of 0.05 mol KOH solution used, the free fatty acid content was given as oleic acid ($M_{C18:1} = 282$).

1.2.2. Thin-layer chromatography. The composition, purity and the more evident changes of the extracted lipids were studied by thin-layer chromatography. Kieselgel 60 (Merck) plates were run in a 80 : 20 : 2 mixture of petroleum ether–diethyl ether–acetic acid solvent system (MANGOLD & MALINS, 1960).

Reagents:

— Saturated $SbCl_3$ solution in chloroform (STAHL, 1969). This reagent gives a characteristic violet colour with sterols, but reacts also with every lipid containing unsaturated bond.

— One % solution of vanillin in alcohol containing 10% sulfuric acid (STAHL, 1969). The vanillin reagent is a developing agent of general use giving colour reaction with organic compounds.

After spraying with either of the two reagents the plates were heated to maximum colour intensity at about 70 °C.

1.2.3. Infrared spectrophotometry. The infrared spectrum of the lipid extracts was taken from their 4 and 20% solutions in carbon tetrachloride using a 0.61 mm NaCl cuvette. In the double lightpath UR-20 (Zeiss, Jena) instrument, the solvent was used as control. Light absorption of the lipid samples was measured along the whole spectrum in the wave number range of 500 cm^{-1} to 4600 cm^{-1}.

1.2.4. Gas chromatography. The fraction containing fats and other lipids obtained by extraction with a chloroform–methanol mixture was hydrolysed

according to the modified method of WACHS (1961). The fraction thus obtained containing fatty acids from glycerides and esters as well as originally free fatty acids is called the total fatty acid fraction (*S*). The other product of chemical fractionation contains the unsaponifiable compounds (hydrocarbons, sterols etc.) (*N*).

Fatty acids of long carbon chain, between C_8 and C_{20}, were determined as methyl esters (CHALVARDJAN, 1964) by capillary gas chromatography. The CS_2 solution of fatty acid methyl esters was chromatographed.

The column used was of 15 m length and 250 μm inner and 800–820 μm outer diameter Pyrex glass column, given a $BaCO_3$ basic treatment and packed with PEG-1000 (polyethylene glycol). Film thickness of the partition phase was 0.16 μm (GROB et al., 1977).

Chromatograms were taken at 180 °C under H_2 carrier gas, using a flame ionization detector (FID). Qualitative evaluation was carried out by comparing the retention times of standard fatty acid methyl esters or added standard methyl esters. For quantitative evaluation the peak regions were integrated and the distribution of individual fatty acids (relative %) was compared (integrator type: Spectra Physics Minigrator).

Changes of the unsaponifiable fraction containing hydrocarbons, sterols, alcohols of long carbon chain were followed also by gas chromatography. Measurements were carried out in capillary column with temperature programming. Length of glass capillary column: 16 m, inner diameter: 250 μm, partition phase: OV-101, film thickness: 0.16 μm, detector: FID ($t = 270$ °C). Temperature program: 50 °C–250 °C, 8 °C min^{-1}, carrier gas: H_2, integrator: Spectra Physics Minigrator.

The fingerprint-like chromatograms obtained from the samples were compared without exact identification of individual components.

The results were evaluated statistically.

$$s^2 = \frac{\sum (\bar{x} - x_i)^2}{n - 1}$$

s^2: value of variance
x : measured value
n: number of parallels

Significance was calculated with the help of Student's *t*-test.

$$\tilde{s}^2 = \frac{(n_1 - 1)\, s_1^2 + (n_2 - 1)\, s_2^2}{n_1 + n_2 - 2}$$

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\left(\frac{1}{n_1} + \frac{1}{n_2}\right)^{1/2} \tilde{s}}$$

On a $P = 0.05$ probability level the results are significant when $t \geq 2.78$.

2. Results

2.1. Characterization of the lipid extracts and their changes as a function of radiation dose and storage time

Each sample — 5 half chickens, 1000 g each — was separated into boneless meat and skin with adipose tissue, the former weighing 890 g and the latter 402 g on the average.

The average lipid content of the meat extracts amounted to 5.15% ± ± 0.5%, while that of the skin and adipose tissue to 42.3% ± 4.3%.

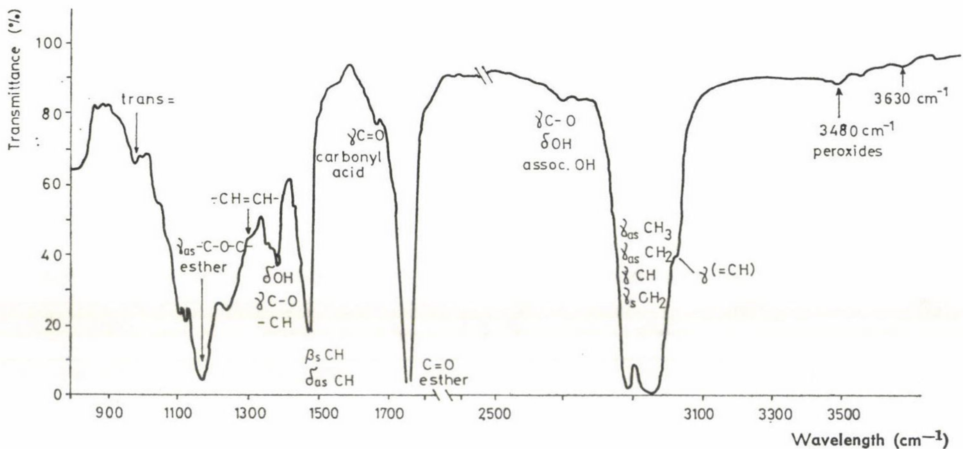

Fig. 2. Infrared spectrum of the lipid extract in 4% carbon tetrachloride solution from non-irradiated broiler meat (0.61 mm NaCl cuvette, control carbon tetrachloride)

Lipid extracts contained beside triglycerides as main components, small amounts of partial glycerides (di- and monoglycerides), free fatty acids, cholesterol and cholesterol ester detected by thin-layer chromatography. On the 2nd day after radiation treatment with 4 kGy no changes were observed in the meat samples, while in the meat samples treated with 50 KGy the quantity of free fatty acids and diglycerides increased. On the 30th and 105th day of storage both the radiation treated and the untreated samples showed a slightly increased free fatty acid and diglyceride content.

In the infrared spectrum of the lipid extracts (Fig. 2) differences were found between the samples at about 1670 cm^{-1} wave number corresponding to the vibration zone of carboxylic acid (C = 0) and at about 3500 cm^{-1} wave number. In quantitative analyses the 20% solutions of lipid extracts were compared.

The extinction values at 1670 cm^{-1} wave number characteristic of free carboxylic acids are shown in Table 1.

Table 1

Extinction values of lipid extracts obtained from the muscle tissue and the adipose tissue of broilers as measured in the absorption zone of free organic acids at 1670 cm^{-1} wave number in the infrared spectrum

(20% carbon tetrachloride solution, 0.61 mm cuvette)

a) muscle tissue

Storage time (day)	Dose (kGy)					
	0		4		50	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
2	0.091	0.0044	0.097	0.0089	0.106	0.0092
30	0.106	0.0125	0.106	0.0053		
150	0.106	0.0053	0.107	0.0095		

b) adipose tissue

Storage time (day)	Dose (kGy)					
	0		4		50	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
2	0.102	0.0089	0.132	0.0182	0.113	0.0061
20	0.114	0.0125	0.123	0.0118		
180	0.123	0.0202	0.127	0.0131		

Results of *t*-tests:

Time (day)	Dose (kGy)	*t*-values	
		muscle tissue	adipose tissue
2	0–4	1.05	2.56
2	0–50	2.55	1.77
30	0–4	0.00	0.91
180	0–4	0.16	0.29

In the case of muscle tissue a non-significant increase was found in the free fatty acids in the 4 kGy irradiated samples as compared to the untreated samples. During storage a similar increase was measured in the control samples, too. No difference between radiation treated and untreated meat samples was measured after 1 month storage. In the case of adipose tissue the difference is greater, thus equalization takes a longer time.

Peroxide content of meat lipid extract is not affected by irradiation with low doses but 50 kGy dose causes an increase (Table 2). In lipids obtained from adipose tissue peroxide content increases with increasing doses, however, a

Table 2

Extinction values of the peroxide compounds in lipid extracts obtained from the muscle tissue and adipose tissue of broilers as measured in the infrared spectrum at 3480 cm^{-1} wave number

(20% carbon tetrachloride solution, 0.61 mm NaCl cuvette)

a) muscle tissue

Storage time (day)	Dose (kGy)					
	0		4		50	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
2	0.071	0.0024	0.069	0.0024	0.082	0.0026
30	0.045	0.0056	0.045	0.026		
105	0.049	0.0020	0.048	0.0012		
150	0.057	0.0076	0.066	0.0036		

b) adipose tissue

Storage time (day)	Dose (kGy)					
	0		4		50	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
2	0.088	0.0066	0.100	0.0076	0.108	0.0062
30	0.095	0.0046	0.102	0.0095		
105	0.095	0.0030	0.092	0.0052		
150	0.102	0.0092	0.108	0.0044		

Results of t-tests:

Time (day)	Dose (kGy)	t-values	
		muscle tissue	adipose tissue
2	0–4	1.04	2.06
2	0–50	6.74	3.83
30	0–4	0.00	1.14
105	0–4	0.72	0.87
150	0–4	1.83	1.03

significant increase was measured in the case of 50 kGy dose. The difference between treated and untreated samples disappears during storage. This observation was substantiated by the results obtained by other peroxid-determination methods and also by sensory tests (KISS et al., 1986).

Changes in the KOH titrated acidity of the lipid extracts as a function of radiation dose and storage time are shown in Table **3**.

Table 3

Acidity of the lipid extract obtained from the muscle and adipose tissues of broilers as a function of radiation dose and storage time

(g oleic acid per 100 g lipid extract)

a) muscle tissue

Storage time (day)	Dose kGy)					
	0		4		50	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
2	2.3	0.30	2.4	0.23	4.6	0.61
30	4.2	0.75	5.6	0.92		
150	5.0	0.70	4.9	0.75		

b) adipose tissue

Storage time (day)	Dose (kGy)					
	0		4		50	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
2	1.9	0.23	2.3	0.26	4.2	0.10
30	3.0	0.46	2.5	0.30		
150	3.5	0.30	3.5	0.30		

Results of *t*-tests

Time (day)	Dose (kGy)	*t*-values	
		muscle tissue	adipose tissue
2	0–4	0.45	1.99
2	0–50	5.87	8.49
30	0–4	2.04	1.58
150	0–4	0.17	0.00

Four kGy which is the optimum radiation dose from the point of view of cell count reduction, does not cause significant increase in the case of meat, but a dose of 50 kGy results in a two-fold increase of the free fatty acid content. Free fatty acid content of adipose tissue increased significantly both upon 4 kGy and 50 kGy irradiation dose. During storage in both the control sample and the irradiated sample increases the free fatty acid content.

2.2. *Analysis of fatty acids obtained by saponification*

The alkaline hydrolysis of the lipid extracts splits off fatty acids from all the glyceride and ester type compounds. The hydrolyzed and the originally free fatty acids form the total fatty acid fraction. The quantity and the com-

Table 4

Quantity of total fatty acid fraction in the lipid extract from the muscle and adipose tissues of broilers as a function of radiation dose and storage time
(mg fatty acid per 100 mg lipid extract)

a) muscle tissue

Storage time (day)	Dose (kGy)					
	0		4		50	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
2	71.5	2.47	72.5	1.65	85.5	2.34
30	70.5	2.88	73.5	2.92		
105	77.5	2.53	80.0	2.81		

b) adipose tissue

Storage time (day)	Dose (kGy)					
	0		4		50	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
2	74.0	2.52	74.5	3.01	75.5	3.11
30	73.5	2.17	72.5	2.69		
105	76.5	2.00	75.5	2.80		

Results of *t*-tests

Time (day)	Dose (kGy)	*t*-values	
		muscle tissue	adipose tissue
2	0–4	0.58	0.22
2	0–50	7.11	1.03
30	0–4	1.27	0.50
105	0–4	1.15	0.50

position of the total fatty acid fraction is given in Table 4. The total fatty acid content extracted from meat increases slightly as an effect of irradiation with 4 kGy. The increase caused by 50 kGy is more substantial. In the case of adipose tissue changes upon radiation treatment are not significant either as a function of dose or of storage time.

The chromatogram of fatty acid methyl esters gained from muscle tissues upon treatment with 4 kGy are shown in Fig. 3. No substantial difference could be observed in the composition of meat fatty acids and that of adipose tissue concerning quality or quantity.

Irradiation did not cause qualitative changes in fatty acids; new peaks did not appear in the chromatograms.

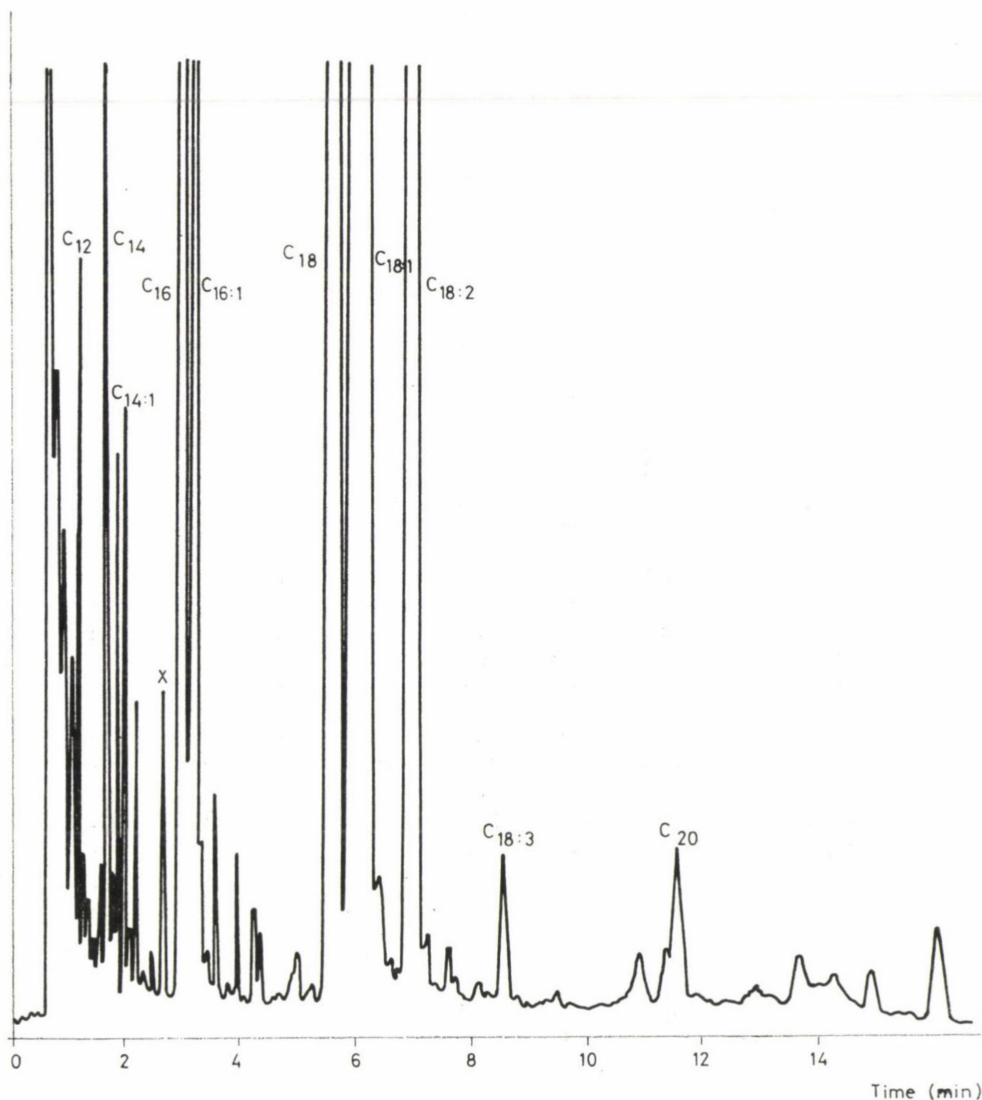

Fig. 3. Gas chromatogram of the fatty acid methyl esters extracted from irradiated (4 kGy) broiler muscle tissue (stored at −18 °C for 105 days)

Quantitative changes caused by radiation treatment and storage of muscle tissue are shown in Table 5. The changes in the case of adipose tissue are very similar to those of muscle tissue, so results are not published in detail.

On the second day after treatment with 4 kGy slight, non-significant changes were observed. Our results are in agreement with the results of LYASOVSKAYA and PIUB'SKAYA (1975) who investigated pork meat and found that 6 kGy dose had no effect on the quantitative composition of polyunsaturated

Table 5

Percentages of fatty acids obtained from the meat tissue of broilers as measured by gas chromatography as a function of radiation dose and storage time
(at −18 °C)

Fatty acid	Storage time (day) 2			30		105	
	Dose (kGy)						
	0	4	50	0	4	0	4
C_{10}	t	t	t	t	0.1	t	0.1
C_{12}	0.1	t	0.1	t	0.2	0.2	0.2
$C_{12:1}$	t	t	t	t	t	t	t
C_{13}	0.1	t	0.1	t	t	t	t
C_{14} x	1.2	1.1	0.8	0.8	0.9	0.9	0.7
$C_{14:1}$ x	0.6	0.4	0.3	0.3	0.3	0.2	0.2
$C_{14:1}$	0.4	0.4	0.2	0.1	0.4	0.2	0.4
C_{15}	0.2	0.2	0.2	0.1	t	0.2	0.3
Y	1.0	0.6	0.1	0.1	0.1	0.2	0.2
C_{16} x	32.4	32.6	28.3	28.9	29.6	27.2	27.2
$C_{16:1}$ xx	9.0	9.6	9.4	9.8	9.5	8.7	7.8
$C_{16:1}$	0.2	0.3	0.1	0.2	0.2	0.1	0.2
C_{17}	t	t	0.1	0.1	0.1	0.1	0.1
C_{18} x	6.4	6.9	2.9	6.0	6.1	6.8	6.9
$C_{18:1}$ xx	38.8	37.6	41.2	40.0	39.0	38.7	39.2
$C_{18:1}$	t	t	t	t	t	t	t
$C_{18:2}$ x	9.1	10.5	11.8	12.2	11.6	12.5	12.9
$C_{18:3}$ xx	t	t	0.2	0.2	0.3	0.2	0.2
$C_{18:3}$	t	t	0.6	t	0.4	0.5	0.5
C_{20}	—	—	0.1	0.2	0.1	0.1	0.2
$C_{over\ 20}$	—	—	—	0.2	0.2	0.4	0.5

x: isomers
t: trace amounts
Y: unidentified compound

fatty acids. Treatment with 50 kGy, on the other hand, caused more substantial changes. The distribution of the main components and the standard deviations 2 days after irradiation are illustrated in Fig. 4.

In the 50 kGy irradiation the percentage of palmitic and stearic acids was lower, while that of $C_{18:1}$ was higher then in the control. The relative percentage of $C_{18:2}$ was not significantly higher in meat but lower in fat. In the changes found in fatty acid composition, apart from the direct hydrolysis of triglycerides, an important role is played by other hydrolytic processes wich increase the breaking down of fatty acids bound in more stable bonds (e.g. in membranes). This is shown by the increase in the relative quantity of $C_{18:2}$ in

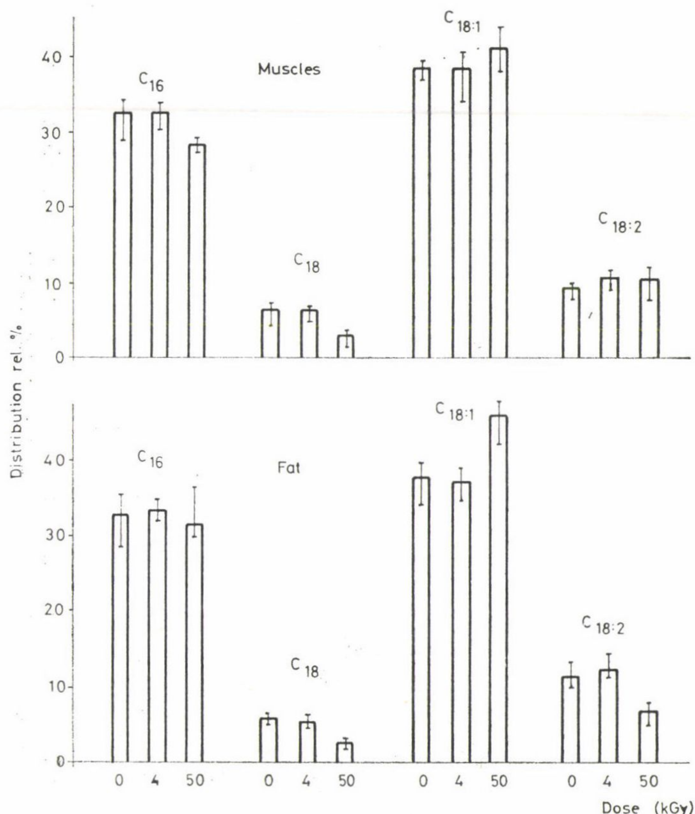

Fig. 4. Relative percentage of fatty acids of irradiated and untreated muscle tissue as measured by gas chromatography on the second day after treatment (stored at −18 °C)

the muscle tissue upon irradiation and storage. In the case of the free, easily extracted depot lipids of adipose tissues, changes are of different character. At the beginning the decrease caused by breaking down is more substantial than the increase as caused by the hydrolytic processes. In about a storage period of one month the differences in the control and the radiation treated samples disappear. The effect of an extremely high radiation dose (50 kGy) on the distribution of fatty acid components of herring was studied by ADAM and co-workers (1982). The authors did not find any significant changes in the amount of mono- and polyunsaturated fatty acids, however pure fatty acids were destroyed as an effect of 50 kGy dose.

2.3. Non-saponifiable compounds

The chromatogram obtained by capillary gas chromatography of the non-saponifiable fraction of the lipids extracted with a chloroform – methanol mixture is shown in Fig. 5.

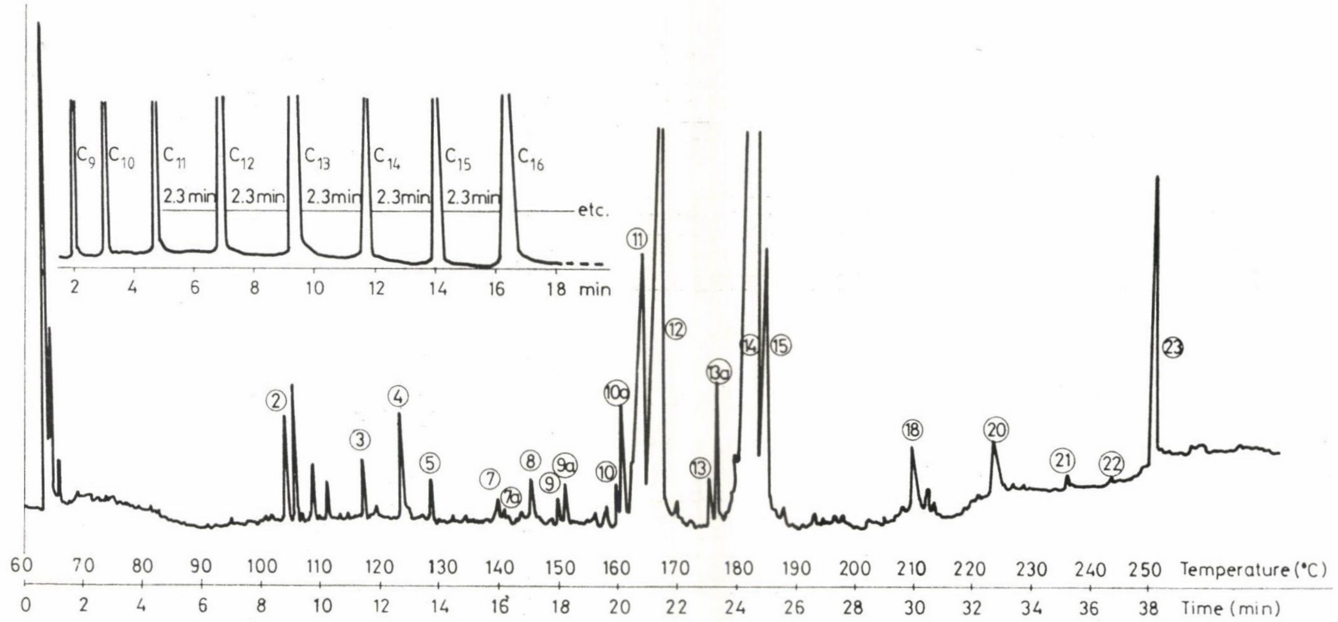

Fig. 5. Characteristic gas chromatogram of the unsaponifiable fraction obtained from the adipose tissue of broilers. For comparison the chromatogram of standard hydrocarbons is shown in the left top corner

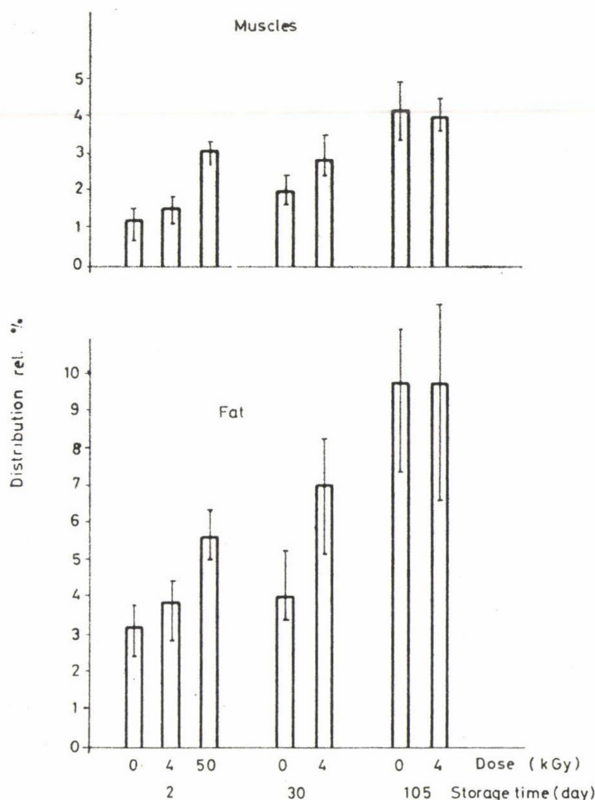

Fig. 6. Relative percentage of hydrocarbons with carbon chains shorter than C_{10} as a function of radiation dose and storage time

The results obtained for hydrocarbons of chains below C_{10} are shown in Fig. 6.

The values for the adipose tissue were significantly higher than those for the muscles which show that the character and extent of changes depends on the fat content of the original tissue and on the function of lipids.

In the case of hydrocarbons of short carbon chain the quantity increases as a function of both storage time and radiation dose. After 105 days of storage the quantity of short-chain hydrocarbons is the same in the control and in the radiation treated samples.

3. Conclusions

A satisfactory reduction in the cell count of frozen broilers and the elimination of salmonellae can be achieved by irradiation with 4 kGy. Qualitative and quantitative changes in the lipids caused by radiation treatment with the

above dose were investigated. The effects of an extremely high dose (50 kGy) were also studied.

The average fat content of the broilers examined was 8.6%. Changes in the muscle tissue and in the skin + adipose tissue were separately studied. On the second day after irradiation no difference was observed in the untreated samples and those treated with 4 kGy by TLC. In the samples treated with 50 kGy the chromatograms showed an increase in the amount of diglycerides and of free fatty acids. The latter change was manifested in the untreated samples during storage as well. As seen from the infrared spectra the quantity of free fatty acids increases upon irradiation to a minor extent, however, the increase measured in the untreated samples during storage is about the same. Peroxide compounds do not show increase upon treatment with 4 kGy, while they definitely increase upon treatment with 50 kGy. Acidity did not increase significantly upon irradiation with 4 kGy but increased upon storage and as an effect of 50 kGy dose.

As seen in the gas chromatograms 4 kGy did not cause any significant changes. Upon treatment with 50 kGy dose the percentage of both palmitic and stearic acid was lower than that in the control. The $C_{18:1}$ content was higher, while $C_{18:2}$ was nonsignificantly higher in meat, but lower in fat. During storage the importance of hydrolytic processes increased and they promoted the solubilization of fatty acids. In longer storage periods these processes lose significance and differences disappear. Hydrocarbon content of the non-saponifiable fraction depends on the radiation dose. Treatment with 50 kGy caused a substantial change.

Summing up the results it can be stated that the radiation dose of 4 kGy, suitable for the practice, did not cause the appearance of compounds that would not have been detectable in untreated samples. During storage similar lipidchemical changes occurred in both the treated and and in the untreated samples. This is in accordance with the statements of MERRITT (1972) who detected the same volatile compounds in irradiated chickens and in untreated samples. Certain changes, on the other hand, were eliminated during storage. This was proven by sensory tests as well (KISS et al., 1986). Quality and quantity of compounds formed depends apart from the radiation dose on the lipid content of the original material, on the quality of the lipids and on their function.

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Acta Alimentaria, Vol. 16 (2), pp. 129—142 (1987)

HIGH-PERFORMANCE LIQUID-CHROMATOGRAPHIC CONTROL OF THE STANDARD SPECIFICATION OF GROUND PAPRIKA

A. CS. PAVISA, F. HAJDU, Á. HOSCHKE, J. BODNÁR and P. BIACS

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

(Received: 5 November 1985; accepted: 8 April 1986)

Classification of commercially marketed ground paprika is based world-wide on the measurement of the overall pigment content as established by spectrophotometry subsequent to organic solvent extraction.

The measurable carotenoid content of ground paprika depends largely on the extracting solvent applied and the wavelength used for determination, thus, to avoid marketing problems it seemed expedient to study the conditions of determination as applied in the different countries (solvent used for extraction, wavelength applied, time of extraction). Investigations were carried out by high-performance liquid chromatography (HPLC) and spectrophotometry. Based on the results the use of a three-component solvent (e.g. chloroform–acetone–isopropanol, 2 : 1 : 1) is suggested for extraction instead of the pure apolar solvents generally applied. Determination is suggested to be made in the 500 to 510 nm wavelength region. The minimum extraction time was found to be 1 h.

Keywords: HPLC, ground paprika, spectrophotometry

The amount of pigments is considered by both the trade and the industry to be the most important parameter of the quality of ground paprika. The price of paprika is determined in many countries among them in Hungary, too, on the basis of this parameter.

The most simple method to establish the carotenoid content of ground paprika is the assessment of the pigment content directly from the extent of light reflection (LUKÁCS, 1982). The various spectrophotometric and chromatographic methods are more exact (ROSEBROOK, 1971; HUNGARIAN STANDARD, 1976; BARANYAI & SZABOLCS, 1976; LANGE, 1976; AOAC, 1980; BARANYAI et al., 1982). These permit of drawing conclusions not only on the amount of the total carotenoids but also on the amounts of individual carotenoid components.

In the classification of ground paprika it is a requirement all over the world to determine by spectrophotometry at the iso-absorption point or in its vicinity, the total carotenoid content. In a great number of countries the so called ASTA value, calculated on the basis of extraction in acetone, is accepted, but extraction with iso-propanol is also widely used. In Hungary benzene is the accepted extraction agent as specified in the relevant standard, however, petroleum ether b.p. 120 was also tested (ANDRE, 1973; HUNGARIAN STANDARD, 1976; WOODBURY, 1977; AOAC, 1980).

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

There is a great variation in the wavelength regions of the accepted methods in different countries (ASTA: 462 nm, Hungarian Standard: 477 nm, etc.). In consequence of this and the difference in extraction solvents it is possible that samples of the same carotenoid content are classified in one country in a lower category than in another country.

Whatever method is used to determine the pigment content the first most important step is to extract the pigment content in an organic solvent.

In selecting the appropriate solvent the structure of carotenoids (carotenoids are a highly variable pigment group consisting of 3–8 isoprenes containing conjugated double bonds; in paprika the major part of the pigment group is present bound to fatty acids of higher C content (C_{12}; C_{14})), their lipophilic character has to be borne in mind. Further, the 8–10% moisture content of marketed ground paprika has to be accounted for (SZABOLCS, 1979; BAUERFEIND, 1981). Therefore, it was presumed that to extract carotenoids it would be more expedient to use polar solvent mixtures instead of purely apolar solvent. However, carotenoids become decomposed to different degrees in different solvents and that has to be kept in mind, too, when selecting solvent.

Taking into consideration all the above points of view it was thought necessary to review the conditions of measurements (solvents, wavelength of the measurement, extraction time) to produce reproducible results of pigment analysis.

High-performance liquid chromatography and spectrophotometry was used in the investigations and the results were evaluated by mathematical statistical methods.

1. Materials and methods

1.1. Materials

The material used in the investigations was industrially produced ground paprika, an identified variety of cultivar Sz-20.

The following solvents of analytical grade were applied: carbon tetrachloride (Interkémia, Hungary), benzene (Interkémia, Hungary), petroleum ether b.p. 120 (Reanal, Hungary), methanol (Reanal, Hungary), acetone (Interkémia, Hungary), chloroform (Finomvegyszer Szövetkezet, Hungary), isopropanol (Reanal, Hungary) and their various mixtures.

1.2. Methods

1.2.1. Extraction. To establish the extracting capacity of the solvents the method described in HUNGARIAN STANDARD (1976) was applied with minor modifications. Samples of 0.5 g were extracted in 100 cm^3 solvent during 0.5, 1 and 1.5 h, respectively.

Extraction was carried out in the dark, under continuous shaking. The extracts were then dehydrated over Na_2SO_4, filtered and an aliquot of the solvent evaporated in an inert medium (N_2 gas, at 30 °C). The evaporated residue was dissolved in acetone and chromatographed by HPLC. The absorption spectra characteristic of the extracting capacity of individual solvents were taken with a double beam spectrophotometer (Specord M-40, GDR).

1.2.2. Separation of carotenoid compounds by HPLC technique. Systems of reversed phase and applying gradient elution (LANGE, 1976; BARANYAI et al., 1982) are extremely suitable to the analysis of hydrolysed compounds present in non-esterified form, but do not help much in the analysis of carotenoids present in paprika in the form of ester. These esters form about 80—90% of the red pigment. Thus, a HPLC technique was applied suitable to the determination of the esterified colour components in reversed phase system under isocratic conditions and thereby to detect the differences between individual solvents.

Analyses were carried out with a Labor MIM type Liquochrom 2010 apparatus, in an reversed phase system under isocratic conditions.

Column packed with 10 μm Chromsil C_{18} (Labor MIM, Hungary)
Column: 250 × 4.6 mm
Eluent: acetone - water (9 : 1)
Flow rate: 1.4 cm^3 min^{-1}
Detection at 438 nm

1.2.3. Identification of carotenoid pigments by thin-layer chromatography (TLC). The identification of the carotenoid compounds separated by HPLC technique was carried out by preparative thin-layer chromatography (VINKLER & KISZEL-RICHTER, 1972). Each carotenoid ester, separated on Kieselgel plates was hydrolyzed and the peaks of carotenoids freed of fatty acid were identified on the basis of their spectrum and their HPLC chromatogram. Identification of free (non-esterified) capsanthin and capsorubin was carried out by standards obtained from the University Medical School, Pécs.

2. Results

2.1. Absorption spectra characteristic of the extracting capacity of the individual solvents

In parallel to the detailed HPLC analyses the absorption spectra of the individual solvents, characteristic of the extracting capacity were studied, too. As seen in Tables 1 and 2 there is a substantial difference between the wavelengths of the red components at maximal light absorption (λ_{max}) values

Table 1

Absorbancy at 510 nm

2. Benzene	0.398 ± 0.008
3. Petroleum ether	0.399 ± 0.006
4. Chloroform	0.375 ± 0.008
5. Acetone	0.440 ± 0.004
6. Isopropanol	0.408 ± 0.009
8. Chloroform–methanol (2 : 1)	0.428 ± 0.006
9. Chloroform–acetone (1 : 1)	0.418 ± 0.007
10. Chloroform–isopropanol (1 : 1)	0.408 ± 0.004
11. Acetone–isopropanol (1 : 1)	0.422 ± 0.008
Chloroform–acetone–isopropanol	
12. 1 : 1 : 1	0.384 ±0.007
14. 5 : 4 : 1	0.390 ± 0.006
13. 2 : 1 : 1	0.412 ± 0.006
15. 1 : 2 : 1	0.422 ± 0.007
16. 1 : 1 : 2	0.410 ± 0.007

Instrument: Specord M-40 (GDR)

and the maximum absorption values in various solvents (509–520 nm and 0.375–0.440, respectively). The absorption values characteristic of the extracting capacity of individual solvents were examined at 510 nm. It was found that the absorption value of carotenoids dissolved in acetone is prominent (0.440), of those dissolved in solvent mixtures of two or three components is about the same (0.410–0.420) and those dissolved in benzene, chloroform and petroleum ether 120, very low (0.375–0.398).

Table 2

Maximum absorbancy of the red components

	λ (nm)
2. Benzene	518
3. Petroleum ether	509
4. Chloroform	518
5. Acetone	509
6. Isopropanol	510
8. Chloroform–methanol (2 : 1)	516
9. Chloroform–acetone (1 : 1)	510
10. Chloroform–isopropanol (1 : 1)	510
11. Acetone–isopropanol (1 : 1)	510
Chloroform–acetone–isopropanol	
12. 1 : 1 : 1	510
14. 5 : 4 : 1	516
13. 2 : 1 : 1	510
15. 1 : 2 : 1	510
16. 1 : 1 : 2	510

Standard error: ± 2 nm
Instrument: Specord M-40 (GDR)

2.2. Characterization of the extracting capacity of individual solvents by the HPLC technique

The solubility of individual pigment components as affected by the different solvents is summarized in Tables 3, 4 and 5 and Figs. 1, 2 and 3.

In studying the solubility of individual components the distribution by quantity of free capsanthin, free capsorubin and their esters were accounted for as functions of individual solvents. (When evaluating the chromatograms chloroform was considered as inner standard because its extracting capacity proved to be constant during extraction periods of 0.5, 1 and 1.5 h, respectively.)

2.2.1. Analysis of pure solvents. Based on the HPLC chromatograms taken after 30 min extraction as specified in the relevant Hungarian Standard great differences were found in the carotenoid dissolving capacity of individual solvents (Table 3, Fig. 1). Taking into account the relative precentage composition of the total and the total red components the almost identical extracting capacity of chloroform and isopropanol could be established, while the dissolving capacity of acetone was found to be lower. (As seen in Fig. 1, free capsanthin dissolves better in chloroform than in acetone.)

To compare the extracting capacity of benzene as specified for use in the Hungarian Standard and of acetone, internationally accepted (related to the total red components) mathematical statistical evaluation was applied. The extracting capacity of the two solvents, based on t test, differed at the $P = 1\%$ probability level ($t = 4.61$). The total pigment content as dissolved in petroleum ether was found relatively high (Table 3) but the proportion of red components was very low (52.4%). The amount of red pigments in the highly apolar CCl_4 and in benzene was found very low, too (37.9 and 49.7%, respectively).

In Fig. 4 and Table 6 the carotenoid dissolving capacity of benzene, acetone chloroform and isopropanol, showing good dissolving capacity already in the first 30 min, was compared at increasing extraction time increased only the extracting capacity of benzene which had a very low capacity in the first 30 min. The dissolving capacity of acetone increased only slightly, while that of chloroform and isopropanol did not change at all.

2.2.2. Analysis of solvent mixtures of 2 and 3 components. Because of the reasons as mentioned in the introduction (ground paprika is a heterogeneous system where the highly lipophilic carotenoids, due to their structure polar to different degrees, have to get through a hydrophilic medium because of the moisture content in the ground paprika) it was found expedient to study the extracting capacity of a highly apolar (CCl_4) solvent of very low dissolving capacity on adding methanol and thereby increasing its polarity (CCl_4–MeOH, 2 : 1) (Fig. 1, Table 3).

Table 3

Correlation between the extracting capacity of individual solvents and pigment content

Solvent	Total pigment (%)		Total red pigment (%)		Total capsanthin (%)		Total capsorubin (%)		Free capsanthin (%)		Free capsorubin (%)		Total capsanthin ester (%)		Total capsorubin ester (%)	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Carbon tetrachloride	47.5	± 4.4	37.8	± 1.7	30.7	± 1.3	7.1	± 0.32	8.0	± 0.45	2.1	± 0.18	22.7	± 1.9	4.9	± 0.25
Benzene	58.8	± 4.2	49.7	± 5.3	40.7	± 2.0	9.0	± 0.42	11.4	± 0.52	3.0	± 0.21	29.2	± 1.8	5.9	± 0.30
Petroleum ether	81.7	± 5.8	52.3	± 2.6	42.7	± 1.8	9.6	± 0.4	7.6	± 0.36	2.7	± 0.30	35.1	± 1.7	6.9	± 0.40
Chloroform	100.0	± 4.5	72.9	± 3.4	60.3	± 2.6	12.2	± 0.56	18.5	± 0.70	3.9	± 0.28	42.0	± 2.1	8.3	± 0.35
Acetone	77.5	± 3.4	64.6	± 1.8	54.0	± 2.3	10.6	± 0.51	13.3	± 0.60	4.0	± 0.22	40.7	± 2.3	6.5	± 0.26
Isopropanol	100.0	± 4.6	70.8	± 2.2	59.8	± 2.4	11.0	± 0.58	17.1	± 0.90	3.3	± 0.19	42.7	± 2.2	7.7	± 0.32
CCl_4–methanol (2 : 1)	63.9	± 5.5	49.3	± 2.6	40.7	± 1.5	8.6	± 0.36	13.0	± 0.58	3.4	± 0.22	27.6	± 1.6	5.1	± 0.30
Chloroform–acetone–isopropanol (2 : 1 : 1)	120.0	± 4.4	87.6	± 3.0	72.5	± 3.2	15.0	± 0.7	23.5	± 0.98	5.9	± 0.31	49.4	± 2.8	9.1	± 0.50

Extraction period: 0.5 h; $\bar{x}$: mean value of three measurements; s: standard deviation

Table 4

Correlation between the extracting capacity of individual solvents and the pigment content

Solvent	Total pigment (%)		Total red pigment (%)		Total capsanthin (%)		Total capsorubin (%)		Free capsanthin (%)		Free capsorubin (%)		Total capsanthin ester (%)		Total capsorubin ester (%)	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
Carbon tetrachloride	84.4	± 4.2	58.9	± 2.0	47.8	± 2.7	11.0	± 0.48	14.8	± 0.46	3.7	± 0.26	33.0	± 2.1	7.3	± 0.32
Benzene	91.0	± 4.5	62.6	± 1.6	52.3	± 2.4	10.3	± 0.51	17.9	± 0.72	4.1	± 0.20	34.3	± 1.5	6.1	± 0.28
Chloroform	100.0	± 4.8	71.7	± 2.3	58.9	± 3.0	12.8	± 0.50	21.3	± 0.88	5.3	± 0.23	37.5	± 0.90	7.4	± 0.31
Acetone	90.0	± 4.0	70.0	± 1.9	57.7	± 2.10	12.2	± 0.56	21.9	± 0.78	5.2	± 0.22	35.8	± 1.0	7.0	± 0.30
Isopropanol	94.1	± 4.9	67.5	± 1.7	55.1	± 2.8	12.3	± 0.49	20.7	± 0.89	4.7	± 0.24	34.3	± 1.80	7.6	± 0.29
Chloroform–methanol (2 : 1)	112.4	± 4.6	80.8	± 2.6	67.5	± 3.2	13.3	± 0.68	23.9	± 0.91	5.4	± 0.31	43.6	± 1.8	7.8	± 0.30
Chloroform–acetone–isopropanol (2 : 1 : 1)	123.1	± 4.9	86.9	± 1.8	71.5	± 3.1	15.4	± 0.30	22.0	± 0.92	5.8	± 0.29	49.4	± 2.1	9.5	± 0.40

Extraction period: 1 h; $\bar{x}$: mean value of three measurements; s: standard deviation

Table 5

Correlation between the extracting capacity of individual solvents and the pigment content

Solvent	Total pigment (%)	Total red pigment (%)	Total capsanthin (%)	Total capsorubin (%)	Free capsanthin (%)	Free capsorubin (%)	Total capsanthin ester (%)	Total capsorubin ester (%)
	x̄ s	x̄ s	x̄ s	x̄ s	x̄ s	x̄ s	x̄ s	x̄ s
Benzene	90.7 ± 3.6	67.7 ± 2.3	55.3 ± 1.9	12.4 ± 0.36	22.0 ± 0.8	5.2 ± 0.23	33.2 ± 1.8	7.1 ± 0.23
Petroleum ether	86.8 ± 3.9	66.7 ± 2.8	53.1 ± 1.8	13.6 ± 0.40	15.3 ± 0.56	4.9 ± 0.22	37.7 ± 1.5	8.7 ± 0.18
Chloroform	100.0 ± 4.5	75.3 ± 3.1	61.3 ± 2.5	13.9 ± 0.62	20.0 ± 0.81	5.3 ± 0.30	41.3 ± 1.6	8.6 ± 0.26
Acetone	82.9 ± 3.8	61.7 ± 2.9	49.7 ± 1.8	12.0 ± 0.71	19.0 ± 0.76	5.4 ± 0.29	30.6 ± 1.9	6.5 ± 0.25
Isopropanol	94.2 ± 4.6	69.1 ± 3.0	55.4 ± 2.0	13.6 ± 0.36	19.9 ± 0.70	5.5 ± 0.19	35.4 ± 1.2	8.1 ± 0.24
Chloroform–methanol (2 : 1)	95.4 ± 4.2	75.8 ± 2.4	62.6 ± 2.4	13.2 ± 0.35	25.9 ± 0.66	6.3 ± 0.28	36.7 ± 1.7	6.8 ± 0.22
Chloroform–acetone (1 : 1)	107.3 ± 4.6	82.0 ± 3.9	68.5 ± 2.7	13.4 ± 3.1	23.6 ± 0.66	5.5 ± 0.33	44.9 ± 2.4	7.9 ± 0.35
Chloroform–isopropanol (1 : 1)	105.1 ± 4.9	87.8 ± 3.8	73.3 ± 2.6	14.5 ± 0.30	22.6 ± 0.7	5.1 ± 0.28	50.6 ± 2.0	9.4 ± 0.40
Acetone–isopropanol (1 : 1)	98.4 ± 3.4	79.9 ± 3.5	65.1 ± 2.4	14.7 ± 0.5	24.1 ± 0.88	4.9 ± 0.19	41.0 ± 1.9	9.8 ± 0.41
Chloroform–acetone–iso-propanol								
1 : 1 : 1	94.8 ± 4.2	72.2 ± 3.1	59.2 ± 2.6	13.0 ± 0.52	25.1 ± 0.8	5.4 ± 0.27	34.1 ± 1.3	7.5 ± 0.30
2 : 1 : 1	114.2 ± 5.1	91.4 ± 2.7	76.8 ± 3.0	14.6 ± 0.60	26.3 ± 0.81	5.6 ± 0.16	50.4 ± 2.2	9.0 ± 0.35
5 : 4 : 1	88.8 ± 4.6	69.5 ± 2.8	56.7 ± 2.3	12.7 ± 0.41	20.3 ± 0.70	4.8 ± 0.30	36.4 ± 1.7	7.9 ± 0.26
1 : 2 : 1	106.0 ± 4.0	82.5 ± 3.5	68.9 ± 3.1	13.6 ± 0.3	24.2 ± 0.88	5.3 ± 0.20	44.7 ± 2.3	8.2 ± 0.20
1 : 1 : 2	91.8 ± 3.9	70.0 ± 3.6	56.0 ± 2.9	14.0 ± 0.30	20.9 ± 0.76	5.4 ± 0.30	35.0 ± 1.3	8.6 ± 0.30

Extraction period: 0.5 h; $\bar{x}$: meen value of 3 measurements; s: standard deviation

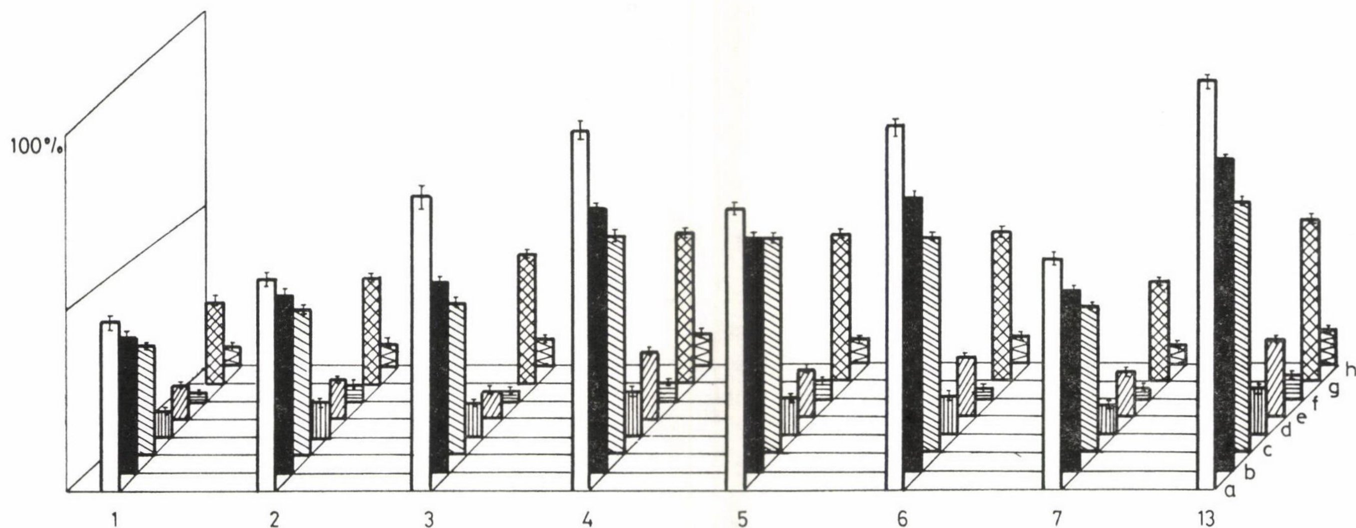

Fig. 1. Correlation between the extracting capacity of individual solvents and the pigment content 1. Carbon tetrachloride; 2. Benzene; 3. Petroleum ether 120; 4. Chloroform; 5. Acetone; 6. Isopropanol; 7. CCl_4–methanol (2 : 1); 13. Chloroform–acetone–isopropanol (2 : 1 : 1) a: total pigment content; b: total red components; c: total capsanthin (free capsanthin and capsanthin esters); e: free capsanthin (non-esterified); f: capsanthin esters; g: free capsorubin; h: capsorubin esters; extraction time: 0.5 h

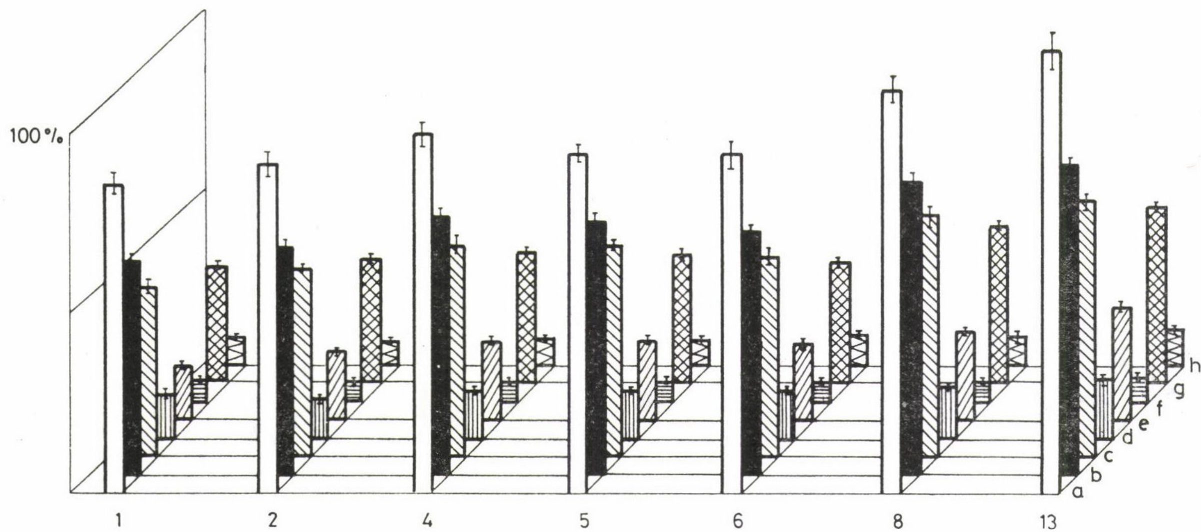

Fig. 2. Correlation between the extracting capacity of individual solvents and the pigment content 1. Carbon tetrachloride; 2. Benzene; 4. Chloroform; 5. Acetone; 6. Isopropanol; 8. Chloroform–methanol (2 : 1); 13. Chloroform–acetone–isopropanol (2 : 1 : 1) a: total pigment content; b: total red components; c: total capsanthin (free capsanthin and capsanthin esters); d: total capsorubin (free capsorubin and capsorubin esters); e: free capsanthin (non-esterified); f: capsanthin esters; g: free capsorubin; h: capsorubin esters; extraction period: 1 h

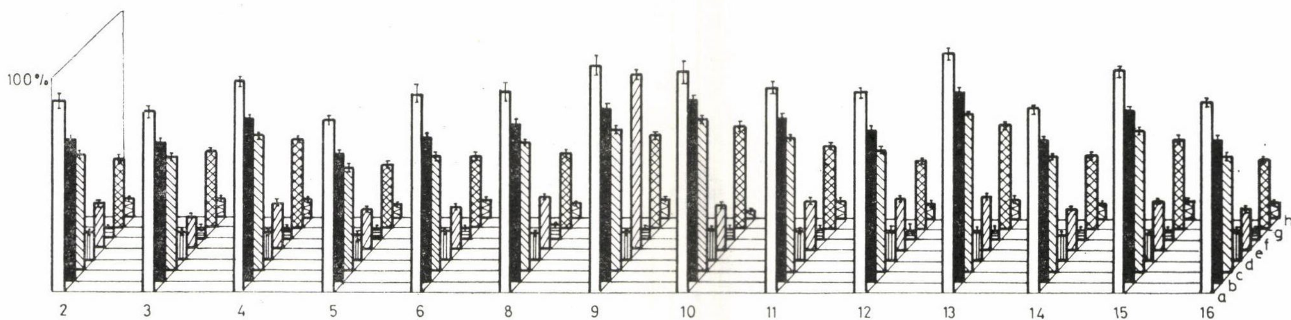

Fig. 3. Correlation between the extracting capacity of individual solvents and the pigment content. 2. Benzene; 3. Petroleum ether; 4. Chloroform; 5. Acetone; 6. Isopropanol; 8. Chloroform–methanol (2 : 1); 9. Chloroform–acetone (1 : 1); 10. Chloroform–isopropanol (1 : 1); 11. Acetone–isopropanol (1 : 1); 12. Chloroform–acetone–isopropanol (1 : 1 : 1); 13. Chloroform–acetone–isopropanol (2 : 1 : 1); 14. Chloroform–acetone–isopropanol (5 : 4 : 1); 15. Chloroform–acetone–isopropanol (1 : 2 : 1); 16. Chloroform–acetone–isopropanol (1 : 1 : 2) a: total pigment content, b: total red components, c: total capsanthin (free capsanthin and capsanthin esters), d: total capsorubin (free capsorubin and capsorubin esters), e: free capsanthin (non-esterified), f: capsanthin esters, g: free capsorubin h: capsorubin esters. Extraction period: 1.5 h

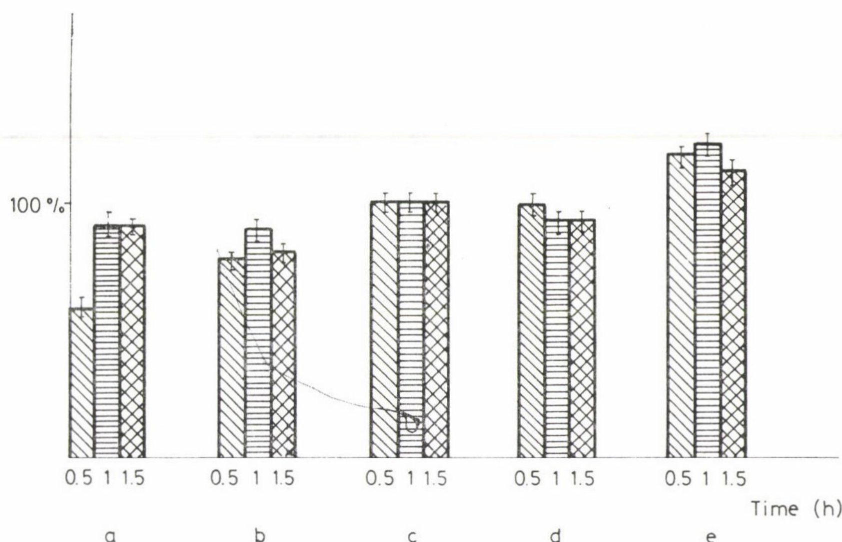

Fig. 4. Correlation between the extracting capacity of some solvents and the extraction period. a: Benzene; b: acetone; c: chloroform; d: isopropanol; e: chloroform–acetone–isopropanol (2 : 1 : 1)

Table 6

Correlation between the extracting capacity of some solvents and the extraction period

Solvent	Extraction period (h) 0,5 — Total pigment content (%) $\bar{x}$ s	1 — $\bar{x}$ s	1,5 — $\bar{x}$ s
Benzene	58.8 ± 4.2	91.0 ± 4.5	90.7 ± 3.6
Acetone	77.5 ± 3.4	90.0 ± 4.0	82.9 ± 3.8
Chloroform	100.0 ± 4.5	100.0 ± 4.8	100.0 ± 4.5
Isopropanol	100.0 ± 4.6	94.1 ± 4.9	94.2 ± 4.6
Chloroform–acetone–isopropanol 2 : 1 : 1	120.0 ± 4.4	123.1 ± 4.9	114.2 ± 5.1

$\bar{x}$: mean value of 3 measurements; s: standard deviation

Comparing by test the extracting capacity of CCl_4 with that of the CCl_4–MeOH, 2 : 1 system in relation to the total red pigment a difference was found between them at P = 1% probability level ($t = 6.38$, degree of freedom = 4).

On examining pure solvents it was found that chloroform, acetone and isopropanol have the highest and fastest dissolving capacity among the solvents tested thus these solvents were used in the mixtures of 2 and 3 components (Fig. 5).

In Figs. 2 and 3 as well as in Tables 4 and 5, it can be seen that both, the 2- and 3-component solvent mixtures were of very high extracting capacity in

accordance with expectations. The mixture chloroform–acetone–isopropanol (2 : 1 : 1) was found particularly good. The dissolving capacity of this mixture was compared by t test with the extracting capacity of benzene or acetone. The mixture was found significantly better than the other two solvents at 1 h and 1.5 h extraction periods.

During 1 h extraction period at the probability level of $P = 0.1\%$, during 1.5 h at $P = 1\%$ level differed the extracting capacity of benzene or acetone from that of the 3-component mixture. (On comparing the extracting capacity of benzene and the solvent mixture $t_{1n} = 20.12$ and $t_{1.5} = 5.56$ on comparing acetone and the mixture $t_{1n} = 13.63$ and $t_{1.5} = 5.61$.)

The observation related to chloroform and isopropanol as described in chapter 2.2.1. was found valid in relation to the chloroform–acetone–isopropanol (2 : 1 : 1) mixture, too, in as much as the very high dissolving capacity found in the first half hour did not change during 1 h or 1.5 h extraction period (Fig. 4, Table 6).

2.2.3. Comparison of the results obtained by spectrophotometry and HPLC technique. From Tables 1, 2, 5 and Fig. 3 it is evident that the results of the two methods differ substantially.

As it can be seen, pigments dissolved in acetone show a very high absorption while as regards the solubility of individual components acetone is not the best solvent (isopropanol, chloroform and two-component and three component mixtures studied gave higher absorption values).

A similar phenomenon may be observed in the case of chloroform. The absorbance obtained in chloroform at 510 nm is the smallest (0.375) at the same time its extracting capacity as judged by the HPLC chromatogram of a 1.5 h extract is almost identical with that of the 2- and 3-component mixtures (Fig. 3).

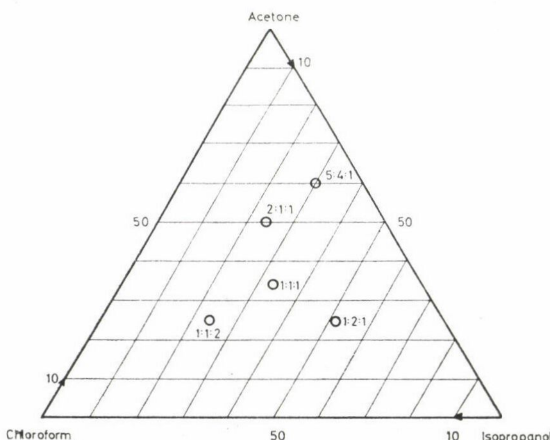

Fig. 5. Analysis of the composition of the extraction mixture

3. Conclusion

The measurable carotenoid content of ground paprika is a function of the solvent used and of the measuring range applied, therefore the aim of this study was to examine the conditions of measurement.

The measurements have shown the results obtained by spectrophotometry and high-performance liquid chromatography to differ substantially. As shown by the spectra (Tables 1 and 2) the absorption of the pigments dissolved in acetone is higher than in any other solvent or solvent mixture studied. At the same time the HPLC chromatograms show that the extraction of acetone is lower than that of chloroform or isopropanol or the solvent mixtures used. This phenomenon can be explained by the circumstance that during the processing of paprika oxidation procedures occur as a result of which the pigments in the final product are not identical with the native components (PHILIP & FRANCIS, 1971). These components and the yellow xanthophylls accompanying the red pigments have different effect on the absorbancy in different solvents. However, the fact that carotenoids are more apt to decompose in acetone cannot be overlooked either. In the evaluation of the HPLC chromatograms only the red xanthophyll components present in the native state were taken into account (Tables 3, 4 and 5).

The measuring range of the generally accepted methods is 460–480 nm while the wavelength showing maximum absorption of the red components is above 500 nm. Thus, it does not seem expedient to measure the total pigment content in a lower wavelength range, particularly in case the amount of the red pigments is of main interest.

In the course of the investigation it was established that during the 30 min extraction period as specified in the HUNGARIAN STANDARD (1976) the highly apolar solvents (CCl_4, benzene, petroleum ether) dissolved a very low level of the red components. In the opinion of the authors at least 1 h extraction period is necessary to approximate the dissolving capacity of chloroform or 2- and 3-component solvent mixtures (Table 6). (In relation to benzene it has to be kept in mind, too, that the mobility of its electrons may contribute to the anyway rapid autooxidation of the carotenoids.)

The 2- and 3-component solvent mixtures were found to extract every pigment group more efficiently than pure, apolar solvents. The extracting capacity of the chloroform–acetone–isopropanol (2 : 1 : 1) mixture is particularly outstanding.

The results are in accordance with the fact that ground paprika is a heterogeneous system containing appr. 10% moisture and thus the lipophil carotenoids (which can be of different polarity depending on the degree of their esterification) are extracted from a medium, to some extent hydrophilic (BAUERFEIND, 1981).

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Acta Alimentaria, Vol. 16 (2), pp. 143—153 (1987)

STUDIES ON PECTIN DEGRADATION

W.H. VAN DEVENTER-SCHRIEMER and W. PILNIK

Agricultural University, Department of Food Science, De Dreijen 12, 6703 BC Wageningen. The Netherlands

(Received: 1 November 1985; accepted: 6 May 1986)

Pectin can be degraded chemically, enzymatically and mechanically. Various depolymerized pectin preparations were prepared and some of their properties are described. Gel filtration chromatography of the degradation products shows the statistical nature of the degradation and correlates with the viscosity average molecular weight. There is a good correlation with gelling strength of the pectin preparations and it appears that breaking strength of gels is more strongly affected by degradation than elasticity.

Keywords: pectin, gelling strength, gel filtration chromatography, degradiation, viscosity

The food technologist is interested in such studies because the functionality of pectin as food additive depends to a large extent on molecular weight. Degradation is a means to compare properties of pectin preparations which only differ in molecular weight. The properties considered in this paper are viscosity, gel-chromatographic behaviour, ion-exchange-chromatographic behaviour and gelling strength. Molecular weight reduction can be achieved by the action of enzymes, by chemical agents and by mechanolysis. When using enzymes a statistical distribution of the molecular weight is only maintained if the specificity of pectindepolymerases is taken into account (PILNIK et al., 1973; PILNIK & ROMBOUTS, 1981).

Endo-polygalacturonase can be used on pectic acid and low esterified pectin; the enzyme is easily obtained as the sole pectolytic activity in cultures of the yeast *Kluyveromyces fragilis* and there is a commercial fungal preparation which is quite pure (Rohament P, Röhm, Darmstadt, FRG).

Endo-pectate lyase can be used on pectic acid and on low methoxyl pectin up to 40% degree of esterification (DE). This enzyme is also easily obtained as sole pectolytic activity from various bacteria.

Pectin lyase needs a very highly esterified pectin as substrate (DE > 70). The enzyme is not easily obtained; it must be isolated from *Aspergillus spp.* cultures or from commercial fungal pectinase preparation in which it occurs together with polygalacturonase and pectinmethylesterase.

Acid hydrolysis gives a statistical depolymerization of pectins of any degree of esterification. However, it always proceeds together with deesterification.

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

Hydroxyl-ion catalyzed pectin depolymerization by β-eliminative splitting of glycosidic linkages next to ester groups (ALBERSHEIM et al., 1960) proceeds statistically with high ester pectin and conditions can be found near neutrality under which no deesterification occurs.

For laboratory experiments the enzymic methods have the disadvantage that only small quantities are obtained unless inconveniently large volumes are used for the reaction and the working-up of the products by alcohol precipitation. The situation is better for the β-elimination which can be carried out in alcoholic suspension.

The most convenient method is mechanolysis (DONGOWSKI, 1970), in which pectin is hydrolyzed in the dry state by mechanical action in a vibrating mill. Quantities sufficient for product consuming gelling experiments can be obtained and the extent of degradation to be achieved is not limited by the requirements of working-up procedures. Pectin of any degree of esterification can be treated without risk of deesterification.

1. Materials and analytical methods

Apple pectins were obtained from Messrs Obipectin Ltd., Bischofszell, Switzerland:

— Purple ribbon pectin DE 34.1%, polyuronide content 87.0%;
— Green ribbon pectin DE 62.2%, polyuronide content 86.6%;
— Brown ribbon pectin DE 72.8%, polyuronide content 84.3%.

DE and uronide content were determined by the titration method (FOOD CHEMICALS CODEX II, 1972) after washing with acid alcohol and neutral alcohol and drying over night in air. Intrinsic viscosity $[\eta]$ was obtained by extrapolating inherent viscosities to zero concentration. Specific viscosities were measured in an Ubbelohde glass viscosimeter at 30 °C and expressed as $(t_s\text{-}t_0)$ per $(t_0 \cdot c)$ where t_s is the flow time of the solution, t_0 is the flow time of the solvent (> 70 s) and c is the concentration in g polygalacturonide per 100 cm³. Measurements were done in a 0.1 mol l^{-1} tris-succinate buffer pH 6.0 with 0.01% thiomersal. The molarity refers to succinic acid; dry tris is added to obtain the desired pH value. This buffer was chosen in preference to others given in literature because solutions showed no change in viscosity during one day at room temperature and several days storage under refrigeration. Thus, six measurements at 0.15 g 100 cm^{-3} concentration spread over 18 hours gave a standard deviation of 0.036 for an arithmetic mean of 6.993. The standard deviation for the same pectin at 0.05 g 100 cm^{-3} concentration was 0.055 for 5.69 as arithmetic mean. Straight lines were obtained in all cases (Fig. 4) which can be expressed as $y = ax + b$. The b is the intrinsic viscosity from which viscosity average molecular weights $\overline{M}v$ were calculated according to OWENS

and co-workers (1946):

$$[\eta] = 1.4 \times 10^{-6} \overline{M}v^{1.34}$$

The Huggins factor was calculated from the equation $K' = $ a per b^2. Optical density was measured at 232 nm at a concentration of 2.5 mg polyuronide per cm^3 distilled water against water. For pectin depolymerized by chemical elimination a molar extinction coefficient of 5500 mol^{-1} cm^{-1} was used (EDSTROM & PHAFF, 1961) and for pectin depolymerized by pectate lyase a molar extinction coefficient of 4800 mol^{-1} cm^{-1} (MACMILLAN & VAUGHN, 1964). From the increase in optical density and the degree of polymerization of the starting material determined by viscosity, the DP of depolymerized pectins was calculated according to ROMBOUTS and co-workers (1970). Multiplication with the average molecular weight of the polyuronide monomer anhydride gives the number of average molecular weight $\overline{M}n$. Gel filtration was done over Sephacryl S 300 (Pharmacia) in LKB columns 2137 (2.6×95 cm) with 5 mg in 0.5 cm^3 tris-succinate buffer pH 6 using 200 cm^3 of the same buffer for elution. The fractions (1.9 cm^3) were analyzed by an automated colorimetric carbazole assay (van DEVENTER-SCHRIEMER & PILNIK, 1976). Sephadex S 500 was also used under the same conditions, but with a smaller column (1.1×60 cm). Ion-exchange chromatography was done over DEAE cellulose columns according to ANGER and DONGOWSKI (1984). Sugar-acid-pectin test gels (400 g) were prepared from brown and green ribbon pectin (65^0 Brix, pH 2.2) and calcium pectinate test gels (300 g) were prepared from purple ribbon pectin (30^0 Brix; 25 mg calcium ions as calcium chloride per 100 g gel, pH 3.0) both according to the FOOD CHEMICALS CODEX II (1972), except for the weight of the gels. Both types of gels were measured with the ridgelimeter (an elasticity measurement) allowing calculations of "jelly grade" of the brown and green ribbon pectin and of "gel power" of the purple ribbon pectin. Jelly grade determination show excellent repeatability. A typical series of 7 gels boiled at various times during one week of a pure brown ribbon pectin gave an average jelly grade of 180.4 with a standard deviation of 3.1. After measuring, the intact gels were placed in a cylindrical extrusion cell of the Allo-Kramer Shear Press (KRAMER & HAWBECKER, 1966) and extruded at a preset piston speed. The surface under the time-force curve recorded during extrusion is a measure of the internal strength of the gels.

2. Experiments and discussion

2.1. Degradation by pectate lyase

The enzyme used was a preparation isolated from *Pseudomonas fluorescens* GK-5 in our laboratory which has a pH optimum at 9.4 and an absolute requirement for calcium ions (ROMBOUTS et al., 1978). The reaction is easily

followed by viscosity and by measuring the extinction at 232 nm, as C5-C4 double bonds are formed by the elimination reaction at the new non-reducing ends. A 1% solution of purple ribbon pectin was prepared in a 0.1 mol pH 7 Tris-succinate buffer solution by mixing a carefully neutralized 2% aqueous pectin solution 1:1 with a 0.2 mol l^{-1} buffer solution. The final solution further contained 0.5 mmol calcium ions (as calcium chloride) per 1000 cm^3. Three hundred units of pectate lyase were added to 5000 cm^3 pectin solution and the reaction mixture was kept at room temperature. On the basis of decrease in viscosity and increase in E 232 measured in 1 : 10 dilution three batches of dry pectin were prepared by adding the double volume of acidified ethanol (70% w/w containing 10% v/v conc. hydrochloric acid), filtering the precipitate through a Büchner funnel with a coarse filter paper (S & S 520 b), washing chloride free with 70% ethanol and finally once with 96% ethanol and once with acetone. For washing the preparations were transferred from the filter into a beaker with enough alcohol to allow thorough stirring by magnetic stirrer. After 10 min stirring the preparations were filtered again. After drying in air over night the pectin preparations were ground in a Culatti-mill (0.7 mm screen). The untreated sample was prepared in the same way without enzyme addition. The properties of these pectins are presented in Table 1. Not shown are DE's which did not change. The depolymerization is also demonstrated by

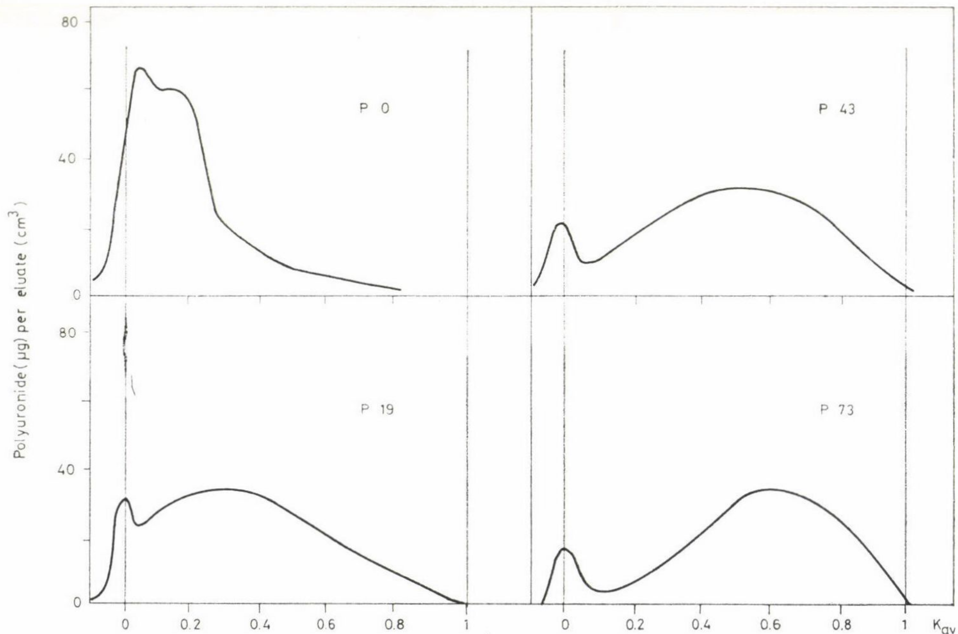

Fig. 1. Gel filtration elution diagrams from Sephacryl S 300 of purple ribbon pectins (DE 34.1%) depolymerized by pectate lyase. Codes indicate hours of treatment. Description of preparations in Table 1

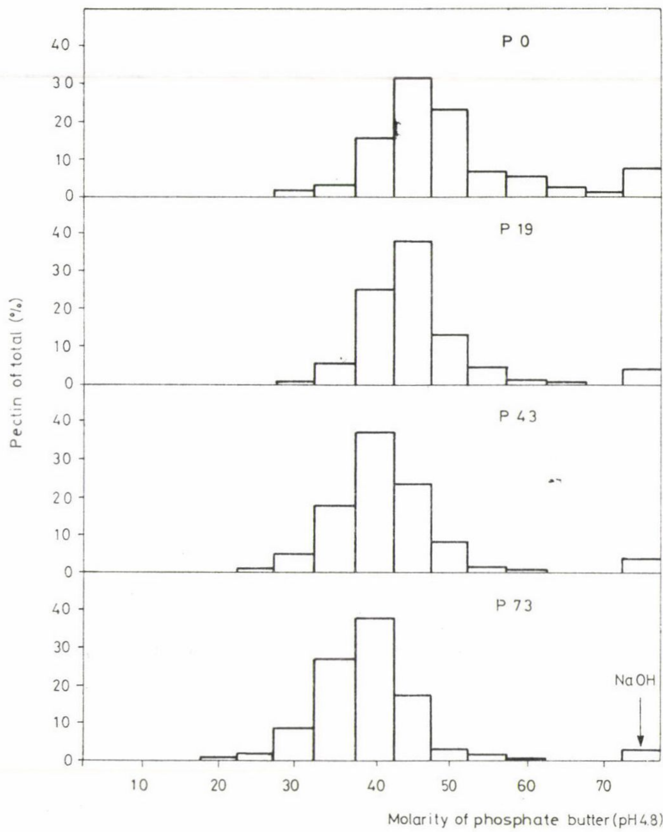

Fig. 2. DEAE-cellulose chromatography of purple ribbon pectins (DE 34.1%) depolymerized by pectate lyase. Codes indicate hours of treatment. Description of preparations in Table 1

Table 1

Properties of pectate lyase degraded low methoxyl pectin (purple ribbon)
(The codes indicate hours of treatment)

Code	[η]	$\overline{\mathrm{M}}$v	ΔE_{232}	$\overline{\mathrm{M}}$n
P 0	4.8	75 330		
P 19	1.65	33 950	0.312	21 444
P 43	1.05	24 230	0.608	12 894
P 73	0.70	17 900	0.870	9 486

[η]: intrinsic viscosity
$\overline{\mathrm{M}}$v: viscosity of average molecular weight
ΔE: increase in extinction
$\overline{\mathrm{M}}$n: average molecular weight

gel filtration over Sephacryl S 300. Figure 1 shows how the elution volume of the bulk of the molecules shifts to the included volume. The elution diagrams of all preparations show a (diminishing) peak of high molecular weight molecules which must be thought to represent molecules not easily attacked by the enzyme. The preparations were also fractionated in relation to their DE by ion-exhange chromatography (DEAE cellulose). Results from a gradient elution are presented in Fig. 2 as block diagrams to allow comparison with fractionations described earlier (van DEVENTER-SCHRIEMER & PILNIK, 1976). It is seen that degraded pectins are eluted at lower buffer molarities which makes them appear to be more highly esterified.

2.2. Chemical eliminative degradation

Brown ribbon pectin was treated under the following conditions: 1% pectin in 0.1 mol l^{-1} tris-succinate buffer pH 6, 90 °C. At specific times the reaction was interrupted by adding acidified alcohol and working-up the precipitate as described above for enzymatic degradation. The properties of the degraded pectins are shown in Table 2. There was a slight deesterification. The gel filtration elution diagrams from Sephacryl S 300 are shown in Fig. 3. In contrast to the enzymatic degradation there is no fraction of the pectin which remains

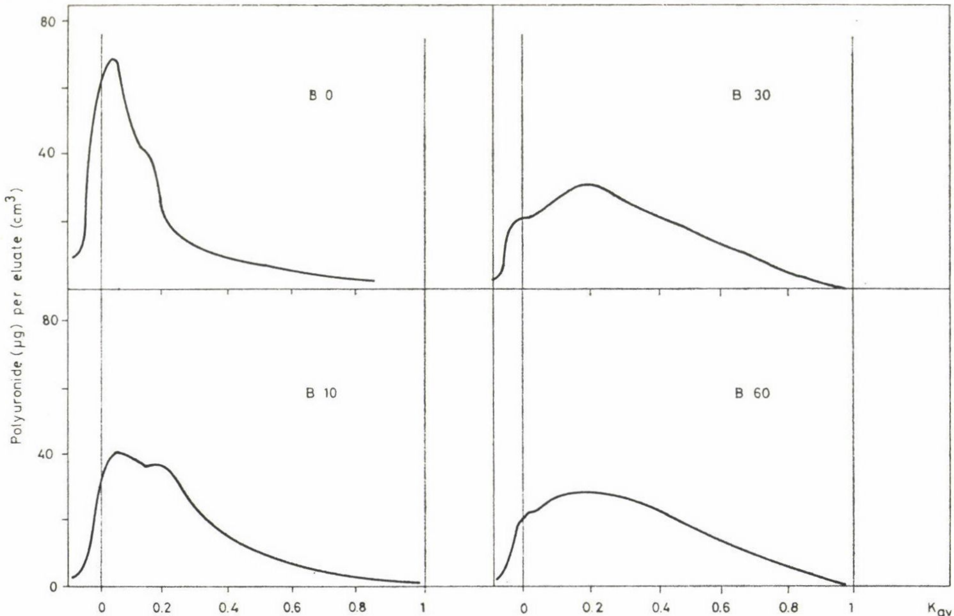

Fig. 3. Gel filtration elution diagrams from Sephacryl S 300 of brown ribbon pectins (DE 72.3%) depolymerized by treatment at 90 °C at pH 6. Codes indicate minutes of treatment. Description of preparations in Table 2

intact. The diagrams in Figs. 1 and 3 confirm the higher molecular weights for the brown ribbon preparations given in Tables 1 and 2.

Table 2

Properties of high methoxyl pectin (brown ribbon) degraded by chemical elimination at 90°C and pH 6

(The codes indicate minutes of treatment.)

Code	DE (%)	[η]	$\overline{Mv}$	ΔE_{232}	$\overline{Mn}$
B 0	72.3	5.10	78 800	—	—
B 10	69.7	2.20	54 350	0.05	57 720
B 30	68.3	1.55	37 720	0.30	23 543
B 60	67.1	1.10	33 180	0.38	19 662

Abbreviations see Table 1.

2.3. Degradation by mechanolysis

Eighteen g of pectin were treated in a vibrating mill (Vibratom SM, Siebtechnik GmbH, Mühlheim, FRG). The volume of the cylindrical jars is 300 cm^3 and is filled to 90% with porcelain cylinders 13×13 mm. The vibration has a frequency of 1420 mm^{-1} and an amplitude of 1.75 mm. After treatment the pectin is present as a very fine powder which must be removed from the cylinders with a fine brush. It is mixed with some ceramic material from abrasion but ash content even after prolonged treatment (24 h) remains less than 1%. For some analyses it may be necessary to centrifuge the solutions used. Table 3 shows the properties of treated pectins. Only DP is changed by the treatment. Figure 4 shows some viscosity curves of the brown ribbon pectin. Both intercept and slope decrease with depolymerization. Accordingly, there is a good relationship between molecular weight and the Huggins factor (Fig. 5). In Fig. 6 some elution patterns from Sephadex S 500 clearly show the depolymerization of the pectins. Similar viscosity average molecular weights (Table 3) give similar elution diagrams. Sugar-acid gels were boiled from the brown and green ribbon pectin series and calcium pectinate gels from the purple ribbon series and gelling strength of the preparations determined as jelly grade and gel power, respectively. The test gels were then extruded through an extrusion cell of the Allo-Kramer Shear Press and the time-force curve during extrusion recorded. Table 4 shows the results of these measurements. Although the sugar-acid gels boiled with depolymerized pectin contained increasing amounts of pectin to obtain (near) standard gels, based on an elasticity measurement, the force needed for extrusion in the shear press diminished. This shows that molec-

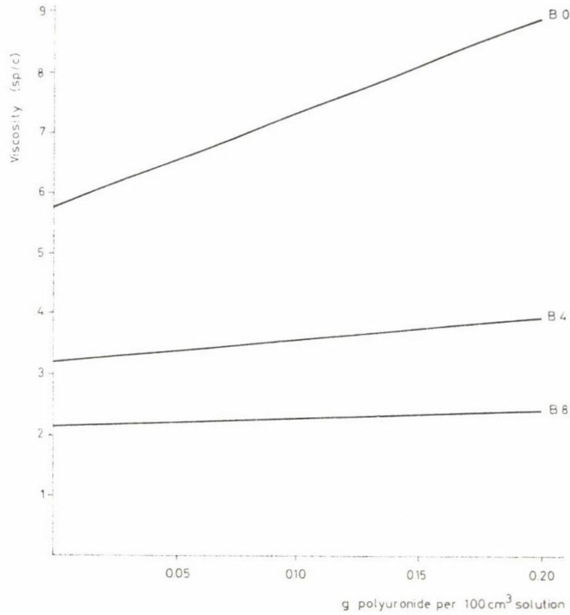

Fig. 4. Specific viscosities of mechanically degraded brown ribbon pectins (DE = = 72.8%). Codes indicate hours of treatment. The equations for these plots are as follows: B0: $y = 15.8x + 5.747$ $(r = 0.99)$; B4: $y = 3.28x + 3.215$ $(r = 0.928)$; B8: $y = 0.52x + 2.265$ $(r = 0.99)$; where y is the reduced viscosity, x the concentration and r the correlation coefficient. Description of preparations in Table 3

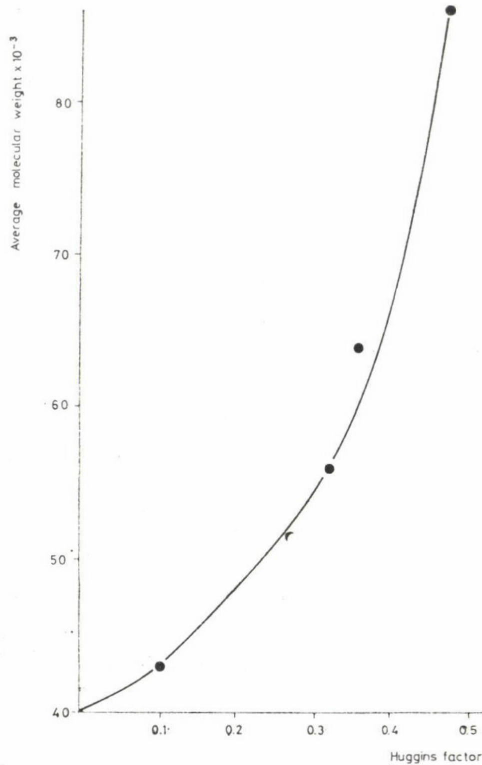

Fig. 5. $\overline{M}v$ vs Huggins factor for brown ribbon pectins degraded by mechanolysis (Table 3)

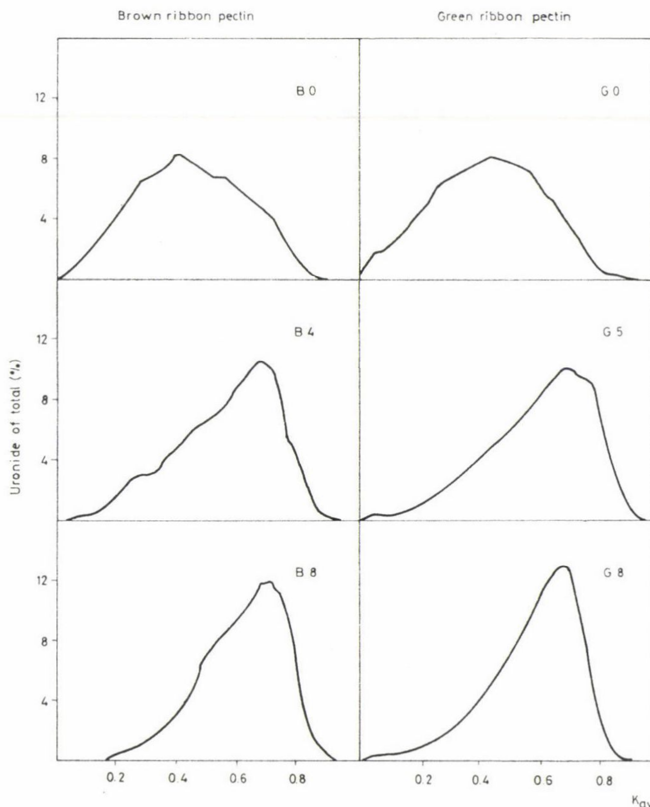

Fig. 6. Gel filtration elution patterns from mechanically degraded brown ribbon and green ribbon pectins. Codes indicate hours of treatment. Description of preparations in Table 3

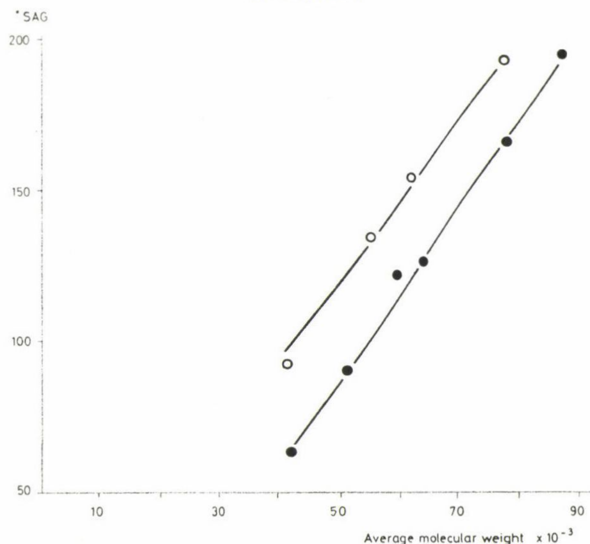

Fig. 7. Jelly grade of green ribbon and brown ribbon pectins degraded by mechanolysis vs $\overline{M}v$. Description of preparations in Table 3. The jelly grade is expressed as °SAG. The equations for these plots are: brown ribbon pectin: $y = 2.738x - 47.5$ ($r = 0.975$); green ribbon pectin: $y = 2.57x - 10.4$ ($r = 0.996$); where x is $\overline{M}v \times 10^{-3}$ and y °SAG. ●——●: brown ribbon pectin, ○——○: green ribbon pectin

Table 3

Properties of pectins degraded by mechanolysis
(The codes indicate hours of treatment.)

Pectin batch	Polyuronide on ash free dry substance (%)	DE (%)	$\overline{M}v$	Huggins factor K'
B 0	84.3	72.8	86 200	0.48
B 1	84 4	72.6	77 400	0.42
B 3	84.1	72.5	63 900	0.36
B 4	84.6	72.5	55 900	0.32
B 6	83.6	72.0	51 400	0.27
B 8	83.9	72.4	42 900	0.10
B 12	84.1	71.9	30 800	
B 48	84.0	72.0	15 000	
G 0	86.6	62.2	79 800	0.58
G 2,5	88.2	61.0	64 100	0.46
G 5	88.4	62.2	55 400	
G 8	88.1	62.3	41 000	
P 0	87.0	34.1	72 600	
P 2,5	87.0	33.6	54 000	
P 5	86.9	33.1	48 400	
P 8	87.0	34.0	33 200	

B: brown ribbon G: green ribbon P: purple ribbon DE: degree of esterification

Table 4

Gelling strength of depolymerized pectins of Table 3

	g pectin used for 400 g test gel	SAG (%) of gel by ridgelimeter[a]	Jelly grade[b]	Extrusion by shear press[c] (mm²)
B 0	1.50	20.8	184	118
B 1	1.70	21.3	165	110
B 3	2.10	22.2	127	90
B 4	2.30	21.5	129	88
B 6	2.70	23.8	85	58
G 0	1.40	21.1	192	116
G 2,5	1.80	20.4	157	95
G 5	2.20	19.7	135	92
G 8	3.00	21.6	92	68

	g pectin used for 300 g test gel		Gel power[b]	
P 0	2.40	14.3	152	97.6
P 2,5	2.40	21.6	121	46.0
P 5	2.40	27.8	87	23.0

[a] describes the elastic properties of a test gel by % sagging under its own weight
[b] describes the gelling strength of a pectin preparation by the quantity necessary to obtain a standard gel
[c] describes the internal strength of a test gel boiled for [a]

ular weight affects inner gel strength (extrusion) more than gel elasticity and confirms previous work (CHRISTENSEN, 1954) and the practical experience that pectins of equal jelly grade can have different breaking strengths. The purple ribbon gels contain all the same amount of pectin, so that such a conclusion is more difficult to derive. Figure 7 shows the good correlation which exists between jelly grades of the brown and green ribbon pectin series and the viscosity average molecular weight. The gel power figures of the purple ribbon pectins do not fit a linear curve.

3. Conclusions

Degradation experiments of pectins show the usefulness of methods like viscosity measurements and gel filtration in connection with the gelling behaviour of pectins. They also demonstrate that breaking strength measurements of gels have greater sensitivity towards viscosity average molecular weight than elasticity measurements.

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Acta Alimentaria, Vol. 16 (2), pp. 155—158 (1987)

SOLANUM AMERICANUM AS A SOURCE OF ANTHOCYANINS FOR USE IN FOODS

P. A. BOBBIO, F. O. BOBBIO and S. C. DE SOUZA

Departemento de Ciencia de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, C. P. 6121, 13100 — Campinas, São Paulo. Brazil

(Received: 6 November 1986; revision received: 26 May 1986; accepted: 28 May 1986)

From the berries of *Solanum americanum*, two anthocyanins were extracted with yields varying from 1.7 to 2.1%. The pigments were identified as malvidin-3-sophoroside-5-glucoside and malvidin-3-(di-caffeoyl-sophoroside)-5-glucoside. The unusually high amount of anthocyanins present in the berries of *S. americanum* makes this plant a potential source of natural pigments for use in foods. Stability of these anthocyanins in the conditions generally found during food processing is under study in our laboratories.

Keywords: *Solanum americanum*, Solanaceae, anthocyanins, food colours

The substitution of artificial colours by natural ones, a desirable objective, suffers from the lack of natural and economical accessible sources outside of grape pomace. Therefore as part of our screening program for new sources of anthocyanins, the berries of *Solanum americanum* were examined for their content of pigments since this annual sturdy shrub is easily grown in poor soil and produces a considerable quantity of small black berries per plant. These berries are eaten by animals and human beings without any reported ill effect, a fact which was taken into consideration for the examination of *S.americanum* as a potential source of anthocyanins.

1. Materials and methods

The berries were collected in Campinas and surroundings in the months of November and December.

Authentic samples of malvidin, malvidin-3,5-diglucoside and malvidin-3-glucoside used as standards were obtained from *Primula japonica* (HARBORNE, 1968) by usual procedures of extraction and acid hydrolysis of anthocyanins.

1.1. Isolation of anthocyanins

The anthocyanins were extracted in the dark with 0.1% HCl in methanol at 5 °C. The crude extract was concentrated at 30 °C under reduced pressure and purified by chromatography on Whatman 3 MM paper with BAW

(n-butanol–glacial acetic acid–water), 6 : 1 : 2 and 1% HCl (conc. HCl–water, 3 : 97) as solvents.

The pigments were obtained from the purified extract by chromatography on No. 1 Whatman paper with four different solvent systems: BAW, 1% HCl, AWH (glacial acetic acid–water–conc. HCl), 15 : 82 : 3 and Bu–HCl (n-butanol–2*N* HCl, 1 : 1, upper phase).

1.2. Identification of individual pigments

The anthocyanins were identified by chemical and spectral analysis fully described in the literature.

Spectral analyses were performed with a Unicam SP 8000 ultraviolet-visible recording spectrophotometer (Pye Unicam, UK).

Acid and controlled hydrolyses were done with 2*N* HCl according to FRANCIS and HARBORNE (1966), CHEN and LUH (1967) and FRANCIS and co-workers (1982) and the resulting products identified by chromatographic and spectral analysis. Peroxide degradation was accomplished by adding 30% hydrogen peroxide to a methanolic solution of the pigment following the method of CHANDLER and HARPER (1961) and the produced sugar identified by GLC of its TMS derivatives, under conditions previously described (BOBBIO et al., 1983).

For the alkaline hydrolysis a methanolic solution of the pigment was treated under nitrogen with a 2*N* solution of NaOH, following the method described by FRANCIS and HARBORNE (1966) and the liberated acid identified by chromatography on silica G plates.

The total amount of pigments was determined by the method described by FRANCIS (1982).

2. Results

In order to avoid hydrolysis of the acyl groups, a lower concentration of HCl (0.1%) was used in the extraction of the pigments.

Paper chromatography of the pigment's extract indicated the presence of two anthocyanins. A very satisfactory separation was accomplished with 1% HCl; the two pigments obtained were designated *A* and *B* with *B* present in very small amounts.

Under UV light both pigments showed dark red fluorescence, indicating glycosidation in both 3C and 5C positions, confirmed by the ratio $E_{440}/E_{\lambda\ max\ vis.}$. On addition of $AlCl_3$ no bathocromic effect was observed in the visible spectra of pigment *A* and *B*, therefore ruling out the presence of free vicinal hydroxyl groups in *B* ring.

The spectral curve of pigment *A* showed two peaks in the UV region at 277 and 320 nm, the latter characteristic of acylated anthocyanins (HAR-

BORNE, 1964). Pigment *B* showed only one peak in the UV region at 276 nm, characteristic of non-acylated anthocyanins.

The properties of pigments *A* and *B* are summarized in Table 1.

Acid hydrolysis of pigments *A* and *B* yielded the same aglycone identified as malvidin by spectral data and by co-chromatography with an authentic sample of malvidin. Glucose identified by paper chromatography was the only sugar produced by total hydrolysis of both pigments. A chromatogram of the

Table 1

Characteristic properties for the anthocyanins of Solanum americanum

Pigment	$R_f \times 100$				Spectral data				
	BAW	1% HCl	AWH	BuHCl	UV fluoresc.	λ_{max} (nm)	$\lambda_{vis.\,max}$ + $AlCl_3$ (nm)	E_{440}/E_{532} (%)	E_{320}/E_{532} (%)
A	26	32	50	28	dark red	277, 320, 532	0	14	98
B	20	50	68	5	dark red	276, 532	0	16	—

acid hydrolysis products of pigment *A*, sprayed with bromocresol green, showed a yellow spot confirming the presence of an acid in this pigment. Alkaline hydrolysis of pigment *A* produced caffeic acid identified by co-chromatography on silica plates with a pure sample of caffeic acid and observing the spots under UV light before and after exposure to ammonia vapours, and by spraying the plates with an acidic solution of $FeCl_3$ (PIFFERI, 1965).

In spite of this identification we failed to find a peak at 291 nm in the spectrum of pigment *A*, as described by POMILIO and SPROVIERO (1972) for anthocyanins acylated with caffeic acid. Moreover, the absorption peak at 320 nm found in pigment *A* is consistent with the spectral values obtained in our laboratory for an authentic sample of caffeic acid (a peak at 326 nm and only a shoulder at 292 nm). The ratio $E_{440}/E_{\lambda\,max\,vis.}$ is indicative of the presence of two molecules of acid per molecule of pigment (HARBORNE, 1964).

Controlled hydrolysis of deacylated pigment *A* and of pigment *B* yielded identical intermediates with characteristics similar to malvidin-3,5-diglucoside (A_1 and B_1) and malvidin-3-glucoside (A_2 and B_2).

Peroxide degradation of deacylated pigment *A* and of pigment *B* yielded sophorose identified by GLC.

The characteristic data for the hydrolysis products of pigments *A* and *B* are summarized in Table 2.

The results are consistent with the identification of pigment *A*, the major pigment in the berries of *S. americanum* as malvidin-3-(di-caffeoyl-sophoroside)-5-glucoside and pigment *B* as malvidin-3-sophoroside-5-glucoside.

The amount of anthocyanins in the berries of *S. americanum* varied from 1.7 to 2.1%, an unusually high amount of anthocyanins.

Table 2

Characteristic properties for hydrolysis products of the anthocyanins of Solanum americanum

		Aglycone	(A_1, B_1) (control. hydrol.)	(A_2, B_2) (control. hydrol.)	Acid (alk. hydrol.)
$R_f \times 100$	1% HCl	—	16 (14)[e]	08 (08)[f]	—
	AWH	—	45 (45)[e]	30 (30)[f]	—
	BuHCl	—	05 (06)[e]	18 (17)[f]	—
	Forestal[a]	64 (64)[d]	—	—	—
	BAW	60 (61)[d]	34 (34)[e]	40 (39)[f]	84 (85)[g]
	PrN[b]	—	—	—	12 (11)[g]
	TAE[c]	—	—	—	37 (37)[g]
Spectral data	UV fluoresc.	—	dark red	no fluoresc.	blue
	$\lambda_{vis.\ max.}$ (nm)	542 (541)[d]	534 (535)[e]	534 (534)[f]	—
	$\lambda_{UV\ max.}$ (nm)	275 (275)[d]	275 (275)[e]	275 (276)[f]	—
	$\Delta\lambda_{vis.+AlCl_3}$ (nm)	0	0	0	—
	$E_{440}/E_{\lambda\ vis.\ max}$ (%)	—	14 (16)[e]	21 (21)[f]	—

[a] Forestal: glacial acetic acid–conc. HCl–water (30 : 3 : 10).
[b] PrN: n-propanol–conc. ammonium hydroxide (1 : 3).
[c] TAE: toluene–glacial acetic acid–absol. ethanol (15 : 3 : 1).
Numbers in parenthesis are values obtained with authentic samples of: [d] malvidin; [e] malvidin-3,5-diglucoside; [f] malvidin-3-glucoside; [g] caffeic acid.

*

One of us (S. C. de Souza) thanks Fundacao de Amparo a Pesquisa do Estado de São Paulo, for a scholarship.

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Acta Alimentaria, Vol. 16 (2), pp. 159—166 (1987)

RADIATION DISINFESTATION AND QUALITY OF DRIED FRUITS

M. WAHID, A. SATTAR, NEELOFAR, S. ATTA, I. KHAN
and D. A. E. EHLERMANN[a]

Nuclear Institute for Food and Agriculture, Tarnab, Peshawar. Pakistan

(Received: 2 January 1986; accepted: 17 March 1986)

Dried apricots, dates, figs and raisins were irradiated at 0.25, 0.50, 1.00 kGy and stored for 12 months at ambient (28–40 °C) and lower temperatures (20 ± 2 °C). The effectiveness of radiation for disinfestation and nature of insects involved were determined. Changes in ascorbic acid and sensory quality, were studied. The insect infestation increased during storage and this increase was much larger at room than at lower temperature. Irradiation dose of 1.00 kGy completely checked infestation in all the dried fruits throughout the storage. Treatment with 0.25 kGy and lower temperature was also effective in controlling the insect population during a storage period of 12 months. *Tribolium castaneum* infested the dates and raisins, while the apricots were infested by *Corcyra cephalonica* and figs by *Cadra cautella*. Ascorbic acid declined during storage and was reduced by radiation especially at higher doses. The results of sensory evaluation revealed higher rating for irradiated than unirradiated controls. It was concluded that radiation processing of dried fruits in combination with storage at reduced temperatures is an effective method of providing the consumer with an acceptable and nutritious produce also outside the season for fresh fruits.

Keywords: irradiation, disinfestation, ascorbic acid, sensoric behaviour, irradiation of dried fruits

A substantial portion of the fruits, about one fourth of the total produce is dried during the glut season by sun-drying. Dried fruits are considered a major source of income and foreign exchange in many countries (INTERNATIONAL TRADE CENTRE, 1973). In Pakistan, almost 1 947 498 tons of fruits are produced on 487 346 acres of land. A major portion of this comes from the northern areas of Pakistan. In addition, more than 50 000 tons of different dry fruits are imported annually from Afghanistan (KHAN et al., 1981).

The spoilage of dried fruits by insect infestation, colour deterioration and chemical changes during storage is a serious problem especially under humid tropical conditions such as prevailing in Pakistan (N. A. S., 1978). Occurrence of extensive losses of food products, during postharvest storage and marketing has stimulated a great deal of research on radiation disinfestation of stored grains and dried fruits.

[a] Visiting IAEA-expert under project PAK/5/15 from Federal Research Centre for Nutrition, Karlsruhe, Federal Republic of Germany; the reported research was initiated during the visit from 1 July to 31 August 1982.

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

Gamma radiation has been used for insect disinfestation of dried fruits and nuts by some workers (BROWER & TILTON, 1970; TALHOUK, 1966; GUERRIERI, 1975 and SCHEGOLEVA, 1963). However, conflicting results have been reported as to the insect involved and optimum doses needed for disinfestation. Present work was undertaken to study the effect of radiation doses in combination with lower (20 ± 2 °C) and room temperature (28–40 °C) storage on the insect infestation and other quality parameters of dried fruits.

1. Materials and methods

Four dried fruits such as apricots, dates, figs and raisins were obtained from the local dry-fruit-market at Peshawar. These were sorted for uniform shape and size and packed in clear polyethylene bags. All the samples were irradiated at doses of 0.25, 0.50 and 1.00 kGy in a Cobalt-60 gamma irradiator (Issledovatel' USSR) having a dose rate of 9 kGy h^{-1} and a maximum–minimum-ratio of 2.5. The samples were stored at lower (20 ± 2 °C) and at room temperatures (28–40 °C).

Percentage insect damage of dried fruits was determined by weight according to COGBURN (1977). The samples were examined during storage for the nature of insects infestation following the method of BLAND (1978). The ascorbic acid was determined by titrimetry according to AOAC (1975). Sensory evaluations were conducted by hedonic ratings on a 9-point-scale (LARMOND, 1977).

2. Results and discussion

The effect of gamma irradiation and storage on the infestation of dried fruits is shown in Table 1. All the samples were examined for insect infestation fortnightly: however, for the sake of brevity the results have been presented only for 3, 6, 9 and 12 months storage period. Initially, the samples were free of any apparent infestation but during storage of 3 months, the insect population reached 18, 4, 5 and 3% in unirradiated apricots, dates, figs and raisins, respectively, kept at room temperature. The infestation increased consistently during storage. The rate of infestation was generally higher in apricots and figs than dates and raisins. The unirradiated samples of apricots and figs were totally infested (100%) by insects after 6 months. The identification of insects revealed that *Tribolium castaneum* (red flour beetle) was dominant and attacked dates and raisins. The apricots were infested by *Corcyra cephalonica* and figs by *Cadra cautella*. A radiation dose of 1.00 kGy completely checked the infestation in all the dried fruits throughout storage at room temperature. Doses of 0.25 and 0.50 kGy decreased insect infestation, but in all cases after

Table 1

Effect of irradiation and storage on the insect infestation in percent

Dried fruits and storage temperature	Storage period (months)	Irradiation dose (kGy)				Insects
		0	0.25	0.50	1.00	
Apricot; room temp.	3	18	10	0	0	*Corcyra cephalonica*
	6	100	80	40	0	
	9	100	100	90	0	
	12	100	100	100	0	
low temp.	3	4	0	0	0	
	6	8	0	0	0	
	9	26	0	0	0	
	12	50	0	0	0	
Dates; room temp.	3	4	0	0	0	*Tribolium castaneum*
	6	32	8	62	0	
	9	100	100	82	0	
	12	100	100	100	0	
low temp.	3	0	0	0	0	
	6	12	0	0	0	
	9	18	0	0	0	
	12	22	0	0	0	
Figs; room temp.	3	5	0	0	0	*Cadra cautella*
	6	100	25	17	0	
	9	100	100	47	0	
	12	100	100	100	0	
low temp.	3	0	0	0	0	
	6	10	0	0	0	
	9	50	0	0	0	
	12	60	0	0	0	
Raisins; room temp.	3	3	0	0	0	*Tribolium castaneum*
	6	58	10	7	0	
	9	100	100	50	0	
	12	100	100	100	0	
low temp.	3	0	0	0	0	
	6	8	0	0	0	
	9	15	0	0	0	
	12	27	0	0	0	

Initial infestation : zero
Room temperature: 28—40 °C
Low temperature : 20 $\pm$ 2 °C
Infestation : ratio of weight of damaged fruits to total weight of sample

12 months of storage at room temperature the insect damage reached 100%. It was observed that lower temperature storage alone (20 $\pm$ 2 °C) also slackened insect infestation. However, the radiation dose of 0.25 kGy coupled with lower temperature completely checked development of all types of insects during the storage period of one year. Although published studies carried out at reduced temperatures are not available, conflicting results have been reported for controlling insects in dry fruits at ambient conditions. BROWER and TILTON

(1970) recommended a dose range of 0.30–0.40 kGy for complete control of insect development in dry fruits. SCHEGOLEVA (1963) reported that a dose of 0.70 kGy was sufficient to kill most insects. PAPADOPOLOU (1963) recommended a dose of 1–2 kGy for complete destruction of Plodia, Cadra and Oryzaephilus species during their various stages of growth and development.

It is known that there is little loss of ascorbic acid during refrigerated storage of food materials. Changes in the L-ascorbic acid were, therefore, studied during storage at room temperature only. The ascorbic acid contents of unirradiated and irradiated dried fruits are presented in Figs. 1–4. The dashed lines indicate the range of vitamin losses during storage and the effect of the maximal dose used, 1 kGy. After drying the samples contained 9.47, 3.54, 16.82 and 3.64 mg per 100 g of ascorbic acid in apricots, dates, figs and raisins, respectively. There was a significant loss of this vitamin during the

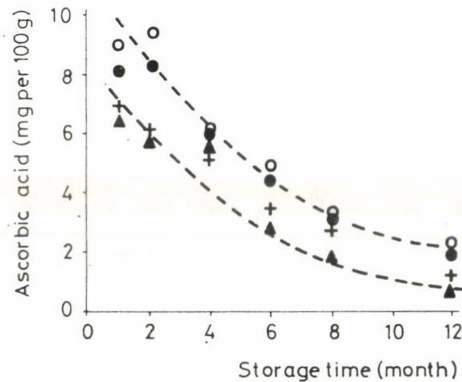

Fig. 1. Effect of irradiation and storage on ascorbic acid contents of dried apricots ○: 0.00 kGy; ●: 0.25 kGy- +: 0.50 kGy; ▲: 1.00 kGy (see text for explanation of curves)

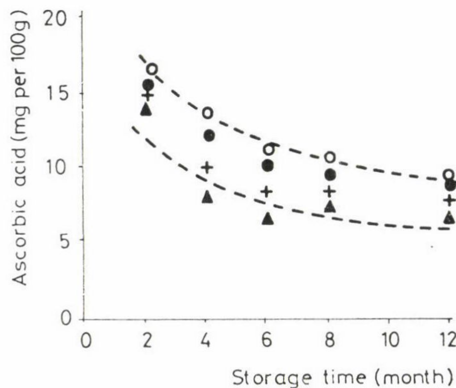

Fig. 2. Effect of irradiation and storage on ascorbic acid contents of dried figs. ○: 0.00 kGy; ●: 0.25 kGy; +: 0.50 kGy; ▲: 1.00 kGy (see text for explanation of curves)

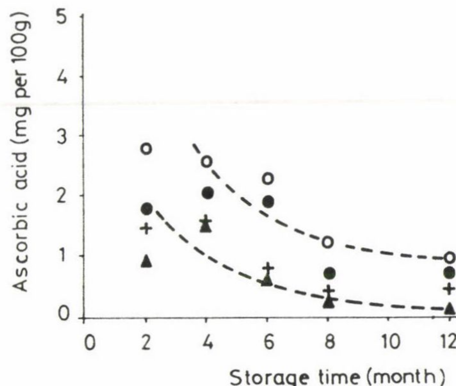

Fig. 3. Effect of irradiation and storage on ascorbic acid contents of dried dates. ○: 0.00 kGy; ●: 0.25 kGy; +: 0.50 kGy; ▲: 1.00 kGy (see text for explanation of curves)

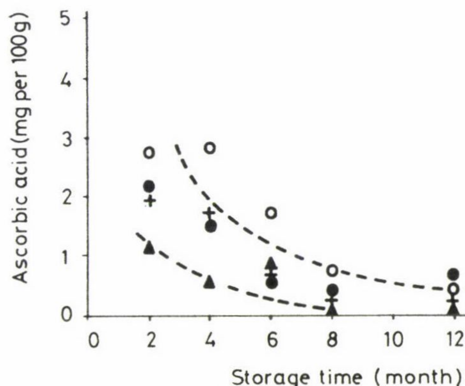

Fig. 4. Effect of irradiation and storage on ascorbic acid contents of dried raisins. ○: 0.00 kGy; ●: 0.25 kGy; +: 0.50 kGy; ▲: 1.00 kGy (see text for explanation of curves)

storage period of 12 months. In the beginning there was no prominent effect of irradiation for dried figs and apricots but during later storage radiation contributed to the loss of ascorbic acid. Higher losses were observed at higher radiation doses while the lower doses had a intermediate effect. As radiation converts some of the vitamin C to dehydroascorbic acid, the reported changes are not necessarily of any nutritional importance and are used here only as a quality index. Ascorbic acid is generally considered radiosensitive. Irradiation also brings about losses in other vitamin contents, like other preservation method of fruits and the degree of loss is dependent upon the type of fruits, conditions of irradiation, e.g. temperature, moisture level and the presence or absence of oxygen (JOSEPHSON et al., 1975). CHOWDHURY and RAHMAN (1973)

reported that ascorbic acid contents were reduced immediately after irradiation, but the loss was nutritionally nonsignificant. At pasteurization doses (1–4 kGy), the losses could vary from little to extensive depending on the food material irradiated. In fresh cherries the loss of ascorbic acid was reported as 2–3%, while in lemon it vas 71–90% with 2–4 kGy doses (Maxie & Sommer, 1968). Similarly significant loss of ascorbic acid was observed in dry fruits by Khan and co-workers (1981).

Table 2

Effect of irradiation and temperature on sensory quality of dry fruits

Dried fruit	Storage condition	Storage period (month) 3		6		9		12	
		Irradiation dose (kGy)							
		0	0.25	0	0.25	0	0.25	0	0.25
Apricot	room temperature	5.2	5.4	4.1	4.6	1.2	1.2	1.0	1.0
	low temperature	6.2	6.1	6.1	6.4	5.3	5.9	4.8	5.3
Dates	room temperature	6.3	5.8	5.0	5.3	4.3	4.5	3.4	4.0
	low temperature	6.4	6.5	6.0	6.0	5.7	6.0	5.2	5.7
Figs	room temperature	5.2	5.4	4.3	4.8	2.1	2.5	1.4	1.7
	low temperature	6.4	6.6	6.2	6.3	5.7	6.1	5.3	5.6
Raisins	room temperature	5.3	5.5	3.0	3.9	1.5	2.1	1.1	1.0
	low temperature	6.6	6.7	6.4	6.6	5.8	6.0	5.7	5.8
Mean	room temperature	5.5	5.5	4.1	4.6	2.3	2.6	1.7	1.9
	low temperature	6.4	6.5	6.2	6.3	5.6	6.0	5.2	5.6

Values are the mean of 6 judgements for overall acceptability for colour, texture and flavour, 1 : extremely disliked, 9: extremely liked.
Initial scores: apricots 7.3, dates 8.4, figs 8.2 and raisins 8.4.
Room temperature: 28—40 °C, low temperature: 20 ± 2 °C.

All the dried fruits were evaluated in a sensory test after every 3 months by trained panelists. The results of overall acceptability for colour, texture and flavour are shown in Table 2. It was observed that the storage period had a significant effect on the appearance of all the samples. The freshly obtained samples had overall acceptability scores of about 8 which decreased to 3 and lower scores in all varieties after 12 months storage at room temperature. The decreases in mean scores were higher in samples stored under room conditions than at lower temperatures. The samples stored at 20 ± 2 °C were found acceptable even after one year and received scores of about 5.5 for all varieties. The irradiation dose of 0.25 kGy had no significant effect on the texture, taste or flavour of any sample. Farkas and co-workers (1974) reported that the eating quality of dates was not affected significantly by an irradiation dose of 5.4 kGy and no off-flavour was observed. Jaddou and Al-Hakim (1978) also reported that irradiation (0.50 kGy) and heating (323 K) did not

affect the flavour of dates during their storage. Similarly PAPADOPOULOU (1963) observed that irradiation with doses of 1–2 kGy showed no deleterious effect on the texture, appearance and nutritive value of irradiated dried figs. Similar findings have been reported by KHAN and co-workers (1982; 1983).

3. Conclusion

As a result of these studies it was concluded that a radiation dose of 1.0 kGy was necessary for complete disinfestation of dried fruits. However, due to increased discolouration, these samples were found unacceptable after 6 months storage. Combination treatment of 0.25 kGy and lower temperature (20±2 °C) was found optimum for longer storage of dried fruits. When the content of ascorbic acid was used as a quality index it showed a considerable loss during storage and an additional loss due to radiation processing. However, radiation processing can extend the period during which dried fruits are available to the consumer thus ameliorating also the resources for vitamins outside the season for fresh fruits.

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Acta Alimentaria, Vol. 16 (2), pp. 167—179 (1987)

SCALE-UP OF THE PRODUCTION OF GLUCOSINOLATE-FREE CANOLA MEAL

L. L. Diosady, C. G. Tar, L. J. Rubin and M. Naczk[a]

Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, M5S 1A4 Canada

(Received: 24 January 1986; accepted: 14 March 1986)

The solvent grinding of canola seed using a semi-pilot-scale Szego mill was investigated in a process for the production of glucosinolate-free meal which is also low in polyphenols. The optimum grinding and contacting conditions for canola seed suspended in methanol containing 5% water (v/v) and 10% NH_3 (w/w) were determined to be 10–20 s contact time with a solvent-to-seed ratio for grinding of 2.5. The ground slurry was diluted to a pre-selected solvent-to-seed ratio (preferably 3.3) and left undisturbed for 5–15 min, then filtered, washed and extracted with hexane. The methanol–ammonia–water solution was more effective in the extraction of glucosinolates and polyphenols on the semi-pilot-scale than in the laboratory. The treatment resulted in a meal with a glucosinolate content below the detection level of the analytical method used. The polyphenol content was reduced by 80%. The oil recovered from the hexane phase is low in phosphorus and can be considered as degummed oil.

Keywords: Szego mill, glucosinolates, canola, extraction

The use of rapeseed as a source of food protein is prevented by the presence of glucosinolates, phenols, phytate, and hull. Canadian plant breeders have developed canola varieties which are low in glucosinolates and erucic acid. However, the canola meal still contains 1–3 mg glucosinolates per gram and about 33% hull and therefore it cannot be used as an ingredient in food products.

The conventional extraction process for rapeseed is an adaptation of soybean technology adjusted for the small seed size, and the high oil and glucosinolates content. None of the many methods for the removal of glucosinolates developed over the past 30 years is in commercial use due to protein losses, poor functional properties, or high processing costs (Maheshwari et al., 1981).

Obviously, an alternative to soybean technology specific to the processing of rapeseed is required. The traditional process utilizes percolating bed extractors in which rapeseed flakes are contacted with hexane. The objective of flaking is to massively deform the seed structure to produce a large surface-to-volume ratio to facilitate solvent penetration. Optimum thickness for the flakes is

[a] Author Naczk is on leave of absence from the Department of Food Preservation and Technical Microbiology, Politechnika Gdańska, Gdańsk, Poland.

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

between 0.2 to 0.3 mm. Rapeseed flakes thinner than 0.2 mm result in less satisfactory oil extraction due to the decreased mechanical strength of flakes, which leads to the compaction of the bed. In order to attain higher extraction rates, a better means of solvent-to-seed contacting must be developed which allows the use of smaller meal particles. The new alkanol–ammonia process developed by our group (RUBIN et al., 1984; RUBIN et al., 1986; DIOSADY et al., 1985; NACZK et al., 1985a) differs in almost every essential step from the conventional process. The alkanol–ammonia–water/hexane treatment simultaneously produces a meal free from glucosinolates and an oil low in phosphorus. The meal produced by this process has excellent water and fat absorption but a low nitrogen solubility index (NACZK et al., 1985). The grinding of the seed in the presence of the solvent was found to be a very effective method for the simultaneous extraction and "detoxification" of the seed. In the case of solvent grinding, the rate of extraction and glucosinolates removal are controlled by the residence time in the mill and the solvent-to-seed ratio. The reduction of the median particle size increases the surface area and decreases the solvent penetration distance resulting in increased mass transfer rates. Excessive particle size reduction requires additional energy that outweighs the advantage of enhanced oil extraction, and subsequently also results in difficulties in separating the meal from the solvent. Hence the size and uniformity of the particles must be controlled.

While there are several mills capable of wet grinding such as the ball, attrition, and Fitz mills, the Szego mill seemed most suitable for the scaling up of the process. Performance of the Szego mill has been investigated extensively in both dry and wet operations. The mill is very effective in slurry grinding (TRASS, 1980; PAPACHRISTODOULOU & TRASS, 1985) and has many distinct advantages over ball and attrition mills such as compactness, high throughput, moderate energy consumption, and low capital cost. In addition, the rolling action of the mill can produce flake-shaped particles, which are advantageous in the extraction or leaching process.

The objectives of the present study are to scale up the process and evaluate the semi-pilot-scale production of glucosinolate-free canola meal by treatment with alkanol-ammonia followed by hexane extraction of the separated meal. The procedure described in our papers and patent had to be altered because of the emulsion formed in the mill by the two-phase solvent system. The performance of the mill is discussed in terms of median particle size and standard deviation of particle size, which are important for the steps following grinding. The glucosinolate and complex phenol content of the meal and the phosphorus content of the oil were compared with those obtained in the laboratory-scale process.

1. Materials and methods

The laboratory-scale preparation of glucosinolate-free meals was carried out as illustrated in Fig. 1, and described previously (RUBIN et al., 1984; RUBIN et al., 1986; DIOSADY et al., 1985.)

Only the first part of the process for treating rapeseed was studied here, that is the treatment of rapeseed on a semi-pilot-scale with alkanol–ammonia–water, as detailed in Fig. 2.

Altex seed (500 g) was ground in the Szego mill with 1250 cm^3 of 10% ammonia in methanol (w/w) containing 5% water (v/v) ($MeOH/NH_3/H_2O$). The contact time was varied by repeated passes of approximately 10 s each, through the Szego mill, a planetary ring-roller mill consisting of three helically grooved rollers which rotate about a motor-driven shaft (TRASS, 1980). In the present study a small mill was used, which had an effective grinding length of 17 cm, an inside diameter of 10 cm, operating at 1300 r.p.m. in continuous grinding with a solvent-to-seed ratio (R) of 2.5. The apparatus for continuous solvent grinding is illustrated in Fig. 3. A feed slurry was prepared in a feed tank by recycling a mixture consisting of canola seed and the solvent, at the selected solvent-to-seed ratio, through the pump and feed lines until a uniform dispersion was obtained. The slurry was then pumped through the mill into a second feed tank at a flow rate of 8 dm^3 min^{-1}. The slurry was sampled and re-ground, or the meal was separated, and extracted separately with hexane. To complete the procedure shown in Fig. 2 the slurry was transferred into a 4 dm^3 beaker and fresh $MeOH/NH_3/H_2O$ solution was added to increase the

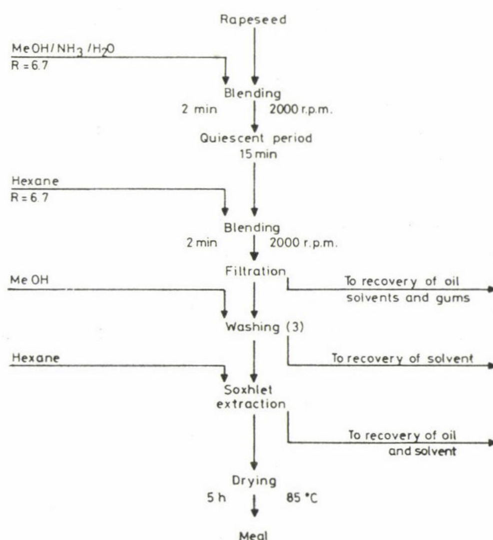

Fig. 1. Flowsheet for the laboratory-scale process. R: solvent-to-seed ratio

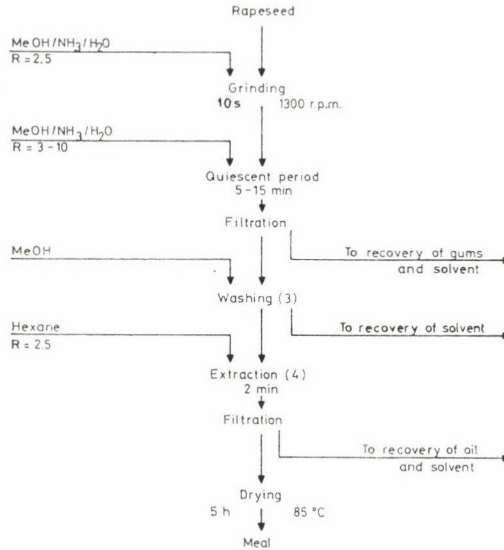

Fig. 2. Flowsheet for the semi-pilot-scale process. R: solvent-to-seed ratio

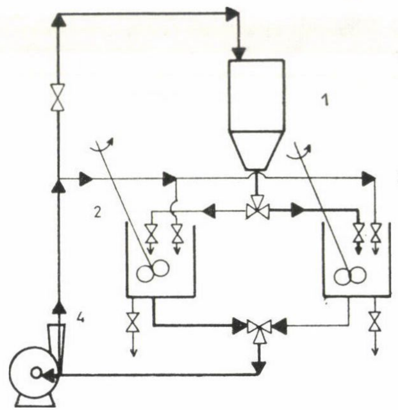

Fig. 3. Apparatus for continuous grinding and extraction ——: grinding; 1: Szego mill; 2: feed tank; 3: receiving tank; 4: pump

solvent-to-seed ratio in steps to 10. After a quiescent period of 5–15 min the meal was separated by vacuum filtration on a Büchner funnel using Whatman No. 41 filter paper, and washed three times with 300 cm³ of methanol. The meal was suspended and stirred four times in hexane for 2 min each time at $R = 2.5$. After the last separation of hexane, the meal was oven dried at 85 °C for 5 h. Prior to glucosinolate and polyphenol determination, the meal was further extracted with hexane using a Soxhlet apparatus. The oil was recovered from the combined hexane solutions by evaporation in vacuo.

A series of grinding experiments were performed in the Szego mill using hexane as the solvent in order to determine the grinding characteristics of the mill.

Particle-size distribution of the ground seed was obtained using a Rotap Shaker and 21 cm diameter Tyler Canadian Standard sieves in the presence of solvent to prevent agglomeration of smaller particles. The ground seed was shaken for 10 min to ensure sharpness of cut.

Proximate analysis was carried out using AACC procedures (AACC, 1976). The total glucosinolate content was measured by the method of WETTER and YOUNGS (1976). The meals containing less than 0.2 mg glucosinolate per g (i.e. below the sensitivity level of the method) will be referred to as glucosinolate-free.

The total phosphorus content in the oil was determined by the AOCS method (AOCS, 1983).

Phytate was determined by the method described previously by NACZK and co-workers (1985a).

Polyphenols were isolated and determined colorimetrically as previously described (DIOSADY et al., 1985).

2. Results and discussion

2.1. Grinding in hexane

The effect of contact time, defined as the time spent by the slurry in the grinding zone, on the particle size distribution is presented in Fig. 4. The feed is indicated as a monosize material, 2 mm in diameter.

Plotting the cumulative undersize on the probability scale versus the logarithm of particle-size, straight lines were obtained. Particle-size uniformity which may be characterized by the standard deviation (σ) is desirable for the steps following grinding. The value of σ may be readily determined from the probability plots using the logarithmic abscissa, by the method of LESCHONSKI and co-workers (1974):

$$\sigma = \frac{\text{particle size at 84\% probability}}{\text{particle size at 50\% probability}}$$

or

$$\sigma = \frac{\text{particle size at 50\% probability}}{\text{particle size at 16\% probability}}$$

Both the median particle size (particle size at 50% cumulative undersize) and the standard deviation of the ground slurry were affected by the contact time. The σ value increased from 1.8 (at 10 s contact time) to 3.8 (at 60 s

contact time), showing a decrease in homogeneity. As expected, the most uniform meal was obtained at 10 s contact time; however, the median particle size was high (575 μm). After 60 s of contact time, a less homogeneous particle size distribution was observed with a median particle size of 125 μm. Since in solid–liquid separations, such as liquid cyclone fractionation, particle size uniformity is very important, a lower contact time, in the range of 10 to 20 s is desirable.

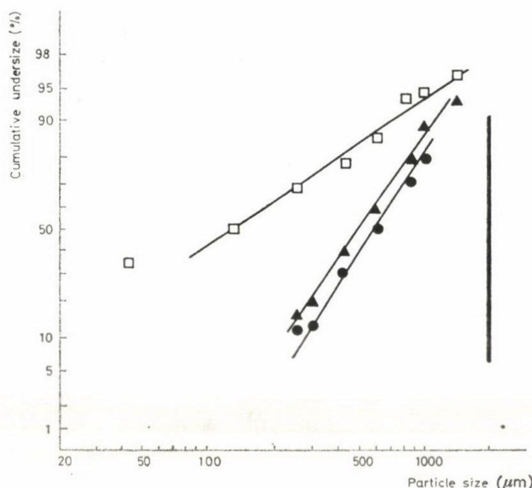

Fig. 4. The effect of contact time on particle size distribution using hexane. $R = 2.1$; rotor speed = 1300 r.p.m; ———: feed; ●: 10 s: ▲: 20 s; □: 60 s

2.2. *Grinding in $MeOH/NH_3/H_2O$ solvent system*

The effect of the solvent-to-seed ratio on the particle size distribution of the ground slurry is illustrated in Table 1. The σ value varied from 3.3 to 6.0, showing wide differences in uniformity. The most uniform meal was obtained at a solvent-to-seed ratio of 2.50; however, the median particle size was 355 μm. The median particle size increased with R from 295 μm ($R = 1.25$) to 460 μm ($R = 2.10$), but further increase in R decreased the median particle size to 325 μm ($R = 3.33$). These fluctuations are the result of different grinding conditions, i. e. paste grinding at low R (< 2.10), and agglomerate and diluted agglomerate grinding at higher R (> 2.10).

The effect of the throughput rate is illustrated in Table 2. The median particle size is almost directly proportional to the throughput from 6.18 to 9.58 $dm^3 \, min^{-1}$. At a flowrate of 8.17 $dm^3 \, min^{-1}$ lower size reduction was observed than at the flowrate of 6.18 $dm^3 \, min^{-1}$. This indicates that the slurry layer thickness in the grinding zone exceeds the optimal thickness, thereby causing considerable quantities of seed parts to bypass the rollers without

Table 1

Effect of solvent-to-seed ratio on grinding[a] in $MeOH/NH_3/H_2O$

Solvent-to-seed ratio (v/w)	Median particle size[b] (micron)	Standard deviation (σ)
1.25	295	6.0
1.67	315	4.6
2.10	460	4.1
2.50	355	3.3
3.33	325	4.8

[a] Grinding conditions: contact time: 60 s
rotor speed: 1300 r.p.m.
flowrate: 8 $dm^3 min^{-1}$
[b] The results are mean values of duplicates.

Table 2

Effect of throughput rate on median particle size[a]

Throughput rate ($dm^3 min^{-1}$)	Median particle size[b] (μm)
6.18	225
8.17	335
9.58	480

[a] Grinding conditions: contact time = 60 s
rotor speed = 1300 r.p.m.
R = 2.5
[b] The results are mean values of duplicates.

participating in the grinding process. At flowrates greater than 9.58 $dm^3 min^{-1}$ this effect is further enhanced.

These experiments showed that the Szego mill gave optimal grinding at a solvent-to-seed ratio of between 2.1 and 2.5 (v/w) Based on the results in Tables 1 and 2 the semi-pilot-scale grinding runs were performed at $R = 2.5$ and at a flowrate of 8 $dm^3 min^{-1}$, as a compromise.

Figure 5 illustrates the effect of contact time on the particle-size distribution for the $MeOH/NH_3/H_2O$ solvent system. In this system the particle-size distribution curves have two distinct regions: a zone of steep slope ($\sigma < 3.0$ at 60 s contact time) down to particle size of about 250 μm, followed by a much flatter region ($\sigma > 9.0$) where little further grinding occurs (from about 250 to 70 μm). The median particle size decreases from 1000 μm at 20 s contact time down to 475 μm at 40 s contact time. At longer contact times the decrease is not as pronounced (355 μm at 60 s and 170 μm at 120 s contact time). Again, similarly to the case of grinding in hexane, a contact time of 10–20 s seemed to be most advantageous, if the coarser material is recirculated.

The whole rapeseed kernel contains four major parts — hull, inner cotyledon, outer cotyledon, and embryo (APPELQUIST & OHLSON, 1972). The physical properties of the different parts such as hardness, tensile strength, compressibility, cohesiveness, abrasiveness, flow characteristics, and the tendency to agglomerate under pressure affect the behaviour of the seed in the grinding zone.

In this process the ammonia in the methanol ruptures the cell walls, thus changing the cotyledon structure and resulting in the rapid grinding of

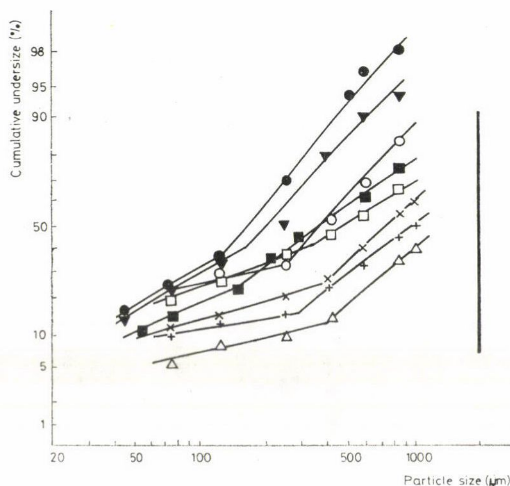

Fig. 5. The effect of contact time on particle size distribution using $MeOH(NH_3)H_2O$. $R = 2.5$; rotor speed = 1300 r.p.m. ———: feed; △: 10s; +: 20 s; ×: 30 s, □: 40 s; ■: 50 s; ○: 60 s; ▼: 90 s; ●: 120 s

meal particles and giving the flatter region in Fig. 5, while the abrasion resistant hulls are ground only at higher contact times giving rise to the steep slope in the larger diameter region of the graph.

This mechanism is confirmed by the protein values determined for the screened fractions after 20 s of contact time in the Szego mill (Fig. 6). The protein concentration of the meal is $43 \pm 0.5\%$ after hexane extraction (on dry basis), which is equal to the protein content of a screened fraction between the 425/600 μm and 600/850 μm sieve cuts. The fine fractions were always higher in protein, indicating that the meal fraction is preferentially ground. At 60 s contact time the presence of a higher proportion of ground hull in the finer fractions was obtained (Fig. 6).

Meals produced by treatment with $MeOH/NH_3/H_2O$ have a higher crude protein ($N \times 6.25$) content than the corresponding hexane-extracted meal (Table 3). The increase of crude protein from 39% to about 50% was mainly

the result of extraction of carbohydrates and other non-protein constituents into the methanol–ammonia phase. The phytate content of the meal was not altered by the $MeOH/NH_3/H_2O$ treatment (Table 3).

In one experiment the glucosinolate level in the $MeOH/NH_3/H_2O$-treated meals was determined after a contact time of 10, 20, 30, and 40 s in the Szego mill followed by dilution to $R = 10$. In all cases the glucosinolate content was less than 0.20 mg per g indicating that a contact time of 10 s is sufficient for glucosinolate reduction. However, the glucosinolate removal at

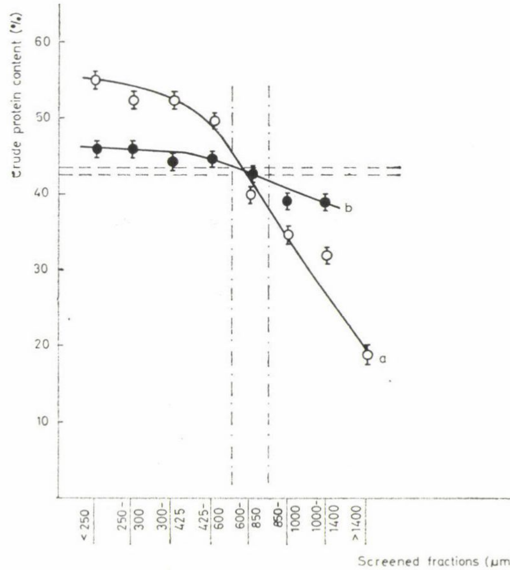

Fig. 6. Crude protein content of the screened fractions ($MeOH/NH_3/H_2O$). a: 20 s; b: 60 s contact time; solvent-to-seed ratio = 2.5; rotor speed = 1300 r.p.m.; ———: protein content; –·–·–·–: median particle size range at 20 s; – – – –: oil-free ground meal

Table 3

Analysis of canola meals

Treatment	Crude protein (%) (N × 6.25) solvent-to-seed ratio 6.7	Phytate (%) solvent-to-seed ratio 2.5	Phytate (%) solvent-to-seed ratio 6.7
Hexane	39.2 ± 0.2	—	3.8 ± 0.1
$MeOH/NH_3/H_2O$ (laboratory-scale)	48.8 ± 0.6	—	4.2 ± 0.2
$MeOH/NH_3/H_2O$ (semi-pilot-scale)	50.9 ± 0.6	3.8 ± 0.2	3.7 ± 0.1

Both crude protein and phytate are calculated on a dry basis.

Table 4

Glucosinolates content of canola meals-$MeOH/NH_3/H_2O$ grinding[a]

Solvent-to-seed ratio (v/w)	Glucosinolates content (mg per g dry meal)			
	semi-pilot-scale		laboratory-scale	
	quiescent period (min)			
	5	15	5	15
2.5	< 0.81 ± 0.05	0.74 ± 0.07	—	—
3.3	< 0.20	< 0.20	0.71 ± 0.03	0.70 ± 0.03
5.0	< 0.20	< 0.20	0.57 ± 0.08	0.40 ± 0.06
6.7	< 0.20	< 0.20	0.31 ± 0.06	<0.20
7.0	< 0.20	< 0.20	—	—
10.0	< 0.20	< 0.20	—	—
Hexane extracted	1.76 ± 0.08			

[a] Grinding conditions: contact time = 10 s
rotor speed = 1300 r.p.m.
flowrate = 8 $dm^3\,min^{-1}$
R = 2.5

The results are mean values of 4 replicates for semi-pilot-scale and 6 for laboratory-scale.

$R = 2.5$ as used in the grinding step was poor, and the meal still contained about 0.8 mg of glucosinolates per g of meal (Table 4). This indicated that the glucosinolate removal required a higher R value than the grinding step. In order to optimize both the grinding and the glucosinolate process, the effect of dilution with $MeOH/NH_3/H_2O$ after grinding was investigated. The seed was first ground with $MeOH/NH_3/H_2O$ at $R = 2.5$ and then fresh $MeOH/NH_3/H_2O$ was added to the slurry in stages until $R = 10$ was attained. The results given in Table 4 show, that extraction with $MeOH/NH_3/H_2O$ at $R > 3.3$ decreases the glucosinolates content in the meal below the detection level of the analytical method of WETTER and YOUNGS (1976) for the quiescent periods of 5 and 15 min. The semi-pilot-scale extraction process was more effective in lowering the glucosinolate level than the laboratory procedure; therefore, a shorter quiescent period than the 15 min found to be optimal in the laboratory may be adequate in the scaled-up process.

The total phenolic acid content, expressed as trans-sinapic acid equivalent, in the hexane-extracted meal and $MeOH/NH_3/H_2O$-treated meals are shown in Table 5. The $MeOH/NH_3/H_2O$ extraction using the Szego mill at $R = 2.5$ removed 59.2% of the phenolic acids initially present in the hexane extracted meal. An increase in R to 6.7 further reduced the phenolic acid content to less than 17% of the value normally found in the meal (1807 mg per 100 g). However, it has previously been shown that $R = 3.3$ is adequate for the removal of glucosinolates (Table 4).

Table 5

Effect of solvent-to-seed ratio on the polyphenols content of the meal[a]

Solvent-to-seed ratio (v/w)	Phenolic acids[b] (mg per 100 g dry meal)	
	semi-pilot-scale	laboratory-scale
2.5	737	—
3.3	703	—
6.7	304	505

[a] The hexane extracted meal contained 1807 mg phenolic acid per 100 g.
[b] Expressed as mg trans-sinapic acid.
The results are mean values of duplicates for semi-pilot-scale and for laboratory-scale.

Table 6

Mass balance for $MeOH/NH_3/H_2O$-hexane process
(as % of dry seed weight)

	Solvent-to-seed ratio (v/w)	Meal (%)	Oil (%)	Gums (%)	Σ %
	2.5	44.7	41.9	8.8	95.4
	3.3	42.2	41.8	9.0	93.0
Semi-pilot-scale	5.0	41.8	41.5	10.8	94.1
	6.7	42.1	41.4	11.2	94.7
	10.0	40.6	40.7	12.7	94.0
Laboratory-scale	6.7	48.5	41.4	9.4	99.7

The seed was first treated with $MeOH/NH_3/H_2O$, then the meal was separated and extracted with hexane in semi-pilot-scale.
The results are mean values of duplicates.

The mass balance of the semi-pilot-scale process varied from 93.0 to 95.4% of the weight of Altex seed used (Table 6). This is about 5% lower than the values obtained for the laboratory process, indicating losses in the system. Since it was difficult to transfer quantitatively the slurries and cakes from one step to another, the observed losses for the operations with the Szego mill were high.

The process also has a significant effect on oil quality. The results presented in Table 7 indicate that an increase of R to a value above 3.3 reduces the phosphorus content of the oil obtained by the semi-pilot-scale process. The best results for phosphorus removal were obtained at $R = 6.7$ for both the laboratory and semi-pilot work. The solvent extraction system produced oil at least equivalent to, or better in phosphorus content than the degummed oils produced by the conventional commercial processes.

Table 7

Phosphorus content of the oil fraction
(semi-pilot-scale)

Solvent-to-seed ratio (v/w)	Phosphorus content of the oil (ppm)
2.5	280 ± 20
3.3	50 ± 1.0
5.0	47.5 ± 2.5
6.7	25.4 ± 1.5

The results are the mean values and standard deviations of 6 replicates

3. Conclusions

The Szego mill proved effective as a grinder and contactor for the processing of canola by the methanol–ammonia–water process developed in our laboratories.

The results suggest that the semi-pilot-scale production of glucosinolate-free meal can be carried out at a lower solvent-to-seed ratio ($R > 3$) and a shorter quiescent period ($t > 5$ min) than with the laboratory preparation. Similar yields of oil and gums were obtained. The compositions of the meals differed only slightly except in the phenolic acids content, for which the Szego mill gave significantly better results at higher R values. Phosphorus is effectively removed at higher R values from the oil resulting in an oil equal or superior to conventionally produced degummed canola oil.

The viability of the two-phase solvent extraction system depends primarily on the technical and economic feasibility of the extraction and solids recovery steps of the process. Research must now be directed toward the pilot scale optimization of the hexane extraction step using innovative equipment, perharps liquid cyclones. A thorough engineering evaluation of the process must include a review of the energy requirements of the system, which may provide an economic advantage to this approach.

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This work was partially supported by Agriculture Canada through the ERDAF program. The assistance of Ms. D. SIDAWAY-WOLF is appreciated.

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IN MEMORIAM K. VAS

Acta Alimentaria, Vol. 16 (2), pp. 183—204 (1987)

K. VAS MEMORIAL MEETING ON FOOD IRRADIATION

ORGANIZED BY

THE HUNGARIAN NATIONAL IUFOST COMMITTEE

and

THE JOINT COMPLEX COMMITTEE ON FOOD SCIENCE OF THE HUNGARIAN ACADEMY OF SCIENCES AND THE HUNGARIAN MINISTRY OF AGRICULTURE AND FOOD

Budapest, Hungary
26 September 1986

OPENING SPEECH

P. A. Biacs[a]

On behalf of the Hungarian Academy of Sciences and its Committee on Food Science, the Hungarian Scientific Society for Food Industry and the Central Food Research Institute of the Ministry of Agriculture and Food, I thank you for accepting our invitation to the memorial meeting which is devoted to Professor Károly Vas who passed away 5 years ago.

We are very much honoured with the participation of the president and general secretary of the International Union of Food Science and Technology, Richard Hall and Jack Kefford, high officers of an organization which was founded with active participation of Professor Vas, being then its executive committee member and chairman of congress advisory committee for a number of periods.

I wish to express our thanks to Paisan Loaharanu, Head of the Food Preservation Section of the Joint FAO/IAEA Division at the International Atomic Energy Agency, who is present to deliver a lecture on Professor Vas' activities, his role in the international development of food irradiation. This is an international cooperation of many countries which was strenghtened by Professor Vas during his official duties spending 6 years in Vienna at this United Nations Organization.

This is a memorial meeting to which the widow and the son, relatives, and friends of the late Professor Vas have gathered from the near Austria to the far Mexico. But it is an occasion when scientists give a survey on the development of food irradiation the subject to that most valuable contribu-

[a] General Director of the Central Food Research Institute, H-1022 Budapest, Herman O. út 15. Hungary.

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

tions were given by Professor Vas. I do hope this meeting will be a milestone on the way we are going together to fulfill his expectations.

Lectures of this meeting are held in English, one of the languages of the international community of scientists, for which he always encouraged young and senior scientist to use. But comments and remarks after lectures could be given in Hungarian, in our mother tongue.

Now I ask Professor Holló, Chairman of the Committee on Food Science, President of our scientific society to hold his lecture with the title:

"Prof. K. VAS — a leading personality
of the Hungarian food science".

PROF. K. VAS — A LEADING PERSONALITY OF THE HUNGARIAN FOOD SCIENCE

J. Holló[a]

Károly Vas was born in Magyaróvár, Hungary on 20 August, 1919. His father, head of the Institute of Dairy Farming, Magyaróvár, was a pioneer in introducing modern food research in Hungary. This family background and home atmosphere, naturally, had a deep impression on the young Károly Vas and a decisive role in the choice of his career.

He obtained his degree in chemical engineering from the Budapest Technical University in 1941, and worked at the Department of Food Chemistry as an assistant till 1948. He received the PhD degree and started research activities at the National Experimental Institute of Agricultural Industry in 1944. Here he had the opportunity in 1947–1948 to accept a fellowship granted by the University of Chicago and University of California, USA, and subsequently, to go on a scholarship to England (Cambridge University, and Low Temperature Research Station, Cambridge). The work performed in latter Institute under the guidance of, and in cooperation with Professor Ingram was particularly decisive in his further scientific activities and professional career.

In 1948 he was appointed head of the Department of Microbiology of the Research Institute for the Canning-, Meat- and Refrigeration Industries. On the basis of his former activities he was granted the Candidate's Degree of Chemical Sciences in 1952 and after defending his thesis on "Physical and chemical methods for protection against microorganisms causing the deterioriation of foodstuffs", he received the Doctor's Degree of Chemical Sciences in 1956.

[a] Member of the Hungarian Academy of Sciences; Director of the Central Research Institute for Chemistry of the Hungarian Academy of Sciences, H-1025 Budapest, Pusztaszeri út 59–67. Hungary.

In 1959 he was appointed full professor and head of the Department of Food Technology and Microbiology of the University of Horticulture. He was elected corresponding member of the Hungarian Academy of Sciences in 1964 and held his inaugural lecture on "Some chemical and physical methods of food processing" in 1967. From 1967 through 1981 to the day of his death on 22 November he worked as a director of the Central Food Research Institute and also held the post of honorary professor at the University of Horticulture, where he was conferred the title of Honorary Doctor of Horticultural Sciences in 1979. He was dedicated to teaching throughout his life, following with interest the progress of his young colleagues year after year. He was always correct and direct in all his activities and manifestations: a precise and straightforward approach and an unambigous, clearcut answer were typical of him. His work as head of institute and department was suspended for the periods 1964–1966 and 1972–1978 when he filled a post at the United Nations, carrying out activities in international science organization, in charge of the Food Preservation Section of the Department of Food and Agriculture of FAO/IAEA.

He made considerable contribution to science organization also as a member of various international societies. He was president of various institutions: Complex Committee on Food Sciences of the Hungarian Academy of Sciences/Ministry of Food and Agriculture; Hungarian Committee of Food Codex; Hungarian National Commission of PUGWASH; member of the presidium of the Scientific Association of the Hungarian Food Industry and president of the Microbiological Section of the same institution; member of the Executive Committee of the International Union of Food Science and Technology (IUFOST); the European Society of Nuclear Methods (ESNA); Division of Applied Chemistry of IUPAC, and the Hungarian Microbiological Society; national representative in the International Union of Microbiological Societies (IUMS), honorary member of the Austrian Association of Food and Biotechnologists, and served on the Editorial Board of Confructa, a scientific journal published in Frankfurt. He was the founding, and highly demanding chief editor of Acta Alimentaria, a scientific journal published in English with the objective of gaining international recognition for results attained in Hungary in the field of food science.

He had only one passion, his work, and fully devoted his life and activities to scientific research. He performed his work with unflagging zeal, driven by a genuine desire for knowledge, planning experiments and collecting information with the precision and profoundness of a true scholar, with a humble appreciation and honour towards science.

From the very beginning of his career, the leading motive of his scientific activities was the preservation of food and food quality and the elimination of deterioration through a better knowledge of the factors causing it. He always

selected the most up-to-date methods. His first activities concerned the deterioriation of milk, dairy products and fats and, with this in view, he was among the first to apply paper chromatography and partition-chromatographic methods to the isolation of organic acids. Ripeness being an essential factor in preservation, he focussed interest on determining the degree of ripeness. For the same reason he was interested in cell density measurements by turbidimetric methods, solidity measurements of tomato puree, determination of equilibrium relative humidity, water activity measurements, micro fermentation tests or the Howard number characteristic of microbial infection. He was among the first to apply biometry, modern mathematical-statistical evaluation of measurement results in Hungary.

Early recognition of the significance of microbiological research in the preservation of foodstuffs also goes to his credit. In addition to its theoretical significance, a closer knowledge of the correlation between the formation, resistance and decay of bacterial spores is a basic condition of the further development of preservation industry technologies. The conversion of resting spores into sensitive cell state prior to food preservation treatment, combined application of sporacid physical or chemical factors determining spore dormancy, germination and resistance may also be credited to the activities of K. Vas and his school.

Among the various factors determining spore germination, the study of the effect of cell concentration, nutrient concentration, temperature and pH value is of fundamental significance. The results of his investigations related to different germinants and the sensitivity of spore germination to salt, initiated by these germinants, indicate that at the initial stage the mechanism of spore germination is not necessarily identical in the various media. K. Vas and co-workers have also shown that a lethal dose of irradiation rendering spores incapable of colony production does not deactivate the enzymes participating in germination and, furthermore, that larger doses of irradiation lead to pseudogermination changes in the spores.

In the investigation of the death kinetics of bacterial spores, the kinetic interpretation different from the exponential is the result of K. Vas and his school, often referred to in the literature. His studies of the irradiation resistance of bacterial spores and the relationship between structural and chemical parameters determining the spore state are also of fundamental importance, and his interpretation of the resistance of the spores of highly irradiation-resistant *Clostridium botulinum* strains is particularly essential.

A study of the combined effect of antimicrobial agents led to both theoretically and practically important results, verifying that irradiation greatly decreases the heat tolerance of the surviving spores, whereas heat treatment followed by irradiation has an additive effect. Based on these results, it could be verified that a scientific combination of sporocid physical and sporastatic

chemical factors gives better protection against bacterial spores, with lower energy requirements, and better product quality can be attained than by separate application of the individual factors.

There is hardly any food product not subjected to microbiological investigation by K. Vas, who studied: tomato puree, salted tomatoes, green peas, pickles, leaf vegetables, beets, fruit juice, fruit-juice preserves, plum juice, sauerkraut, strawberries, grapes, winter pears, peaches, cultivated mushrooms, malting barley, boiled potatoes, salami, fish and by-products.

K. Vas was an internationally acknowledged outstanding figure also in applying ionizing irradiation to food preservation. His activities in project organization and scientific diplomatic efforts offered an invaluable contribution to the standardization of the international trade of irradiated foodstuffs. Through his activities we acquired international recognition in the field of the irradiation of spices, enzyme products, dehydrated products and by practical introduction of the whole procedure.

In the early 1950-s K. Vas raised the idea of microbiological production of enzymes of industrial significance. Subsequently he sought to elucidate the mechanism and kinetics of enzyme production by fermentation, and as a result, the practical application of enzyme products obtained by a controlled technology has been realized: with the use of glucosoxydase, lactase, chimosine, endopolygalacturonase, endopolymethylgalacturonase, pectinelyase, and pectatelyase preparations in the production of cheese, fruit juice and tomato concentrates considerable improvement in quality and much lower energy input could be attained. Investigations were carried out under his guidance to study enzyme complexes in the mature tissue of fruits and vegetables, determining product quality and, especially, aromatic changes during storage.

At a public session of the General Assembly of the Hungarian Academy of Sciences in 1981, K. Vas gave a profound analysis of the current state of food research in Hungary, supplying results attained so far and outlining future tasks ahead of us. On the basis of this assessment, the Complex Committee of Food Sciences submitted a proposal to the Presidum of the Academy, the preparation of which he closely followed and directed with enthusiasm even when confined to bed by his mortal disease. It is particularly sad that he has not lived to see how the cause and further development of modern food industry research has been promoted by the Academy, indicating also the significance and predominant role of the food industry and research in our national economy.

We shall only live up to his memory if, following the route outlined by him, we do our utmost to expand the horizons for high level food science research and further development of the food industry he dedicated his life to.

INTERNATIONAL ACHIEVEMENTS OF PROF. K. VAS, HEAD OF THE FOOD PRESERVATION SECTION, JOINT FAO/IAEA DIVISION (1964–66; 1972–78)

PAISAN LOAHARANU[a]

It is an honour for me to speak on the international achievements of Dr. Károly Vas who was my former supervisor, my predecessor and my friend. I am speaking not only on my own behalf but also on behalf of the many colleagues and friends of Dr. Vas, who enjoyed working with him during his two assignments with the Agency.

The international achievements of Dr. Vas were many, including a post as an Executive Member of the International Union of Food Science and Technology (IUFoST). I shall, however, limit my talk to his international achievements while he was Head of the Food Preservation Section of the Joint FAO/IAEA Division from 1964 to 1966 and from 1972 to 1978. During these periods, he devoted his energy and efforts to further the cause of food irradiation which had brought this technology to the headline today. The following represents a summary of his achievements in food irradiation:

Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food (JECFI)

A major international undertaking which had paved the way for further development of food irradiation was the demonstration of the safety and wholesomeness of irradiated food. Dr. Vas was intimately involved in the organization and convening of all meetings starting from 1964, i.e.

— Joint FAO/IAEA/WHO Expert Committee on the Technical Basis for Legislation of Irradiated Food, Rome, 1964. This meeting recommended the general principles under which the production and use of irradiated food may be permitted. Also, it recommended the procedures that should be adopted for the evaluation of the wholesomeness of irradiated food.

— Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food, Geneva, 1967. This meeting recommended temporary approval (5 years) of irradiated wheat and potatoes.

— Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food, Geneva, 1976. This meeting recommended unconditional approval of irradiated wheat, potatoes, strawberries, papaya and chicken. Irradiated rice, onion and fish were recommended provisional approval. This

[a] Head, Food Preservation Section, Joint FAO/IAEA Division International Atomic Energy Agency, 1-1400 Vienna. Austria.

Committee also recognized that food irradiation is a physical process comparable to heat and refrigeration for food preservation.

— Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food, Geneva, 1980. This meeting concluded that ionizing energy treatment of food commodities with an overall average dose of 10 kGy (1 Mrad) causes no toxicological hazard; hence, toxicological testing of food so treated is no longer required. Irradiation also introduces no special problems with regard to nutrition and microbiological safety of treated food.

International Project in the Field of Food Irradiation (IFIP), Karlsruhe, Federal Republic of Germany

IFIP should be given full credit for co-ordinating and developing data on the wholesomeness of irradiated food since its inception in 1970. After the conclusion of the 1969 JECFI, IFIP was established by 19 countries initially under the aegis of FAO and IAEA through their Joint FAO/IAEA Division and the OECA. Dr. Vas played an active role in liaison with IFIP to develop a much needed data on the wholesomeness of irradiated food throughout IFIP's life-span, i.e. until 1980.

Codex General Standard for Irradiated Foods and Recommended Code of Practice for the Operation of Radiation Facilities Used for the Treatment of Food

To assist Member States to establish their legislation on food irradiation and to facilitate trade in irradiated food, Dr. Vas initiated actions as early as 1977 to develop a draft Codex Standard for Irradiated Food and a draft Code of Practice mentioned above. The two drafts were elaborated under the Codex procedure and were adopted by the Codex Alimentarius Commission in December 1979. The Codex Standard and Code of Practice were later amended to incorporate recommendations of the 1980 JECFI. The Codex General Standard for Irradiated Foods and the Recommended Code of Practice were adopted at final step in July 1983. Thus, foods treated by ionizing radiation up to an overall average dose of 10 kGy (1 Mrad) were recommended by the Codex Standard. The recommended sources of radiation include ^{60}Co, ^{137}Cs, electron at a maximum energy of 10 MeV and X-rays at a maximum energy of 5 MeV.

Regional Co-operative Agreement on Research, Development and Training Related to Nuclear Science and Technology (RCA)

A number of Governments in Asia and the Pacific had decided to establish the RCA in 1972 to strengthen the regional co-operation on nuclear science and technology. Under the supervision of Dr. Vas, Radiation Preservation of

Fish and Fishery Products (RPF) was the first project to launch RCA activities in 1973. Seven countries in the region, i.e. Bangladesh, India, Indonesia, Republic of Korea, Pakistan, Philippines and Thailand collaborated under the scope of this project from 1973 to 1978.

RPF had provided an excellent model for other regional projects organized under the RCA. It had generated significant amount of data on technological and economic feasibility of radiation preservation of fish. RPF, together with its successor — Radiation Preservation of Dried Fish Indigenous to Asia, had attracted the attention of several Governments in Asia. After the Japanese Government's accession to RCA in 1978, this Government decided to fund activities on food irradiation in the region under a new scope, i.e. Asian Regional Co-operative Project on Food Irradiation (RPFI), from 1980 to 1984 at a total cost of US $ 226 000. Eleven countries in the region participated in the RPFI.

Research and development on food irradiation conducted under the RPFI demonstrated that several countries in the region were ready to transfer the technology to local industry at the termination of the project in 1984. This type of activity is being conducted under the sponsorship of the Australian Government for 3 years from 1985 to 1987 at a total cost of US $ 260 000. Eleven countries are also participating in the second phase of RPFI (Phase II). It should be noted that commercial/demonstration irradiators for treating food and non-food products are under construction or are in an advanced stage of planning in Bangladesh, Malaysia, Republic of Korea, Pakistan and Thailand.

The success of food irradiation under the RCA had prompted the Agency to initiate similar activities in other regions. A Food Irradiation Programme for Latin America (LAFIP) has recently been initiated. Plans are underway to establish regional activities on food irradiation in Africa, Europe and the Middle East.

International Facility for Food Irradiation Technology (IFFIT), Wageningen, The Netherlands

Dr. Vas was actively involved in the establishment of IFFIT as a center for training and feasibility studies on food irradiation. After several years of negotiation, IFFIT was established on 1 January 1979 under the sponsorhip of FAO, IAEA and the Ministry of Agriculture and Fisheries of the Netherlands. IFFIT has organized 6 general training courses on food irradiation, one of which was regional (Latin America) and one specialized course on public health aspects, supplied training facilities to 20 research fellows from 16 countries and was involved in the evaluation of the quality of trial shipments of

irradiated mangoes, spices, avocadoes, shrimps, onions and garlic from developing Member States. A total of 150 scientists from 42 countries have participated in the 7 IFFIT training courses which have been held during the past 5 years. Data from technological feasibility studies carried out by research fellows, and from the trial shipments, are contained in 46 IFFIT Reports produced during the past 4 years.

Food Irradiation Newsletter (F.I.N.)

Dr. Vas was the founder of our Food Irradiation Newsletter. Based on his personal interest and compilation of data on clearances on irradiated food by national authorities in the 1970's, he decided to publish such a list in the F.I.N. starting in 1977. The list became highly popular as national authorities, food industry, mass media, etc. often referred to it as a source of information. At present, 32 countries have approved over 40 irradiated food items or groups of related food for consumption either on an unconditional or restricted basis. The Newsletter itself has become very popular as the distribution list expanded from approximately 150 at the time of its inception to over 1000 at present.

Commercialization of Food Irradiation

The untimely death of Dr. Vas had deprived him from witnessing the success of his own work. Although he was fully aware of the commercial scale irradiation of potatoes in Japan starting 1974 and some commercial activities in the Netherlands and South Africa in the late 1970-s, he would have been very pleased to learn that 18 countries are now using the technology to treat a variety of foods and ingredients according to Table 1. In addition, more than 10 other countries are constructing commercial/demonstration irradiators for treating food and non-food items in their countries (Table 2).

Conclusions

The foundation on food irradiation which was built on the initiative and ingenuity of Dr. K. Vas is beginning to produce a positive impact. His foresight and plans have resulted in an international standard for irradiated food which is being considered favourably in many countries. An increasing number of countries are using food irradiation on a commercial scale. Dr. Vas' dream of food irradiation as a method for reducing food losses, facilitating international trade and improving public health of food, has become a reality.

Table 1

Commercialization on food irradiation

Nation	Company (city)	Food item (tonnage)	Starting date
Belgium	IRE (Fleurus)	Spices (350 t/y), Dehydrated vegetables (700 t/y), Deep-frozen foods (2000 t/y)	1981
Brazil	EMBRARAD (Sao Paulo)	Spices, Dehydrated vegetables	1985
Chile	CCHEN (Santiago)	Onions (200 ~ 300 t/y) Potatoes (50 ~ 100 t/y), Spices & dehydrated vegetables (20 ~ 30 t/y)	1983
China	Nuclear Research Institute (Shanghai)	Potatoes	1985
Denmark	Riso National Laboratory	Spices	1986
Finland	KOLMI-SET Oy (Ilomantsi)	Spices	1986
France	Conservatome (Lyon)	Spices (500–600 t/y)	1982
	Caric (Paris)	Spices, poultry (300 t/y)	1986
German Dem. Rep.	Cent. Inst. Isotop. Radiat. Res. (Weideroda)	Onions (600 t/y) Garlic (4 t/y)	1983
	Queis Agric. Coop. (Spickendorf)	Onions (4000 t/y)	1986
	VEB Prowiko (Shoenebeck)	Enzyme Solution (300 t/y)	1986
Hungary	AGROSTER (Budapest)	Spices (200 t/y) Wine cork (100 m^3)	1982
Israel	Sorvan Radiation Ltd. (Yavne)	Spices (120 t/y)	1986
Japan	Shihoro Agricultural Co-operative (Hokkaido)	Potatoes (20 000 t/y)	1973
Korea, Rep. of	Korea Advance Energy Research Inst. (Seoul)	Garlic powder	1985
Netherlands	GAMMASTER (Ede)	Spices (100 t/y) Frozen products Poultry, spices Dehydrated vegetables Rice, dehydrated blood Egg powder Packaging materials	1978
	Pilot Plant for Food Irradiation (Wageningen)	Spices	1978
Norway	Institute for Energy Technology (Kjeller)	Spices (500 t/y)	1982
South Africa	Nuclear Development Corporation ISO-STER	Fruits, meats, onions Potatoes Spices Dehydrated vegetables	1981
	High Energy Processing	Fruits	1982
USA	Radiation Technology, Inc.	Spices (500 t/y)	1984
	Isomedix, Inc.	Spices (500 t/y)	1984
	Radiation Sterilizer, Inc.	Spices (500 t/y)	
USSR	Odessa Port Elevator RDU (Odessa)	Grains (400 000 t/y)	1983
Yugoslavia	Ruder Boskovic Institute (Zagreb)	Black pepper	1985

Table 2

Commercial facilities (single and multipurpose) under construction or being planned
(September 1986)

Member states	Number of facilities	Location
Australia	1	Brisbane
Bangladesh	2	Dhaka, Chittagong
Canada	1	Quebec
China	4	Langzhou, Beijing, Zhen Shen, Zhenzhou
Cuba	1	Havana
France	4	Bretagne, Marseille, Orsay-Cedex, Orleans
Hungary	1	Budapest
Israel	1	Yavne
Italy	1	Fucino
Korea, Republic of	2	Seoul
Malaysia	1	Kuala Lumpur
New Zealand	1	Auckland
Pakistan	1	Lahore
Poland	2	Poznan, Przycucha
Thailand	1	Bangkok
USA	5	Washington, Ames (10), Oklahoma, Honolulu, Florida
Vietnam	1	Hanoi
Total	30	

PROF. K. VAS — PIONEER OF FOOD IRRADIATION RESEARCH AND DEVELOPMENT IN HUNGARY

J. FARKAS[a]

It is my great honour to commemorate Professor Vas and his pioneering activities on food irradiation research and development in Hungary, since I was fortunate to join him soon after my graduation when he was starting to set up the first food irradiation laboratory in this country in 1957, at the Institute for Research in Canning, Meat Packing and Refrigeration, Budapest, where he served then as head of the Microbiology Department. The founding director of that Institute, the late Dr. Gábor Török, was also a far-sighted

[a] Professor and Director of the Institute of Food Technology, University of Horticulture and Food Industry, H-1118 Budapest, Ménesi út 45. Hungary.

leading food scientist with broad scientific and practical interest, and he wholeheartedly supported Dr. Vas' inventive approaches in exploring future food processing techniques.

Of course, our limited resources did not allow large investments to establish a large radiation source, however, a 250 kV, 15 mA X-ray machine has served very well the needs of laboratory experiments in the first few years, while co-operations with the Central Research Institute for Physics and the Plastics Research Institute, Budapest, gave us accesses also to their electron accelerators and a small panoramic ^{60}Co source.

It was characteristic of Dr. Vas that he could turn his attention simultaneously to basic and applied aspects of research topics. While he was e.g. devotedly interested in the basic mechanism of radiation resistance of microbial cells, he was able to select at the same time also practical fields of potential application areas of the process. Thus, our first research topics have covered studies on effects of ionizing radiations on yeasts and bacterial spores including the mechanism of their radiation damage (VAS & FARKAS, 1961), but also the radiation resistance of some insects and mites of great importance to several stored products was also one of the early research targets (TÖRÖK et al., 1959). At the same time, radiation decontamination of spices, particularly paprika, was already under investigation during those early years of this pioneering food irradiation laboratory.

In 1959, when the Central Food Research Institute was founded, our laboratory continued to work in the new Institute, which has remained the chief workplace of Hungarian food irradiation research until the present days. Although Dr. Vas has been promoted soon to the University of Horticulture, to the Chair of Food Technology and Microbiology, he never ceased to co-operate with us even during his years of full professorship at the University, or during his subsequent work at the IAEA, serving for us as a source of inspiration, scientific guidance and support.

From the early days of the Hungarian food irradiation research, he has built up systematically a well-trained scientific team dealing with this subject. Ha has used every opportunity to help his young associates to receive grants and fellowships, which have provided excellent opportunities to them for work for some time in leading food irradiation research centers abroad, e.g. in Karlsruhe, Wageningen, Seibersdorf, or Langford. Thus, within several years, and with the highly esteemed assistance of the IAEA, a number of young Hungarian scientists have received valuable post-graduate training, and have developed life-long motivation and helpful international contacts. In the course of years several of them became internationally recognised experts in this challenging field of research and development.

This scientific internationalism has facilitated a co-operation already in the early phase of food irradiation development also with some of our Eastern

European partner institutions, e.g. in the Soviet Union, Czechoslovakia, Poland and Bulgaria, whose leading experts have considered Professor Vas also as an international authority.

Although Professor Vas has devoted his broad interest and seemingly inexhaustible energies to several other fields of food science and technology, and has had commitments far beyond our specific area of work, due to his legendary diligence, managing abilities and enthusiastic leadership, this teamwork has resulted a significant output, and at the end of the sixties, when he has returned to the Central Food Research Institute and has been appointed to be its Director, the aforementioned studies have provided valuable information on feasibility of radiation treatment of almost all commodities, which could be good candidates for this process in the Hungarian national economy (Kovács et al., 1968; Vas, 1972). His inventive mind gave the initial idea and impetus to a number of non-conventional radiation applications, too, where Hungarian studies were then among the pioneering ones, such as the radiation decontamination of enzyme preparations (Vas, 1960; Vas & Proszt, 1965), controlling germination of malting barley (Farkas et al., 1963a), or increasing juice yield of grapes by irradiation. His great interest in combined preservation methods has resulted research projects on combination processes in food preservation already in the late fifties and early sixties (Vas & Farkas, 1960; Farkas et al., 1963b), and resulted in a scientific school of thought (Vas, 1981) which keeps together Dr. Vas' former students independent of their subsequent administrative positions or affiliations.

His deep interest in the storage physiology of fresh fruits and vegetables has provided an initiative to another fruitful area of radiation research of fresh commodities (El-Sayed et al., 1974; Kovács & Vas, 1974a; 1974b), although the enzymological aspects of food irradiation whose importance he has stressed very much (Vas, 1966) received wantonly low attention subsequently. Typical of him was his rational approach thinking always in quantitative terms (Vas, 1983) and demanding always a statistical design and biometrical evaluation of experiments, including shelf-life studies (Vas, 1971) and sensory testing of products (Farkas et al., 1961; Kovács & Vas, 1974), as well as understanding the eminent importance of proper dosimetry both in radiation research and radiation processing (Vas et al., 1978; Vas, 1983).

Thanks to his high reputation, his tireless efforts and project-organizing activities, time was ripe at the end of the sixties to establish a food irradiation pilot plant in Budapest under the aegis of the Central Food Research Institute. This pilot plant was one of the earliest among such facilities in Central and Eastern Europe. It is utilizing the potentialities of a rock cellar system in Kőbánya, an industrial district of Budapest. This facility is entirely a Hungarian construction and it served very well the up-scaling several food

irradiation applications in the meantime. It has presently approx. 4.4 PBq ^{60}Co activity, and it is functioning since the last four years as an independent service irradiator company, called AGROSTER, backed-up by the Ministry of Agriculture and Food and the National Committee for Technical Development. Another semi-commercial irradiation facility which enabled upgrading the experiments of the Central Food Research Institute into larger scale storage and marketing trials with irradiated onions, is a bulk cargo irradiator, which has been constructed by the Institute of Isotopes of the Hungarian Academy of Sciences and it has been operated over several harvesting seasons at a large agricultural co-operative in Rákóczifalva.

Dr. Vas has professed and actively adopted the principle that science does not consist only of the acquisition of knowledge and observation of phenomena but it also means that the results have to be made public property. As founder, editor or editorial board member of various periodicals and journals he contributed to the propagation of scientific research at home and abroad. He encouraged always and assisted selflessly his co-workers and students to communicate their experiences with others. This attitude has contributed greatly to the fact that Hungarian authors published about 600 papers during the last thirty years in the field of food irradiation. Personally, I consider the launching in 1972 Acta Alimentaria, a periodical in English language, as one of the most far-reaching achievements of Professor Vas as chairman of the Committee on Food Science of the Hungarian Academy of Sciences. This journal which he has edited so devotedly for its first ten volumes, i.e. until his premature death in 1981, this journal became in 1984 an international quarterly under the chief editorship of Professor Holló. There is no doubt that Professor Vas had an unusual flair for editing which, coupled with his considerable scientific learning, immense patience and attention to detail was of inestimable value both to the Journal and to its contributors, as those of us who have submitted papers for his editorial scrutiny, are well aware. This periodical has provided and continues to provide an excellent forum also for the Hungarian food irradiation research. We are proud that such articles are well received by the international scientific community.

During the nineteen-seventies, again as a result of Prof. Vas' scientific diplomacy and far-sighted incentives, Hungary was able to join the International Project in the Field of Food Irradiation (IFIP, Karlsruhe). In this very successful international cooperation on wholesomeness testing of irradiated food, which played a decisive role in providing scientific information to the Joint FAO/IAEA/WHO Expert Committee's conclusions on safety of irradiated food, a milestone in the whole development of the process, our membership was justified by an "in-kind" contribution of the Central Food Research Institute, executing wholesomeness testing of irradiated spices for several years.

Professor Vas' diplomatic skill and promoting work helped significantly even the implementation of radiation sterilization of medical supplies in Hungary, when a commercial radiation sterilization plant was set up at the MEDICOR Co., Debrecen, under the sponsorship of the IAEA, the United Nations Development Programme and national governmental offices.

I tend to consider it as an almost symbolical fact that one of his last achievements in science policy was his leadership in a national ad hoc working group on food irradiation. This working group has prepared a report in 1981 on status and perspectives of this process for the National Committee for Technical Development. The report (VAS, 1981a) summarized domestic and international experience in food irradiation, and suggested the establishment of a central industrial-scale food irradiation plant in Budapest. On the basis of this report, the Hungarian authorities agreed to introduce this technology on an industrial scale within the next five years.

I believe, the world-wide progress in food irradiation, as it was so excellently illustrated by Mr. Loaharanu, is a rewarding monument also for Professor Károly Vas, this outstanding scientist and man, with an extraordinary width and depth of scientific knowledge, who so devotedly put himself behind national and international development in this field. We, his pupils and co-workers, who had the happy privileges of his friendship and of working under him, remember him with respect and gratitude, and are happy that were fortunate enough to strive for the same goals under his able leadership. We shall serve best his memory when we try to continue contributing to further scientific progress and technical development with devotion and honesty, as could be learned from him.

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Abstracts

of papers presented at the

SCIENTIFIC MEETING IN MEMORIAM K. VAS

organized by

THE SECTION OF MICROBIOLOGY, BIOTECHNOLOGY AND HYGIENY OF THE HUNGARIAN SCIENTIFIC SOCIETY FOR FOOD INDUSTRY (MÉTE)

and

THE FOOD MICROBIOLOGICAL WORKING GROUP OF THE JOINT COMPLEX COMMITTEE ON FOOD SCIENCE OF THE HUNGARIAN ACADEMY OF SCIENCES AND MINISTRY OF AGRICULTURE AND FOOD

Budapest
25 November, 1986

SCIENCE ORGANIZATION ACTIVITY OF K. VAS

P. A. Biacs[a]

K. Vas was member of the Hungarian Academy of Sciences, professor of the University for Horticulture, director of the Central Food Research Institute, an outstanding personality of Hungarian food science. He had many sided science organization ability and did a lot in the interest of co-ordinating, developing the Hungarian food science and in creating foreign relations. At the Hungarian Academy of Sciences he brought into being a forum (1967) for the researchers working at different parts of food science, where in each month research results are regularly presented, discussed and mutually evaluated at Scientific Colloquia. In 1971 he founded "Acta Alimentaria" quarterly, journal of food science which is published jointly by Akadémiai Kiadó, Budapest and D. Reidel Publishing Company, Dordrecht and Boston. The journal formerly introduced Hungarian research results and today is an international journal with international Editorial Board.

In order to promote the co-operation between food research and food economic management he organized the *Food Scientific Complex Committee* (1976) including the Hungarian Academy of Sciences and the Ministry of Agriculture and Food, which has at present 8 Working Groups. In the interest of Hungarian science, in social organizations he utilized the possibilities of direct work connections, as member of executive committee in the *Hungarian Scientific Society for Food Industry* (MÉTE) and first chairman in the

[a] Central Food Research Institute, H-1022 Budapest, Herman O. út 15. Hungary.

Microbiological Section. To promote the international relations of Hungarian food researchers he took part in the activities of several international organizations, organized scholarships and participations at international conferences and arranged also their organizations in Hungary.

He was master of Hungarian research generations. His unbroken love of work, working ability, professional enthusiasm, thoroughness, accuracy may serve as model example. His memory may only be reserved if the treasure he left to us is fully increased.

SPORE RESEARCHES OF K. VAS AND MAIN RESEARCH TRENDS IN CONNECTION WITH BACTERIAL SPORES OF FOOD INDUSTRIAL IMPORTANCE

J. Farkas[a]

K. Vas in collaboration with his excellent colleague, Gizella Proszt performed internationally recorded research work with bacterial spores highly resistant against antimicrobial effects, between 1954 and 1958. With this, as in case of several other research activities he created a national scientific school.

His spore researches extended to the effect of water activity on the spore formation velocity, and to the germination and heat resistance of spores as affected by pH, some antibiotics and other antimicrobial agents. He also studied the role of cell density and nutrient concentration which influences spore germination.

The lecture commencing with K. Vas' activities surveyed the scientific results, which in the last 30 years lead mainly to the better acknowledgement of connections of structural and physico-chemical factors determining spore resistance and dormancy and which mean valuable information for the representatives of several fields from molecular-biologists to food technologists.

MICROBIOLOGICAL BIOCHEMICAL RESEARCHES BETWEEN 1967–1986

A. Halász[b]

From our microbiological, biochemical research activities three subject fields were chosen. The single cell protein (SCP) production on unconventional carbon resource, on hydrocarbon substrate was one of the world wide actual

[a] Institute of Food Technology, University for Horticulture and Food Industry, H-1118 Budapest, Ménesi út 45. Hungary.
[b] Central Food Research Institute, H-1022, Budapest, Herman O. út 15. Hungary.

research themes of the 60s. The microbiological utilization of methane raised — in addition to gas phase carbon source — also the problem of the probable accumulation of intermediate metabolites and the auto-inhibiting effect of the same. It was stated that in the C_1-metabolism of facultative methane utilizing bacteria, alcohols and aldehides of longer carbon chain were also produced. Certain metabolites in the highest concentration we detected did not result inhibition of cell growth, and the long apparent generation time may be attributed to substrate limitation.

Regarding the human essential amino acid requirement, the methionine concentration of yeasts is low. Methionine enriched yeast strains were successfully prepared with weak mutagens (UV radiation and nitrite treatment). The increased sulphate demand was utilized at mutant selection. Our examinations to methionine antagonist showed higher sensitivity in mutant than in parent strain. Addition of methionine homologue resulted colonies of higher diameter which is the result of better methyl donor provision.

In the strains rich in methionine the lipoic acid concentration increase was noticed and both components decreased in fermentations faster aerated. Comparing sulphate and methionine as sulphur source, the former proved to be more favourable. Our results prove that in the mutants we selected, the methionine content increase may be attributed to the more effective sulphate reduction.

Our results showed that the successful cellulose destruction demands the proportional presence of certain components of the cellulase enzyme complex.

The cellulase complex of four cellulase producing mould strains were investigated in detail. Cellulases excreted into the medium were characterized by isoelectric focusing (IEF), SDS-gel electrophoresis and immunoelectrophoresis. Each specific cellulase activity was determined both from the enzyme complex and in the preparative IEF-fractions. It was found that the cellulases gained by disruption of mycelia may be separated into less fractions with IEF and their molar mass distribution falls to higher molar mass range. The specific enzyme activities of mycelia extracts are lower in comparison to the enzyme secreted into the medium. The endoprotease activity values and the characteristics determined by immunoelectrophoresis indicate that cellulases within the cell are generated as a proenzyme of high molar mass from which the active form originates with the assistance of protease.

FOOD IRRADIATION IN HUNGARY AND ITS INTERNATIONAL EFFECT

I. Kiss[a]

Researches on the field of food irradiation were initiated in Hungary by Professor K. Vas nearly 30 years ago. Having started with systematical researches already in 1958, Hungary's quick join into the international research work was provided.

Author surveyed K. Vas' activities from the aspects of the elaboration and introduction of food irradiation technologies into practice.

Researches launched at the Central Food Research Institute with a so called Stabil 250 industrial X-ray equipment. The first irradiation pilot plant was built in 1968 and installed in 1970 in Budapest. The plant was completed by a suspension conveyor system in 1971. Its whole activity is 2.2 PBq Co-60 with 0.4 kGy/ton/h capacity. In 1971 a Co-60 laboratory radiation source of 0.7 PBq activity was also installed. In 1979 — to the initiatives of the Central Food Research Institute — by the support of the National Committee for Technical Development (OMFB) — an onion irradiation target equipment was built first in the world at Rákóczifalva (with an output of 0.5 kGy/ton/h). Between 1980–81 AGROSTER Food Irradiation Enterprise was created (4.5 PBq activity at present).

In the meantime the scientific work has extended to basic and applied researches as well. The feasibility of food irradiation was investigated practically to all kind of foods. Nearly 600 publications contain the results. A special laboratory was set up in the Central Food Research Institute to carry out the wholesomeness studies of irradiated food with biological tests.

The continuous experimental marketing tests of irradiated potatoes, onions, various fruits, mushrooms, frozen chicken, spices, cereal products, etc. also aimed at the introduction of research results into practice.

Hungarian researchers utilize their theoretical and practical experience not only in Hungary but also internationally (IFFIT, IAEA) by giving professional expertise and receiving scholars.

The high knowledge and school creative activity of Professor Vas had an imperishable role in the research results achieved in Hungary and internationally acknowledged.

[a] Central Food Research Institute, H-1022 Budapest, Herman O. út 15. Hungary.

BIOTECHNOLOGICAL RESEARCH IN SERVICE OF THE FOOD INDUSTRY

K. Zetelaki–Horváth[a]

In a 23 year research period, technologies for the production of 6 enzymes [glucose oxidase, constitutive and inducible polygalacturonase (PG) enzyme complexes, endo-PG, pectin lyase and lignocellulase], mycelial biomass for food purpose (by 3 microfungi and 2 edible mushrooms) have been developed and patented. In the biotechnological research of the above technologies, directed by K. Vas, agitation and aeration showed a significant effect on the respiration, enzyme and biomass production and the energy requirement of different fungi. In the production of a pectolytic enzyme complex, the increase of agitation speed resulted in an increased pectin lyase synthesis at the expense of pectin esterase.

Kinetics of growth and product formation of fungi has extensively been studied for protein production and enzyme synthesis, as well as for the prediction of enzyme formation in continuous culture.

In the case of downstream processes a pilot scale cell disruption method for the extraction of intracellular enzymes was succesfully developed.

With the use of the macerating endo-PG, solubilization of vegetables and fruits has been investigated. Particle size of the enzymatically disintegrated tissues has been determined by dispersoid analytical method. From the enzymatically solubilized vegetables and fruits, fibrous cocktails of nutrition physiological value have been produced.

In the case of protein production, amino acid content of the fungal mycelia as well as biological values have been determined.

Concerning fermentations, besides the use of batch and continuous submerged methods, semi-solid semi-continuous and solid-continuous fermentation methods have been developed for the utilization of lignocellulosic agricultural wastes.

Enzyme kinetics of the produced enzymes have also been the subject of several researches.

The aim of the above research established by K. Vas (to improve the quality and shelf-life of food products, to increase the economy of food processing to produce new foods) were solved through extensive microbiological, biochemical and biotechnological studies.

[a] **Central Food Research Institute, H-1022 Budapest, Herman O. út 15. Hungary.**

MICROBIOLOGICAL ECOLOGY AND MICROBIOLOGICAL CONTROL OF FOODS

I. FÁBRI[a]

The microbial ecology was established by K. Vas in the Hungarian food sciences in the 1950s. K. Vas was in close connection with several well-known food microbiologists; among them his best friend was Prof. M. Ingram, founder and president of the International Commission of Food Microbiology and Hygiene. By means of adoptation, the results of food microbial research improved or started the microbiological research investigations and quality control in several branches of the food industry in Hungary in the 1950s. Microbial ecology is essential in developing the products or the production, moreover to accomplish the preventive food control, the so called Hazard Analysis Critical Control Points (HACCP)-system.

The actual work and the future tasks are the following: 1) to establish the microbial criteria of the microbiologically sensitive foods and their HACCP-system, 2) to develop the appropriate mathematical-statistical methods for the elaboration of methods and specifications and to work out the inspection and quality control of food chain to assure the safety, shelf-life and quality of food products, 3) to introduce the rapid methods to increase the "in plant control" efficiency, 4) the economic calculation of control cost/benefit ratio, 5) training and education.

The Hungarian food microbiologists are in connection with several international food microbiological organizations, e.g. the Joint FAO/WHO Codex Alimentarius Commission, the ICMSF, ICFMH, ISO: International Commission of Microbiological Specification of Foods, International Committee on Food Microbiology and Hygiene, International Standardization Organization, etc. These scientific contacts and the work of young talented experts will be helpful in fulfilling the programme.

[a] Veterinary and Food Control Centre, H-1095 Mester u. 81. Hungary.

Acta Alimentaria, Vol. 16 (2), pp. 205—208 (1987)

BOOK REVIEWS

Toxicologie et securité des aliments

R. DERACHE (Ed.)

Technique et Documentation, Lavoisier and APRIA, Paris, 1986; 594 pages

The work team consisting of 21 authors — including the editor — serves the readers with a comprehensive survey on the toxicology and safety of food. The book is a volume in a series initiated by the Association pour la Promotion Industrie-Agriculture (APRIA) providing information on the technical and production problems of the industry related to agriculture. The volumes published so far treated milk products, food analysis, preservation and storage of cereals, plant and animal protein, additives and ingredients and problems related to the quality of foods.

Food toxicology relies to a large extent on other disciplines. These are: structural chemistry, biochemistry, biophysics, genotoxicity, complex pathology including cancerogenity, analysis of contaminants, nutrition biology.

The chain of nutrition forms an extremely large unity from production to the consumer every link of which being of toxicological significance.

The book treats first the basic substances of nutrition (carbohydrates, fats, proteins, vitamins, minerals, water) from a biochemical aspect. The second subject treated comprises the main types of food materials (meat, fish, egg, milk, fats, cereals, vegetables and fruits, sugar, sweets, drinks). After giving a short survey of digestion and absorption, followed by the discussion of energy and nutrient requirement, the specific significance of the proportion of different nutrients is stressed. The subject is treated in a correct manner understandable even to people not versed in problems of biology. The chapter was compiled by B. CARRERA.

The chapter on the mechanism of toxic effect begins with an exposition (profile of food consumption, physical and chemical properties of foods, absorption) continues with toxicokinetics (metabolic transformations, changes in toxicity within the organism, secretion) and ends by the toxicodynamic phase containing the mechanism of toxic effect. The author of this chapter is PH. DERACHE.

Toxicity — in the most general sense — means the capacity to produce pathological effects in the living organism. That is why "in vivo" toxicological tests are extremely important as surveyed in the next chapter by R. GLOMET. Selection of test animals, determination of the dose and the experimental period, recording of the experiments, acute, subacute-subchronic and chronic observations and certain specific tests (sensibilisation of the skin, cancerogenity, reproductivity, morphological and laboratory analyses), are discussed.

In the chapter on rapid mutagenicity tests R. DERACHE stresses that these tests, carried out on bacteria, yeasts, maybe drosophila or rodents, carry information on cancerogenity. The results thus obtained call for most severe criticism. Particular care has to be taken when interpolating the results to men since mutagenicity is only one of the causes of malignant tumors.

The significance of the analysis of toxic residues and their limitations are demonstrated on pesticides by R. MESTRES. The concept of the permissible maximum quantity of residue and the acceptable daily intake (ADI) is determined, emphasizing, however, that no direct relationship exists between the two.

The next chapter describes the native toxins in foods, first the antinutritive ones, then the toxic compounds, carcinogens and substances of hormonal effect. The author is S. MITJAVILA.

The chapter on metals, written by CL. BOUDENE, treats the presence of lead, cadmium, mercury, arsenic, tin, aluminium in foods, their absorption, the interaction with

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

other elements, with nutrients, their toxicity and the ways and means of preventing poisoning.

PH. DERACHE and R. DERACHE surveys poisonous fungi. Mushrooms may be dangerous themselves, while some yeasts, moulds, etc. form mycotoxins and contaminate with them otherwise edible food materials. Their detection in foods is more (aflatoxin, patulin, zearalenon, achratoxin) or less (trichotecenes, rubratoxin, etc.) reliable.

The next part of the book treats also toxic substances of biological origin: toxins produced by bacteria and bacterial contamination. The authors PH. LAFONT and J. LAFONT discuss botulism, salmonellosis, C. perfringens, B. cereus, E. coli, V. parahaemolyticus, Y. enterocolitica, and diseases caused by these microorganisms.

The chapter on additives brings the book back to the chemistry aspect of foods. G. DE SAINT BLANQUET and G. PASCAL decribe the history of additives, discuss the conception of additive, describe the technology of use, the ADI value and finally the toxicology of individual groups.

Nitrites and nitrates form environmental problem in the more general sense of the word and are connected to modern agricultural production and food technology. Although the reactivity of NO_2^- ion is known many problems are still not elucidated, as for instance the relation of NO_2^- to myoglobin, hemoglobin and some of the vitamins, the Perigo effect and its consequences as regards botulism, the actual conditions of nitrosamine synthesis. The concentration of nitrates increases in the environment, therefore a reduction in nitrite or nitrate incorporations remains a desire, however, the direct toxic effect: methemoglobinaemia can be prevented. The chapter was written by P. FRITSCH and G. DE SAINT BLANQUET.

G. BORIES introduces aromatic polycyclic carbohydrates and products of pyrolysis. These substances are potential cancerogens therefore it is essential to develop reliable analytical methods for their detection, to get to know the process of formation of heterocyclic amines in the digestive system, their stability, absorption and metabolism.

The residues of medicaments used in animal therapy form a problem of public health. A toxicological, allergetic, bacterial resistance awaking effect, cancerogen effect have to be reckoned with. The chapter was compiled by A. G. RICO.

The next chapters treat technical problems: food preservation by heat, irradiation, chemical agents (fumigation, ethylene and propylene oxide, phosphine, organophosphates); materials in contact with food (wood, glass, ceramics, metal, plastics). The chapters were written by R. DERACHE and R. LEFAUT, respectively.

P. LEROY treats the sweetening agents (saccharin, cyclamates, aspartam, acesulfam, steviozide, thamuatine) including those of substituting character (sugar alcohols, monosaccharide-sugar mixture).

Aroma preparations are described by B. LE BOURHIS. Toxicological testing, (including carcinogenicity) of natural, identical with natural and synthetic aroma substances ensures the safety expected in this field.

The toxicity of alcohol, alcoholism and its biological effects are discussed by V. LAMBEUF. Relations between cancer and food are treated by M. BÉRAUD and R. DERACHE. The latter author examines the correlations according to the localization of cancer (esophageal, stomach, large intestine, liver), to individual food components and to extraneous materials in food. It treats also anticancerogen substances (phenols, indols, aromatic iso-thiocyanates, flavons, protease inhibitors).

The last two chapters sum up food legislation in France and Europe. The specifications on food quality, control, safety of consumers, sanitary requirements are put down in France in the laws of August 1, 1905; July 8, 1965 and July 21, 1983. (R. A. DEHOVE). In the final chapter R. DERACHE gives an account of the legislations concerning about 200 million people of OEEC: coordination of legislation, additives, regulations related to contaminants, fundamental principles of toxicology ("Red Book").

The book is useful for specialists in toxicology or people interested in it. It is an aid to toxicologists inasmuch it gives an account of the connected fields, the manifold system relations. A more detailed study is supported by the references given at the end of every chapter.

GY. BÍRÓ

Proceedings of the IUFoST International Symposium on Chemical Changes During Food Processing

Published and distributed by the Instituto de Agroquímica y Tecnologia de Alimentos (CSIC). Jaime Roig, 11. 46010 Valencia. Spain; 1986, two volumes, 373 and 149 pages

With the objective of promoting the exchange of information on the latest advances experienced in the field of the chemical changes occurring in food components and characteristics during processing, an international symposium was held in Valencia in November 1984. The Symposium was sponsored by the International Union of Food Science and Technology (IUFoST) and organized by the Instituto de Agroquímica y Technología de Alimentos (IATA) of the Spanish Research Council (CSIC).

The two volumes mentioned above gather the 43 papers and the 2 plenary lectures presented at the Symposium. Volume I contains papers on chemical changes, classified into the following 6 sections: *Processing changes in nutritional value of foods* (meat, fish, mussels, etc., 8 papers). *Processing changes in animal proteins* (electric stimulation, curing, freezing, etc.; 6 papers). *Chemical changes in processing of fermented foods* (bread dough, cheeses, etc.; 7 papers). *Processing changes in fruit and fruit juices* (orange, apricot, peach, etc.; 8 papers). *Chemical changes during pickling* (olives and cucumber pickles; 3 papers). *Post-harvest changes in fruit and vegetables* (enzymes, sugars, phytohormones, etc.; 3 papers).

Volume II contains the 8 papers presented dealing with advanced methodology for the evaluation of chemical changes and the two plenary lectures. Some topics covered by this group of papers are: characterization of enzymes, identification of oligosaccharides and starches, determination of protein quality, identification of microorganisms. The subjects of the plenary conferences are: Evaluating the safety of food processes and Biotechnology. A challenge for the food industry.

As the above list of titles illustrates, the Symposium covered most of the relevant topics in the area. Although the meeting was run in Spanish and English, the Proceedings have been prepared totally in English to favour its diffusion.

A special care has been paid to the quality of the edition. Composition and printing are excellent, what facilitates reading.

L. Durán

Quantity Food Sanitation

K. Longrée & G. Armbruster

John Wiley & Sons, New York, 1987, 4th Edition, 452 pages

The Quantity Food Sanitation, 4th Edition gives complete coverage of the field: the important reservoirs of the various microorganisms and pathogens causing foodborne illnesses; conditions leading to contamination of ingredients and menu items while being prepared, stored, and served; conditions favouring multiplication of contaminating microorganisms in food; and appropriate measures of control.

This 4th Edition of Quantity Food Sanitation presents detailed information on: the microbiological considerations in connection with the specific categories of conventional, commissary, ready-prepared, and assembly-serve food service systems; the methods for educating food service personnel in food sanitation; the time-temperature relationship and the control of microbial multiplication when freezing, defrosting, chilling, hot-holding, cooking and transporting prepared menu items; an introduction to the basic facts on microorganisms and microbiological terminology.

The book has 15 chapters. They are as follows: food spoilage; some basic facts on microorganisms important in food sanitation; foodborne illnesses (poisonous plants and animals, agents for which food serves as a simple vehicle of transmission, pathogens multiplying profusely in foods and capable of causing outbreaks of acute gastroenteritis); reservoirs of microorganisms causing foodborne gastroenteric outbreaks: people, animals, environment, food supply; control: procurement of sound food supply and appropriate storage of purchased items (agencies and organizations concerned with protection of food supply, microbiological criteria, the role of the dietitian, foodservice manager, or other buyer); contamination of ingredients and menu items in the foodservice establishment; control: preventing contamination of cooked ingredients and menu items in the areas of preparation, service, and storage; multiplication of bacterial contaminants in ingredients

and menu items (effect of food as substrate); multiplication and survival of bacterial contaminants in ingredients and menu items (time-temperature relationships); microwave heating; time-temperature control: preventing multiplication and achieving death of contaminants in ingredients and menu items; microbiological considerations in connection with some specific categories of foodservice systems; educating foodservice personnel in food sanitation; appendix (manager, training and certification programs in food protection, regions, temperature conversion table).

Food handlers, dietitians, and students will find it indispensable as a reference guide, or text.

I. Varsányi

ANNOUNCEMENT

RADIONUCLIDES IN THE FOOD CHAIN

A conference entitled "Radionuclides in the Food Chain" will be held November 2—5, 1987 at the Laxenburg Conference Center near Vienna, Austria. This conference will bring together scientists, industrial managers and policy makers from eastern and western countries to discuss the factors involved in the analysis and management of radionuclides in the food chain. The scientific and policy perspectives discussed at this meeting will contribute to the worldwide harmonization of exposure standards for radionuclides in foods.

The program has been developed by a Scientific Advisory Council composed of leading scientists representing several countries and diverse disciplines. The conference sessions include:

1. a review of fundamental information on radioactivity and radiation including environmental pathways critical to man,
2. consequences of radionuclides release to health, safety and the environment comparative effects of radionuclides and other contaminants of food and water supplies,
4. risk management of food and water supplies,
5. regulatory and control programs,
6. the final session will address the development of guidelines for safety evaluation of food and water after nuclear accidents.

The symposium is designed for an international audience from all relevant disciplines, including those working in the food industry, government agencies, universities and international organizations.

The program is being sponsored by the International Life Sciences Institute (ILSI) in association with the International Institute of Applied Systems Analysis (IIASA). Cosponsors include the International Radiation Protection Association and other international organizations. For more information contact:

Ms. Lili C. Merritt
International Life Sciences Institute (ILSI)
1126 Sixteenth Street, N. W.
Washington, D. C. 20036
U.S.A.
Telephone: (202) 659-0074

SCIENTIFIC CONGRESS ON PROTEINS IN FOOD AND NUTRITION

Vienna, September 8th and 9th, 1988

Organized by

Österreichische Gesellschaft für Ernährungsforschung,
Deutsche Gesellschaft für Ernährung and
Schweizerische Gesellschaft für Ernährungsforschung.

Please address enquiries to:

Secretariat of the Scientific Congress on Proteins in Food and Nutrition

c/o VIENNA Medical Academy
Alser Strasse 4, A-1090 VIENNA, Austria
Phone: (0222) 42 71 65, cables: Medacad Wien
Telex: 134743 medak a

UoR — SCI INTERNATIONAL SYMPOSIUM ON FOOD ACCEPTABILITY

University of Reading 14th—18th September 1987

To honour the work of Dr. Roland Harper

This symposium will deal with food acceptability in its broadest interpretation and we hope that the programme will be of interest to food scientists, psychologists, market researchers, nutritionists and home economists.

It is intended that the symposium should highlight the 'state of the science' in 1987, as viewed by industry and academia, so the majority of papers and all the posters will be contributed rather than invited. For this reason, exact details of the programme cannot be provided at this early stage. However, there has been an excellent response so far, with papers being offered by some of the foremost scientists in our field.

The symposium is jointly organised by the Department of Food Science and Technology, University of Reading (U. K.), and the Food Group (Sensory Panel) of the Society of Chemical Industry (U. K.)

All enquiries, and offers of papers and posters, should be addressed to:

Dr. David M. H. Thomson,
UoR/SCI Symposium,
Department of Food Science and Technology,
University of Reading,
Whiteknights,
PO Box 226,
READING RG6 2 AP,
U. K.

RECENTLY ACCEPTED PAPERS

Change in individual carotenoids and vitamin C on processing and storage of guava juice
PADULA, M. & RODRIGUEZ-AMAYA, D. B.
Certain serum and liver lipid parameters as affected by edible fats of different quantity and quality
J. N. ZSINKA, Á, FÖLDES, V. & PERÉDI, J.
Mathematical modelling in food engineering research
HALLSTRÖM, B. & TRÄGÅRDH, C.
VIth Conference on Food Science

NOTICE TO CONTRIBUTORS

General. Manuscripts in English or Hungarian should be typed double-spaced on one side of the sheet and should not exceed 20 pages.

Title. The title should be concise and informative. It must be followed by the authors' names and the address(es) of the institute(s) where the work was carried out.

Summary. The article should be preceded by a summary (not exceeding 150 words) giving a self-explanatory compendium of the essence of the paper. At the end of the summary some 4 keywords should be put in alphabetical order.

Text. The article should be divided into the following parts: *Introduction* beginning on a new page without a title containing a survey of related literature and the objectives of the work, followed by *Materials and methods; Results; Conclusions.*

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

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

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ACTA ALIMENTARIA

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ACTA
ALIMENTARIA
An International Journal of Food Science

Editor
J. HOLLÓ

Volume 16 **September 1987** **Number 3**

Akadémiai Kiadó
Budapest

D. Reidel Publishing Company
Dordrecht & Boston

ISSN 0139—3006
CODEN ACALDI

ACTA ALIMENTARIA

An International Journal of Food Science

Sponsored by the Joint Complex Committee on Food Science of the Hungarian Academy of Sciences and Ministry of Agriculture and Food.

Editorial office:

Central Food Research Institute, H-1525 Budapest, Herman Ottó út 15, Hungary

Acta Alimentaria is a quarterly journal in English, publishing original papers on food science. The main subjects covered are: physics, physical chemistry, chemistry, analysis, biology, microbiology, enzymology, engineering, instrumentation, automation and economics of foods and food production.

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KULTURA, Hungarian Foreign Trading Company
P.O. Box 149, H-1389 Budapest 62, Hungary

For all other countries:

D. REIDEL PUBLISHING COMPANY
P.O. Box 17, 3300 AA Dordrecht, Holland

Publication programme, 1987: Volume 16 (4 issues).
Subscription prices per volume: Dfl. 266,—/$ 111.00 including postage.

Second class postage paid at New York, N. Y. USPS No. 756-270. U. S. Mailing Agent: Expediters of the Printed Word Ltd., 515 Madison Avenue (Suite 917), New York, N. Y. 10022, U. S. A.

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ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

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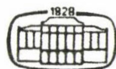

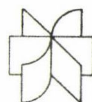

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Acta Alimentaria, Vol. 16 (3), pp. 209—216 (1987)

CHANGES IN INDIVIDUAL CAROTENOIDS AND VITAMIN C ON PROCESSING AND STORAGE OF GUAVA JUICE

M. PADULA[a] and D. B. RODRIGUEZ-AMAYA

Faculdade de Engenharia de Alimentos e Agricola Universidade Estadual de Campinas, 13.100 Campinas, SP. Brasil

(Received: 25 September 1985; revision received: 25 June 1986; accepted: 8 July 1986)

To gain an insight of the degradation of different carotenoids in processed fruits, changes in individual pigments were followed on thermal processing and storage of guava juice. A significant five-fold increase in *cis*-lycopene (from 1.2 μg per g) was observed on processing. A slight but insignificant decrease in *trans*-lycopene was also observed. Both isomers decreased on storage. The small amount of β-carotene (3.7 μg per g) remained unchanged: thus, the vitamin A value was also maintained. No epoxide derivatives of acyclic lycopene were detected either on processing or storage. On the other hand, no *cis*-isomers of bicyclic β-carotene and zeinoxanthin were found, but their epoxides were detected.

In relation to vitamin C, losses of 11% on processing and 46% after 10 months of storage were observed.

Keywords: carotenoids, degradation on processing and storage, guava juice

The degeneration of carotenoids, with concomitant loss of color and vitamin A value, is a common problem in the processing and storage of fruits. Research efforts have been dedicated to this subject, but most of the studies involved the determination of the total carotenoid content by measuring the absorbance at a specific wavelength. Information on individual carotenoids, however, is necessary to understand better the mechanism of the degradation process, verify susceptibility differences between carotenoids and assess more accurately the vitamin A value.

In Brasil, drastic reduction of the carotenoid content was observed in commercial brands of guava juice distributed domestically (PADULA et al., 1983). Thus, the transformation of individual carotenoids on processing and storage of guava juice was investigated in the present study. Since guava is a rich source of vitamin C and loss of this vitamin, along with color loss, is used as an evaluating parameter of processing and storage effects, the vitamin C content was also determined.

[a] Present address: Instituto de Tecnologia de Alimentos Avenida Brasil, 2880 13.100 Campinas, SP. Brasil

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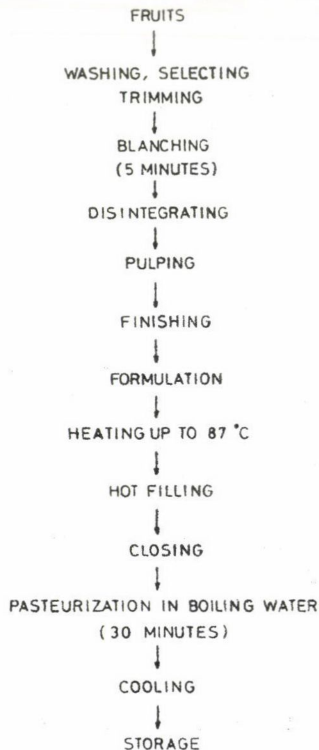
1. Materials and methods

1.1. Materials

Ripe guavas of the cultivar IAC-4 were harvested from a commercial plantation and transported immediately to our laboratory. The fruits were stored in a cold room at 10 °C overnight and processed the following day.

1.2. Processing and storage of the juice

The juice was processed according to standard procedures. The fruits were washed, selected, trimmed and blanched for 5 minutes with direct vapor injection. The fruits were then disintegrated hot using a Rietz disintegrator. To remove seeds and fibrous pieces of skin, a pulper was utilized where the puree passed through 0.154 and 0.0838 cm perforated screans, successively. The °Brix was adjusted to 7.0 and pH to 3.7 with the addition of water and citric acid, respectively. The resultant juice was heated in a steam-jacketed kettle until a temperature of 87 °C was reached. Hot-filling in 500 cm³ bottles was done immediately and the bottles were hermetically sealed. Heat treatment was accomplished by immersing the bottles in boiling water for 30 minutes,



a time period purposely made longer than the usual 12 to 15 minutes necessary for this type of product. The bottles were promptly cooled to 37 °C. These operations are summarized in **Fig. 1**.

The bottles were stored at room temperature, simulating conditions in supermarkets with regards to light exposure. Two and 3 bottles were taken at random after 0, 1, 4, 7 and 10 month storage for carotenoid and vitamin C determinations, respectively.

1.3. Carotenoid and other determinations

The carotenoids were extracted with cold acetone in a blender and filtered, these operations being repeated until the residue was devoid of color. The pigments were transferred to petroleum ether, saponified overnight with 10% KOH in methanol at room temperature, washed free of alkali and concentrated. Separation was initially accomplished in a MgO–Hyflosupercel column developed with a gradient of ethyl ether and acetone in petroleum ether, followed by rechromatography on a neutral alumina columm. Since alumina has been reported to degrade carotenoids, chromatography in this adsorbent was monitored on TLC (i.e. analysis of sample before and after separation on column), showing no artifact formation. Identification was undertaken on the basis of the chomatographic behaviour on column and thin layer plates, visible absorption spectra and specific chemical reactions (e.g. iodine-catalyzed isomerization, epoxide tests, acetylation, methylation). Quantitation was based on the maximum absorbance. Details of the analytical procedure has been described previously (RODRIGUEZ et al., 1976; PADULA & RODRIGUEZ-AMAYA, 1986). Necessary precautions to prevent degradation or alteration of the pigments during analysis were taken (e.g. protection from light and high temperature, short analysis time). The vitamin A value was calculated according to the NAS–NRC (1980) ratio of 0.6 μg-carotene to 1 IU. The contribution of γ-carotene, the only other carotenoid with provitamin A activity, was nil.

All other determinations were done according to AOAC procedures (AOAC, 1980). Vitamin C was determined using the AOAC method No. 43.061.

The results were statistically evaluated by analysis of variance and Tukey's test was conducted to compare the medium values.

2. Results and discussion

Tables 1 and 2 show the changes in the carotenoid and vitamin C contents of the guava juice on processing and storage.

Lycopene decreased from 31.0 to 26.0 μg per g (13.5%), but the statistical analysis showed this decrease to be insignificant. *Cis*-lycopene increased from

Table 1

Effect of processing on the carotenoids (μg per g), vitamin A value (IU per 100) and vitamin C (mg per 100g) content of guava cultivar IAC-4 juice

Carotenoid / Vitamin	Fresh juice	Processed juice
β-carotene	2.7±0.5 (a)	2.7±0.0 (a)
ζ-carotene	Trace (a)	0.2±0.0 (b)
γ-carotene	Trace (a)	0.1±0.2 (a)
Zeinoxanthin	0.8±0.3 (a)	0.8±0.1 (a)
Cis-lycopene	1.2±0.6 (a)	7.8±0.4 (b)
Lycopene	31.0±2.1 (a)	26.8±1.3 (a)
5,8-epoxy-3,3',4-trihydroxy-β-carotene	2.9±0.2 (a)	0.3±0.1 (b)
5,6,5',6-diepoxy-β-carotene	Trace (a)	Trace (a)
Total	38.6±3.3 (a)	38.7±2.1 (a)
Vitamin A	450±94 (a)	450±0.0 (a)
Vitamin C	73.5	65.2

Determination of carotenoids: analysis of 2 samples of the fresh juice and 2 bottles of the processed juice.

Determination of Vitamin C: three bottles of juice were homogenized and two samples were taken for analysis, no difference being observed between the duplicate samples.

The carotenoids were in trans-form, except *cis*-lycopene.

Trihidroxy-β-carotene was tentatively identified. Approximative value calculated on the basis of the β-carotene absorptivity.

Values in the same horizontal line not showing the same letter are significantly different ($P \leq 0.05$).

1.2 to 7.8 μg per g (a six-fold increase) during processing. In terms of the total carotenoid content, however practically no change was observed, demonstrating the inadequacy of this value as a parameter for evaluating processing effects.

During the 10-month storage, lycopene decreased by 25% and *cis*-lycopene by 63%. Beta-carotene content, on the other hand, remained stable during processing and showed only a slight, insignificant tendency to decrease on storage. This meant that the vitamin A value was also practically maintained.

The pigment destruction observed here was much less drastic than that noted in commercial juices (PADULA et al., 1983), although the heat treatment had already been excessive.

In aseptically packaged guava juice, CHAN and CAVALETTO (1982) observed 9.0 and 11.5% loss of total carotenoid, as measured by the absorbance at 468 nm, on thermal treatment at 93 °C for 26 and 38 seconds, respectively.

From the tables, it can be seen that no *cis*-isomers of the bicyclic carotenoids β-carotene and zeinoxanthin were detected on processing and storage, but trace of their epoxides (5,6,5',6'-diepoxy-β-carotene and 5,8-epoxy-zeinoxanthin) were observed. On the other hand, no epoxy derivatives were detected of the acyclic lycopene, which obviously suffered *cis*-isomerization

Table 2

Changes of the carotenoids (μg per g), vitamin A value (IU per 100g) and vitamin C (mg per 100g) content during storage of the processed juice of guava cultivar IAC-4

Carotenoid/Vitamin	Storage time (month)				
	0	1	4	7	10
β-carotene	2.7$\pm$0.0 (a)	2.4$\pm$0.3 (a)	2.5$\pm$0.4 (a)	2.5$\pm$0.1 (a)	2.5$\pm$0.1 (a)
ζ-carotene	0.2$\pm$0.0 (a)	0.2$\pm$0.0 (a)	0.3$\pm$0.1 (a)	0.2$\pm$0.0 (a)	0.3$\pm$0.1 (a)
γ-carotene	0.1$\pm$0.2 (ab)	ND (a)	0.3$\pm$0.0 (b)	0.3$\pm$0.0 (b)	0.2$\pm$0.0 (ab)
Zeinoxanthin	0.8$\pm$0.1 (a)	1.0$\pm$0.2 (a)	0.6$\pm$0.3 (a)	1.3$\pm$0.3 (a)	1.2$\pm$0.1 (a)
Cis-lycopene	7.8$\pm$0.4 (a)	7.9$\pm$0.3 (a)	6.6$\pm$0.6 (a)	3.5$\pm$0.4 (b)	2.9$\pm$0.5 (b)
Lycopene	26.8$\pm$1.3 (a)	25.3$\pm$0.7 (ab)	-22.6$\pm$1.6 (ab)	22.2$\pm$1.7 (ab)	20.0$\pm$1.3 (b)
5,8-epoxy-3,3',4-trhydroxy-β-carotene	0.3$\pm$0.1 (a)	2.9$\pm$0.1 (b)	0.6$\pm$0.1 (a)	1.8$\pm$0.1 (c)	1.6$\pm$0.0 (c)
5,6,5',6'-diepoxy-β-carotene	Trace (a)	ND (a)	0.2$\pm$0.1 (a)	0.1$\pm$0.1 (a)	NA (a)
5,8-epoxy-zeinoxanthin	ND (a)	ND (a)	Trace (a)	Trace (a)	Trace (a)
Total	38.7$\pm$2.1 (ab)	39.7$\pm$1.2 (a)	33.7$\pm$1.3 (abc)	31.9$\pm$2.4 (bc)	28.9$\pm$1.8 (c)
Vitamin A	450$\pm$0.0 (a)	400$\pm$47 (a)	417$\pm$70 (a)	417$\pm$11 (a)	417$\pm$12 (a)
Vitamin C	65.2	56.4	43.3	41.8	35.4

Determination of carotenoids: analysis of 2 bottles of the processed juice.
Determination of vitamin C: three bottles of juice were homogenized and two samples were taken for analysis, no difference being observed between the duplicate samples.
The carotenoids were in *trans*-form, exept *cis*-lycopene.
Trihydroxy-β-carotene was tentatively identified. Approximative value was calculated on the basis of the β-carotene absorptivity.
Values in the same horizontal line not showing the same letter are significantly different ($P \leq 0.005$).
ND: Not detected.

This suggests that the two types of carotenoids (cyclic and acyclic) undergo different degradation routes or different reaction rates, the cyclic and acyclic carotenoids going through the isomerization and epoxidation reaction, respectively, so rapidly as to preclude detection of the products.

This difference in behaviour of cyclic and acyclic carotenoids has been confirmed in mango puree where the major pigment is β-carotene and in papaya where lycopene predominates. Results of this work will be published separately.

It is widely presumed that carotenoids in general suffer *cis*-isomerization on thermal processing. VALADON and MUMMERY (1981), however, observed that despite the heating in acid conditions involved in the canning of oranges, only two of more than thirteen carotenoids underwent *cis*-isomerization. *Cis*-violaxanthin transformed from the 15-*cis*-structure to the 9-*cis*-form. All-*trans* flavoxanthin decreased slightly while its epimer chrysanthemaxanthin increased. All the other carotenoids maintained their all-*trans* structure. In view of their results and ours, greater attention should be given to the isomerization and epoxidation reactions, especially as a function of the carotenoid structure.

No explanation can be given at the moment for the slight tendency of ζ-carotene to increase on processing. As for the tentatively identified pigment, 5,8-epoxy-3,3',4-trihidroxy-β-carotene, the fluctuation could be simply due, at least in part, to the difficulty in extracting completely such a highly polar pigment.

In dried tomato powder *cis*-lycopene formed mostly during processing, could either revert back to *trans*-lycopene or oxidize to colorless fragments (LOVRIC et al., 1970; BOSKOVIC, 1979). In the case of the guava juice, *cis*-lycopene, which was also mostly produced during the thermal processing, decreased gradually during storage, presumably by oxidation. No evidence of reversion to the more stable *trans*-isomer could be observed. A reexamination of the evidence presented for *cis-trans* reisomerization (LOVRIC et al., 1970; WONG & BOHART, 1957), however, showed that such a process occurred under conditions which disfavored oxidation (e.g. vacuum, N_2, CO_2 packed samples).

With regards to vitamin C, a loss of 11% was seen on processing and 46% during the 10-month storage under ambient conditions. General properties (pH, °Brix, acidity %, total and reducing sugars) were also determined but no changes were observed.

In contrast to the situation with the carotenoid composition, loss of vitamin C on processing and storage guava pulp, puree, juice and concentrate had been studied by many workers (JAIN & BORKAR, 1968; 1970; 1971; MURALIKRISHNA et al., 1969; BREKKE et al., 1970; SANCHEZ et al., 1970; FODA et al., 1970; HEIKAL et al., 1972; DIAZ DELGADO & VILLALOBOS CRUZ, 1974; KHURDIYA & ROY, 1974; MARTIN et al., 1975; KATO et al., 1976; SALOMON et al., 1976; 1977; NIP, 1979; ITO et al., 1980; CHAN & CAVALETTO, 1982). The results,

however, were highly variable. Losses of 0–67% on processing and 0–97% after 6 months of storage had been reported. Differences in results are to be expected as a function of fruit variety, type and processing conditions, storage time and conditions, but discrepancies were noted even under similar conditions.

3. Conclusions

Heat processing (pasteurization) provoked a slight but insignificant decrease in *trans*-lycopene (from 31.0 μg per g to 26.8 μg per g, a five-fold increase in *cis*-lycopene (from 1.2 μg per g to 7.8 μg per g) and all 11% loss of vitamin C. The β-carotene content remained stable, thus the vitamin A value was also maintained.

Ten months of storage resulted in losses of 63%, 25% and 46% for *cis*-lycopene, *trans*-lycopene and vitamin C, respectively. No epoxide derivatives of the acyclic lycopene and no *cis*-isomers of the bicyclic β-carotene and zeinoxanthin were observed, demonstrating that pigment structure influences the rate or route of degradation.

*

The authors acknowledge with gratitude the financial support given by the Fundação de Amparo a Pesquisa do Estado de São Paulo. Thanks are due to the Conselho Nacional de Desenvolvimento Científico e Tecnológico for a graduate fellowship granted to the first author.

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Acta Alimentaria, Vol. 16 (3), pp. 217–223 (1987)

CERTAIN SERUM AND LIVER LIPID PARAMETERS AS AFFECTED BY EDIBLE FATS OF DIFFERENT QUANTITY AND QUALITY

(RAT EXPERIMENTS)

Á. J. N. Zsinka[a,c] V. Földes[a] and J. Perédi[b]

[a]National Institute of Food Hygiene and Nutrition,
H-1097 Budapest, Gyáli út 3/a. Hungary
[b]Vegetable Oil and Detergent Research Institute,
H-1106 Budapest, Maglódi út 6. Hungary

(Received: 2 February 1986; accepted: 11 June 1986)

Groups of 20 sexually mature male Wistar rats (National Institute of Food Hygiene and Nutrition, OÉTI) each were fed synthetic rations containing 5% or 20% edible fat of different P/S ratio (sunflower oil, lard, cocunut oil), otherwise of equal nutritive value. The diet was given during 6 weeks, isoenergetically.

The total lipid, triglyceride, total cholesterol and free fatty acid content of the serum and the liver was determined and the following conclusions were drawn:

– On feeding with normal synthetic diet of adequate (5%) fat content the lipid parameters tested did not change as an effect of fats of different fatty acid composition (P/S ratio).

– On feeding a diet of high fat content (20%) the diet containing sunflower oil of high P/S ratio reduced the total lipid and tryglyceride level of the serum in relation to the diet of 5% fat. At the same time the total lipid, total cholesterol and triglyceride content of the liver increased about twofold. Lard and coconut oil did not cause a similar change.

Keywords: edible fats, isoenergetic synthetic diet, serum and liver lipids

There are substantial differences in the quality and quantity of edible fats and these depend on the kind of fatty substance available and on the dietary customs based on it. Many papers have been published on the correlation between population groups living according to different dietary habits and their physical condition, among them fat consumption and vascular diseases, mainly ischemic heart disease (IHD). The World Health Organization (WHO, 1984) recommends on the basis of morbidity and mortality statistics the limitation of the consumption of fat, particularly fat rich in saturated fatty acids and cholesterol. Lately doubts have emerged as to the soundness of the dietary recommendations (Stallones, 1983; Fanu, 1984). Brisson (1984) does not consider a radical change of dietary habits favourable because the usefulness of this is not sufficiently proven while the consumption of fats rich in unsaturated fatty acids may have disadvantageous effects, too. The negative correlation between the low cholesterol level in the serum, developed as an effect of dietary factors, and certain tumours is reflected in statistical

[c]Present address: Central Food Research Institute
H-1022 Budapest, Herman Ottó út 15.

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

investigations (KARK et al., 1980; WILLIAMS et al., 1981) and in results of animal experiments (CARROL, 1981; REEBUCH, 1981; CLINTON, 1984). On analysis of the data it appeared that in these experiments generally large amounts of certain unsaturated fats were onesidedly fed to the animals (CARROL, 1984) the reduction of which is anyway indicated. Later KRITCHEVSKY (1984) found that the development of tumors is promoted not only by a large amount of fat but mainly by overdosage of total energy. It was KRITCHEVSKY (1984), too, who observed in experimental atherosclerosis, that rather the structure of triglycerides than the proportion of unsaturated fatty acids in the fat consumed causes atherogenic effect.

On the basis of these unsettled problems we considered it indicated to investigate the correlation of certain parameters of fat consumption and lipid metabolism, with special reference to fats most generally consumed in Hungary (lard, sunflower oil) and to coconut oil rich in medium chain triglycerides (MCT), in Hungary used only for dietotherapeutic purposes, while elsewhere widely consumed.

1. Materials and methods

Wistar (National Institute of Food Hygiene and Nutrition, OÉTI) sexually mature male rats (153 ± 7 g) were used in the experiments. Each group consisted of 20 rats and was fed during 6 weeks isoenergetically with the diets as seen in Table 1.

Table 1

Percentage composition and energy content of synthetic diets

	I	II	III	IV	V	VI
Casein	20	20	20	20	20	20
Starch	55	55	55	40	40	40
Sunflower oil	5	—	—	20	—	—
Lard	—	5	—	—	20	—
Conconut oil	—	—	5	—	—	20
Salt–vitamins	5	5	5	5	5	5
Sawdust	15	15	15	15	15	15
Energy content						
J per 100 g	1465	1465	1461	1775	1775	1750
Cal per 100 g	350	350	349	424	424	418
Cal from fat	46.3	46.3	46.2	186	186	192
		13%			44%	

Calculated P/S quotients of polyunsaturated/saturated fatty acid of fats

	P/S	MCT(%) ($C_8C_{10}C_{12}$)
Sunflower oil	5.2	—
Lard	0.24	0.5
Coconut oil	0.03	57.8

The groups were pair fed, (all groups were given the amount of feed eaten by the group of lowest consumption) and the energy intake was similar for each group. The residue, if any, was weighed in air-dried state and the average ratio and energy consumed was calculated taking it into account.

The body mass of each rat was weighed weekly and the change of body mass was calculated for 6 weeks. At the end of the 6th week, after 18 hours starvation blood was drawn from the abdominal aorta of the rats in ether narcosis. Their organs: liver, kidney, heart, spleen, were weighed and the relative weights of organs were calculated. From the serum and the liver extracted according to FOLCH and co-workers (1957), the following lipid parameters were determined: total lipids according to ZÖLLNER and KRISH (1961), total cholesterol by Gödecke's enzymatic-test, triglycerides by extraction-test (Reanal, Budapest) and the free fatty acid content by the method of DOLE and MEINERTZ (1960). For each group the averages and the standard deviations were calculated. Inter-group differences were evaluated by analysis of variance (SACHS, 1984).

2. Results

Table 2 contains the data on feed and energy consumption of the rats.

Table 2

Average diet and energy consumption

	I	II	III	IV	V	VI
Diet consumption g per day per rat	17.0	16.9	17.0	13.5	13.7	13.7
Energy intake						
J per day per rat	249	247	249	240	241	241
Cal per day per rat	59.5	59.3	59.5	57.2	57.2	57.3

As it can be seen practically there was no difference between groups. Table 3 shows the changes in body mass.

Table 3

Changes in body mass

	I	II	III	IV	V	VI
Change g per rat in 6 week	109 ± 17	127 ± 14	101 ± 18	108 ± 16	120 ± 17	113 ± 14

Practically there was no significant difference between the groups. However, the group consuming lard showed slightly greater increase in body mass than those fed the other two kinds of fat.

The relative weights of liver are shown in Table 4.

Table 4

Relative liver mass

	I	II	III	IV	V	VI
g per 100 g body mass	2.75 ±0.24	2.69 ±0.20	2.75 ±0.26	2.98[a] ±0.21	2.70 ±2.27	2.81 ±0.30

No significant difference was found between groups. However, the values of the group consuming 20% sunflower oil were slightly higher than those of the group consuming lard. The values obtained in the serum are summed up in Table 5.

Table 5

Lipid levels in the serum

	I	II	III	IV	V	VI
Total lipid (g dm^{-3})	3.0 ±0.34	2.46 ±0.27	2.8 ±0.84	2.31[a] ±0.83	3.0 ±0.37	2.32 ±0.6
Triglyceride (mmol dm^{-3})	0.74 ±0.21	0.58 ±0.17	0.63 ±0.22	0.40[a] ±0.11	0.74 ±0.12	0.54 ±0.09
Total cholesterol (mmol dm^{-3})	2.13 ±0.56	1.7 ±0.47	2.18 ±0.32	1.92 ±0.4	2.08 ±0.2	1.82 ±0.41
Free fatty acid (mmol dm^{-3})	0.65 ±0,08	0.86 ±0.13	0.64 ±0.08	0.55 ±0.14	0.72 ±0.16	0.52 ±0.12

[a] I–IV and IV–V $P < 0.05$

On increasing the fat content of the diets to 20%, in the case of sunflower oil the total lipid and triglyceride content in the serum was significantly reduced as compared to the values obtained with 5% sunflower oil.

Table 6 contains data on the lipid content of the liver.

Table 6

Lipid content in the liver

	I	II	III	IV	V	VI
Total lipid (mg per g)	71.7 ±20	68 ±19	62 ±12	170[ab] ±31	78 ±21	71 ±33
Triglycerides (mg per g)	15.6 ±2.25	15 ± 4.8	14.8 ± 3.8	39.5[ab] ± 4.9	21 ± 3.8	19.7 ± 3.2
Total cholesterol (mg per g)	9.9 ± 1.9	7.9 ± 3.2	9.5 ± 2.3	22[ab] ± 6	9.4 ± 3.6	11.3 ± 3.1
Free fatty acids (mmol g^{-1})	18.4 ± 7.3	10.8 ± 3.8	21 ± 5	22[c] ± 4.5	12.3 ± 3	29 ± 7.1

[a] I–IV $P < 0.05$ [b] IV–V, VI $P < 0.05$ [c] IV–V $P < 0.05$

Increasing sunflower oil content to 20% (apart from the free fatty acids) caused substantial increase in every parameter.

3. Conclusions

Under the given experimental conditions the lipid parameters of Wistar sexually mature male rats differed only with different kinds of fat of the diet contained extremely high fat level. When the rats were fed the normal adequate diet of 5% fats content differences in the parameters were not observed in spite of the great differences in the P/S ratio of the fats. On increasing the fat content of the diet four times (to 20%) the effect of the quality of fats (Keys et al., 1965; Hegsted et al., 1965; Lyapkov, 1981) asserted itself in the lipid parameters of the serum: consumption of sunflower oil of high P/S ratio caused a significant reduction in the total lipid and triglycerid level and the total cholesterol content showed also a decreasing tendency even if not significant. The other two kinds of fat did not cause substantial differences during the six weeks feeding period.

In the liver the onesided and high sunflower oil consumption increased significantly the total lipid, triglyceride and total cholesterol content to about twofold of that with 5% fat containing diet. Fats of lower P/S ratio did not increase the lipid content of the liver. These results are in accordance with the findings of Triscari (1978) who observed a 30% increase in cholesterol synthesis in the liver of rats upon the feeding of linoleic acid ($C_{18:2}$) in comparison with the effect of saturated fatty acids. Ramesha (1980) found upon feeding rats with 10% unsaturated fatty acid containing oil an increased amount of cholesterol and bile acid excretion while ^{14}C-acetate was built in the liver cholesterol in increased quantities.

Presumably, in our experiments increased lipid synthesis appeared because of feeding a large amount of unsaturated fatty acid, at the same time with increasing lipid transport increased the triglyceride uptake of the liver, too, and as a sign of damaged function probably decreased the lipid-release of the liver. Because of the heavy load on fat metabolism this was thrown off its balance as demonstrated in increased lipid accumulation in the liver. In the case of other fats of the same quantity (rich in saturated fatty acids) this effect was not observed.

Of the medium chain triglycerides – present in large amounts in coconut oil – storage is minimal. They burn rapidly and produce more heat (Geliebter, 1980) or because of rapid burning form ketosis instead of lipid accumulation. The breakdown of ^{14}C-glucose to CO_2 and in lipids decreased if MCT formed 55% of the total energy intake (Lavau & Nashim, 1978). Stewart (1978) found that on feeding calves during 24 weeks on a diet containing 30% MCT as the source of energy the cholesterol in the serum, in the liver and in the adipose tissue decreased.

Our results show that high fat consumption – even in the case of otherwise adequate nutrient intake – may cause in a very short time disturbance in fat metabolism. Onesided high fat (sunflower oil) consumption has a disturbing effect on the liver even if – as in these experiments – it affects the level of serum lipids advantageously.

Thus, the investigation of the effect of fat mixtures of different fatty acid ratios, of the reversibility of lipid accumulation in the liver and of the lipid transport seems indicated in feeding experiments with diets complying to the requirement or with diets divergent from the requirement.

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Acta Alimentaria, Vol. 16 (3), pp. 225–230 (1987)

CERTAIN SERUM AND LIVER LIPID PARAMETERS AS AFFECTED BY DIFFERENT FAT MIXTURES

(RAT EXPERIMENTS)

Á. J. N. ZSINKA[a,c] V. FÖLDES[a] and J. PERÉDI[b]

[a]National Institute of Food Hygiene and Nutrition,
H-1097 Budapest, Gyáli út 3/a. Hungary
[b]Vegetable Oil and Detergent Research Institute,
H-1106 Budapest, Maglódi út 6. Hungary

(Received: 5 February 1986; accepted: 11 June 1986)

Groups of 20 sexually mature male Wistar rats (National Institute of Food Hygiene and Nutrition, OÉTI) were fed isoenergetically during 6 weeks on normal synthetic diet containing 20% edible fat or fat mixture of different P/S ratio and fatty acids of different chain length. Effect of the diet on the serum and liver lipids was investigated and the following conclusions were drawn:

– Depending on the kind of fat given significant differences were not observed among the serum lipid levels as compared to the values in control rat fed with normal ratio (5% fat).

– The total lipid, total cholesterol and tryglyceride content of the liver was significantly increased in rats fed a diet with 20% sunflower oil. On the effect of fats of similar quantity containing different medium chain triglycerides (MCT) such as: coconut oil rich in C_8 C_{10} C_{12} MCT, P/S = 0.03; mixture of 67% coconut-oil + 33% sunflower oil P/S = 0.82; mixture of 67% oil rich in C_8 C_{10} MCT + 33% sunflower oil P/S = 0.33; generally decreased the quantity of liver total lipid and triglyceride contents.

– The free fatty acid content of the liver diminished significantly as an effect of coconut-oil rich in C_8 C_{10} C_{12} MCT and of the fat mixtures. Upon feeding the rats on fat mixture rich in C_8 C_{10} MCT the free fatty acid content of the liver was in accordance with the control values.

– The total liver lipid content was significantly lower upon consumption of the fat mixture rich in C_8 C_{10} MCT than with the mixture containing C_8 C_{10} C_{12} MCT of the more advantageous P/S ratio. Thus, probably the length of the carbon chain and/or the quantity was of decisive effect.

Keywords: fats of different P/S, serum and liver lipids

In earlier experiments (J. N. ZSINKA et al., 1987) it was shown that in sexually mature male rats fed for 6 weeks with normal synthetic diet the consumption of 20% fat, substantially exceeding their fat requirement, caused changes in some of the serum and liver lipid parameters, depending on the kind of fat given. In accordance with a number of data in the literature, large quantities of fat of high unsaturated fatty acid content (in the present experiment sunflower oil) generally reduced the level of serum lipids while substantially increasing the total lipid, total cholesterol and triglyceride content of the liver. Thus, the problem arises whether the change in the lipid content of liver is

[c]Present address: Central Food Research Institute,
H-1022 Budapest, Herman Ottó út 15.
Hungary

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

caused, beside overfeeding with fat, by the difference in the proportion of saturated and unsaturated fatty acids in the fat mixtures or the accumulation of lipids occurs in every variation.

1. Materials and methods

As in the earlier experiments (J. N. ZSINKA et al., 1987) groups of 20 sexually mature male Wistar rats (National Institute of Food Hygiene and Nutrition, OÉTI) (194 ± 8 g) were fed isoenergetically for 6 weeks with synthetic diet, complying with the requirements of the rats, but containing 20% of fats of different P/S ratio.

The control group was fed with a normal ratio (Laboratory Animals Institute, LATI, Gödöllő). In Table 1 data of the diets and the P/S ratio of the fats used are presented.

Table 1

Composition and energy content of rat diets

	Diets				
	I	II	III	IV	V
Type of diet	LATI normal		synthetic	normal	
Fat(%)	5	20	20	20	20
Fat composition	—	sunflower oil	coconut oil[a]	33% sunflower+ 67% coconut oil	33% sunflower+ 67% MCT oil[b]
P/S[c]	—	5.2	0.03	0.82	0.33
Energy content					
J per 100 g	1680	1894	1860	1869	1869
Cal per 100 g	400	451	443	445	445

[a]Diet III contained 58.0% medium chain triglycerides (C_8 C_{10} C_{12}) in the coconut oil, diet IV contained 40% of these fatty acids in the fat mixture

[b]The MCT oil contained 99% C_8 C_{10} fatty acids, thus the MCT content of the fat mixture was 66%. (The fatty acid composition of the fats was determined by gas chromatography in the Vegetable Oil and Detergent Research Institute, Budapest.)

[c]Proportion of polyunsaturated to saturated fatty acids

Except for the fats, the composition of the diets was the same. The amount of ratio consumed and changes in the body mass were measured (the leftovers were weighed back). At the end of the 6th week, after 18 hours starvation blood was drawn from the abdominal aorta of each rat. From the serum and the homogenized liver (according to FOLCH et al., 1957) the total lipid content (ZÖLLNER & KRISCH, 1961), the triglycerides with Reanal extraction test and the total cholesterol by the enzyme test of Gödecke and the free fatty acids by DOLE and MEINERTZ (1960) method, was determined. Group averages and standard deviations were calculated; significant differences were determined by analysis of variance (SACHS, 1984).

2. Results

The average diet and energy consumption was listed in Table 2.

Table 2

Diet and energy intake

	Diets				
	I	II	III	IV	V
Diet g per rat per day	15.0	13.0	13.2	13.0	13.1
J per rat per day	248	246	245	244	245
Cal per rat per day	59.0	58.6	58.4	58.6	58.2

As it can be seen the energy consumption of the groups was similar. In Table 3 the body mass gain is presented.

Table 3

Gain in body mass

	Diet				
	I	II	III	IV	V
Gain in body mass (g) in 6 weeks per rat	142 ± 23	188[a] ± 28	142 ± 28	150 ± 32	148 ± 28

[a]II–I, III, IV, V $P < 0.05$

Increase in body mass of the group consuming sunflower oil substantially exceeded that of the control group. Fats of medium chain triglycerides (MCT) content did not affect much the increase in body mass.

Table 4 contains the serum lipid levels.

Table 4

Lipid parameters in the serum

	Diets				
	I	II	III	IV	V
Total lipids ($g\ dm^{-3}$)	3.44 ±0.55	3.55 ±0.72	3.35 ±1.3	3.8 ±0.76	3.59 ±0.63
Total cholesterol ($mmol\ dm^{-3}$)	1.71 ±0.2	1.52 ±0.26	1.64 ±0.21	1.69 ±0.16	1.75 ±0.43
Triglycerides ($mmol\ dm^{-3}$)	0.59 ±0.13	0.55 ±0.22	0.69 ±0.2	0.49 ±0.19	0.61 ±0.19
Free fatty acids ($mmol\ dm^{-3}$)	0.81 ±0.12	0.74 ±0.12	±0.57[a] ±0.13	0.61[a] ±0.12	0.56[a] ±0.13

[a]I, II–III, IV, V $P < 0.05$

As an effect of fats containing MCT out of the serum lipids the level of free fatty acids decreased compared to the effect of normal ratio and of the diet with 20% sunflower oil. Other serum parameters showed a good agreement with the values known from the literature.

Changes in the lipid content of the liver are shown in Table 5.

Table 5

Lipid content of the liver

	Diets				
	I	II	III	IV	V
Total lipids (mg per g)	89.6 ±14	176[a] ± 20	72.3[ae] ± 9.3	83.7 ±10	73.8[ae] ± 9
Total cholesterol (mg per g)	6.5 ± 1.3	14.2[b] ± 2.3	6.6 ± 1.9	7.2 ± 1.3	6.7 ± 0.91
Triglycerides (mg per g)	17.6 ± 7.2	61.3[c] ± 13	10.9[ce] ± 2.7	16 ± 5	12.1[ce] ± 3.1
Free fatty acids (mmol per g)	17.1 ± 4	22.8[d] ± 8.2	11.2[d] ± 3	11.7[d] ± 2.9	16.2 ± 5.8

[a]I–II, III, V $P < 0.05$ [b]I–II $P < 0.01$ [c]I–II, III, V $P < 0.01$ [d]I–II, III, IV $P < 0.05$
[e]IV–III, V $P < 0.05$

Differences appearing in the liver were substantially higher than in the serum. The significant increase manifesting itself in the total lipid, total cholesterol and triglyceride contents of the liver as an effect of sunflower oil did not appear on consumption of fats rich in MCT. The total lipid and triglyceride values as affected by diets III and V were even lower than the control values while the total cholesterol values in all three groups resembled the control values.

Of the oils rich in MCT the mixture of coconut oil and sunflower oil the total lipid and triglyceride values were higher than upon the consumption of the other two oils containing MCT.

3. Conclusions

Similarly to earlier observations (J. N. Zsinka et al., 1987) it was found that under the experimental conditions applied the about fourfold increase in the diet of sunflower oil of high P/S ratio significantly increased the liver lipid concentration in comparison to that of rats fed with normal diet. Coconut oil, rich in saturated medium chain fatty acids and the fat mixtures containing C_8 C_{10} MCT oil did not induce similar increase but a decreasing tendency was caused by them. Except for fats containing MCT, significant changes were not observed in the serum values. The MCT containing fats caused reduction in the

free fatty acid content of the serum as compared to the control values. The lipid levels in the serum corresponded to the values found in the literature.

Results obtained support the conception according to which the absorption and oxidative decomposition of medium chain fatty acids is extremely rapid and in consequence the triglyceride formation is very low. GELIEBTER (1980) found that on burning medium chain fatty acids more heat is developed that in the case of long chain fatty acids. If in this experiment 50% of the energy content of the edible fat came from medium chain fatty acids the depot fat in the experimental animals was 20% lower than in animals fed with fat rich in long chain fatty acids. LAVAU (1978) found, on feeding rats for 4 weeks with a diet containing up to 55% of its energy MCT fat, that both the body mass and the depot fat diminished, even in comparison with the group fed on a diet of low fat content. On feeding rats for 8 weeks with MCT fat the body mass was 10% lower than that of the group given corn oil.

The C^{14} glucose in CO_2 and in the lipids is about one third of that of the group consuming diet of low fat content. LEVATER (1982) feeding a diet of 12% MCT for 9 weeks found lower triglyceride and cholesterol content in the liver than on feeding with lard. The lipid composition of the cell membrane did not change. STEWARD (1978) found lower cholesterol level in the serum and liver of calves when 30% of energy was provided by fat containing MCT instead of tallow.

In the present experiment the differences in the liver lipids took a short time to appear (6 weeks). The question arises whether shorter or longer periods bring about similar progressing changes and these appear also in serum lipid values. The advantageous effect of fats of MCT content on lipid accumulation is shown by fat mixtures, too. The fat mixture containing 66% C_8 C_{10} (V) exerts a better effect than the mixture (IV) containing 39% C_8 C_{10} C_{12} (of which only 8.1% is C_8 C_{10}). Based on this it is presumed that fatty acids of shorter chain length transform into triglycerides at a lower ratio than those containing 12 carbon atoms. The metabolism of the latter is similar to those of long chain fatty acids (DEMARNE, 1977). Thus, in these experiments it was not the P/S ratio decisive from the point of view of lipid accumulation in the liver, which approximated in diet IV the desirable P/S value, but the length of the carbon chain and/or its quantity dominated. Sunflower oil of P/S = 5.2 ratio increased lipid accumulation in the liver. Further investigation of the quantity and quality of fatty acids optimal in relation to the liver seems indicated beside their effect on the serum lipids.

It would be interesting to know the changes of these lipid parameters if beside fat consumption other factors of the diet would also change in the pathological direction. Further experiments are desirable to clarify the effect of excess fat intake with different quality on the development of disturbances in fat metabolism.

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Acta Alimentaria, Vol. 16 (3), pp. 231–237 (1987)

DETERMINATION OF MUTAGENIC COMPOUNDS IN FOOD USING A SPECIFIC BIOLOGICAL SYSTEM

S. MIERTUŠ[a], H. VOJTEKOVÁ[c], J. ŠVORC[a] and E. ŠTURDIK[b]

[a] Department of Analytical Chemistry;
[b] Department of Biochemical Technology, Faculty of Chemical Technology, Slovak Technical University, 812 37 Bratislava. Czechoslovakia
[c] Institute of Food Research 825 09 Bratislava. Czechoslovakia

(Received: 19 February 1986; accepted: 15 July 1986)

We have used SOS inducing potency of genetically manipulated bacteria *E. coli* for the identification and determination of mutagenic compounds in foods. Basic analytical parameters of the method, namely linearity range, threshold value of detection and sensitivity were determined. Comparing analytical parameters such as threshold value of detection and sensitivity with those of classical analytical methods used up to date, we found a similar quality of both approaches. Main advantage of the presented bioanalytical method is the possibility of direct analysis of true samples of food without previous separation. This has been demonstrated by determination of 5-nitrofurylacrylic acid in wine and of nitrovin in chicken liver.

Keywords: food analysis, mutagenicity, nitrovin, 5-nitro-2-furylacrylic acid, SOS chromotest

Many contaminants in the environment act as mutagens and carcinogens in certain test systems. Several compounds used also in food industry or in other industrial branches are distinquished;by mutagenic potency e.g. N-nitrosoderivatives, aromatic hydrocarbons, nitrofurns (FISHBEIN, 1979; GROENEN et al., 1980).

Nitrofurans also belong to the potential mutagens and carcinogens. They were and partly still are used;in clinical and veterinary medicine, as additives to foods and feed mixtures for animals (McCALLA, 1983). After discovering their mutagenic effects, their use was restricted. For example, 5-nitro-2-furyl-acrylic acid (NFAA) was used as wine stabiliser till 1978 (FARKAŠ, 1975). However, several nitrofurans are still used, e.g. nitrovin is used as a growth stimulator of farm animals (ATKINSON, 1971).

The detection of these substances in samples of foods by traditional analytical procedures can sometimes be complicated due to complexity of the true samples and because of the limited specificity to certain mutagenic substances. The success of the analysis often depends on sample preparation, isolation method and on the purity of compounds studied.

The aim of our work was to study the possibilities of detecting mutagenic compounds in food, by a new bioanalytical method, the SOS chromotest

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D. Reidel, Dordrecht

(Hofnung, 1982; Ohta et al., 1984). The SOS chromotest is based on genetically manipulated bacteria *E. coli* which produce the enzyme β-galactosidase to an increased extent after genetic damage to DNA. The advantage of this test is quantitative response of mutagenic activity to the amount of mutagen. We have recently made direct use for analytical purposes of this dependence (Miertuš et al., 1987).

In the present work we have verified the linearity of the response (production of β-galactosidase enzyme) to the amount of mutagens, the extent of linear dependence, and the threshold value of detection of the method. Furthermore, we have studied the possibilities of determining mutagenic compounds in true samples of foods, namely NFAA in wine and nitrovin in chicken meat, stomach and liver. Here we present the analysis of a model liver sample. Model samples were prepared to verify the influence of matrix on the analysis.

1. Materials and methods

1.1. *Materials*

The chemicals used in this work were obtained from Slovakofarma Hlohovec, and Chemapol Prague (Czechoslovakia). Enzyme substrates *o*-nitrophenyl-β-D-galactopyranoside (ONPG) and *p*-nitrophenyl phosphate disodium (PNPP) were purchased from Lachema, Brno (Czechoslovakia).

1.1.1. Bacterial strain. Strain *E. coli* K-12, recombinate PQ 37 was put at our disposal by Dr. M. Hofnung (Institute Pasteur, Paris) and is gratefully acknowledged.

1.1.2. Media and buffers. Bacteria were cultured in LB medium (Miller, 1972) supplemented with ampicillin at 20 μg cm^{-3}. The *Z* buffer is as described by Miller, (1972) The *T* buffer is 1 mol dm^{-3} TRIS adjusted to pH 8.8 with HCl.

1.2. *Methods*

1.2.1. SOS chromotest. The test consists of colorimetric assay of enzymatic activities after incubation of the tester strain in the presence of various amounts of test compounds. A detailed procedure was published by Hofnung (1982). Briefly, an exponential-phase culture grown to A_{600}=0.2 in LB medium with ampicillin at 37 °C was diluted 1 : 10 into fresh medium. Fractions (0.6 cm^3) were distributed into glass test tubes containing 10 μl to 60 μl of the diluted compound to be tested. After 2-h incubation at 37 °C with shaking, β-galactosidase and alkaline phosphatase activities were assayed.

1.2.2. Enzyme assays. 1.2.2.1. Alkaline phosphatase – The *T* buffer (0.9 cm^3) was added to 0.1 cm^3 of cell culture. Cell membranes were disrupted by adding two drops of 0.1% sodium dodecyl sulphate solution and two

drops of chloroform and mixing vigorously. Tubes were equilibrated at 28 °C. The reaction was started by addition of 0.2 cm^3 of PNPP (4 mg cm^{-3} in T buffer) and stopped by addition of 0.34 cm^3 of 2 mol dm^{-3} HCl. After 5 min 0.34 cm^3 of 3 mol dm^{-3} TRIS was added to restore the colour which was measured spectrophotometrically at 420 nm.

1.2.2.2. β-galactosidase – The protocol was the same as for alkaline phosphatase except that T buffer was replaced by Z buffer, ONPG replaced PNPP, and the reaction was stopped with 0.65 cm^3 of 1 mol dm^{-3} Na_2CO_3.

Enzyme activities were expressed as units calculated according to MILLER (1972):

$$\text{Unit} = 1000\ (OD_{420} - 1.75\ OD_{550})\ /\ t\ v\ OD_{600} \qquad (1)$$

where t is the time (min) of reaction and v is the volume (cm^3) of culture used in the assay. The ratio R for β-galactosidase and alkaline phosphatase activity is calculated from the absorbances (A_β and A_p) measured at 420 nm, namely $R = A_\beta / A_p$. In order to standardize the conditions of the measurement the ratio R_c is normalized by dividing through the ratio (R_0) obtained at the absence of the mutagen. The factor I_f is thus expressed as $I_f = R_c/R_0$. The dependence of the induction factor I_f vs. amount of mutagenic substance (n) is linear for a certain interval. The SOS-inducing potency (SOSIP) is calculated as a slope from the linear range of $I_f = f(n)$ and represents the change of the induction factor I_f per nmol of the tested substance.

2. Results

2.1. Basic analytical parameters of the method

The typical dependence $I_f = f(n)$ on three chosen nitrofurans are presented in Fig. 1. Nitrofurans were dosed in 0.1–2 nmol amounts of the substance and nitrovin in 0.05–0.5 nmol amounts, respectively. The whole measured range was linear.

The linear dependence $I_f = f(n)$ can be expressed by the equation of the straight line $I_f = a + bn$. However, it follows from the definition of I_f that if mutagen is not present in the system ($n = 0$) the I_f value is equal to one. That is why, the dependence $I_f = a + bn$ can be substituted by the new one $I_f = l + b'n$. Using the F test we have proved the validity of this substitution.

At the same time we have determined the threshold value of detection and the sensitivity of the method. The difference of the signal offered by the mutagenic compound from the signal of the background is the criterion for the calculation of the threshold value of detection. For the limit value of the signal, i.e. of the induction factor, it follows:

$$I_m = k\, I_f(\text{o})$$

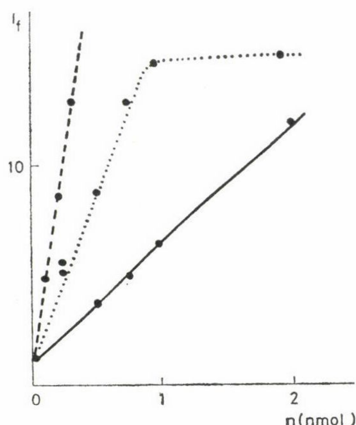


Fig. 1. Dependence of induction factor I_f on amount (n) for three selected nitrofurans. ———: NFAA, ...: methylester NFAA, ------: nitrovin

when $k = 3$ (i.e. a threefold higher value than that of the background) and the significance value level $\alpha = 0.02$; I_f (o) is the value of the induction factor for $n = 0$ (the value of the background). For the threshold value of detection of the amount of substance containing mutagen it follows

$$n_M = \frac{3\, I_f(o) - 1}{b'} = \frac{2}{b'}$$

The threshold value of detection expressed in the units of substance amount can be converted into the mutagen concentration in the sample. Thus, it was necessary to know the maximal volume of the sample which can be added to bacteria without the decrease of the signal. The I_f dependence on sample volume for the amide NFAA is shown in Fig. 2. The same dependence is obtained for other studied nitrofurans. It can be seen from the measured dependence that the maximal sample volume which can be added to the bacteria without decreasing of the signal is about 60 μl. This value was used for calculations of concentration threshold value: $c_M = n_M/V_M$ (see Table 1).

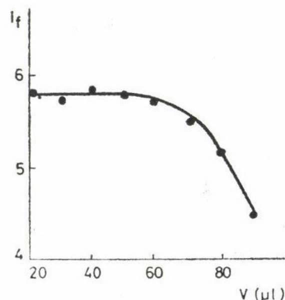
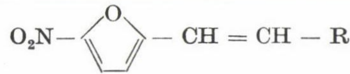


Fig. 2. Dependence of induction factor I_f on added volume (V) of measured sample at constant amount of mutagen ($n = 0.2$ nmol of amid NFAA)

Table 1

Threshold value of detection (n_M), threshold value of concentration (c_M) and sensitivity (S) *of the method for three chosen nitrofurans*



No	R	S ($nmol^{-1}$)	n_M (nmol)	$c_M \times 10^5$ ($mol\ dm^{-3}$)
1	— COOH	4.8	0.42	0.69
2	— $COOCH_3$	14.4	0.14	0.23
3	>C=N-NH-C(NH)-NH_2 · HCl	38.7	0.05	0.09

The sensitivity of the method was defined in the original paper (Quillardet et al., 1985) as the lowest amount of mutagen tested. However, the sensitivity according to analytical definition is rather defined as a change of signal caused by the change of amount i.e. $\Delta I_f / \Delta n$ and it is identical with the SOSIP value – the slope of dependence of $I_f = f(n)$. The threshold values of detection and the sensitivity of the method for three chosen nitrofurans are shown in the Table 1.

2.2. Determination of nitrofurans by SOS chromotest directly in foods

In the next section of this work we determined NFAA in wine and nitrovin in chicken liver.

NFAA was determined directly in wine (Račiansky výber) diluted with distilled water in the ratio of 1 : 1. NFAA was added in the concentration range of 2×10^{-7}–1×10^{-6} mol dm^{-3}. The control contained only the diluted sample of wine (without the addition of NFAA). In Fig. 3 the dependence of induction factor I_f on substance amount (n) of NFAA is shown. For comparison the analysis was made also in the absence of wine. Four parallel determinations were made in all cases. The values of slopes of the dependence $I_f = f(n)$ have been calculated. We also tested the congruence of arithmetical mean of slopes

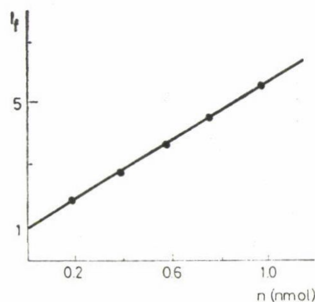


Fig. 3. Dependence of induction factor I_f on amount of NFAA *(n)* in sample of wine

found both in the presence and in the absence of wine. The slopes of dependence $I_f = f(n)$ were congruent in both cases. The values of R_0 ratios were also congruent, which means that the wine without NFAA did not show any mutagenic activity. The threshold value of detection for NFAA in wine is 2 mg NFAA per dm^3. For conservation purposes the NFAA was used in the concentration range of 10–20 mg dm^{-3} of wine (FARKAŠ, 1976).

We proceeded similarly with the determination of nitrovin in chicken meat, stomach and liver. Here, we present the results of the analysis of liver, while metabolic processes are taking place in this organ. However, the metabolic processes need not be always completed.

The 10^{-2} mol dm^{-3} sample of nitrovin in DMSO was diluted 10-times by adding the homogenate of chicken liver (prepated by homogenization of 10 g liver with 5 cm^3 of distilled H_2O) and then diluted with distilled water to the corresponding concentration. The control contained the sample of liver homogenate diluted in the same way, without nitrovin. The dependence of the induction factor I_f on substance amount (n) of nitrovin is shown in Fig. 4. The slight inhibition of the mutagenic effect of nitrovin observed in the presence of liver homogenate is probably caused by constituents present in the liver. The values of $R_{(0)}$ ratios of the activities of β-galactosidase and alkaline phosphatase were congruent both in the presence and in the absence of liver homogenate, thus the matrix did not show any mutagenicity without nitrovin.

The threshold value of detection for nitrovin is 0.3 mg kg^{-1} of liver. The obtained results show the possibility of direct determination of nitrovin in chicken liver.

3. Conclusions

The possibility of analytical use of response of genetically manipulated bacteria *E. coli* to the presence of mutagenic substances was verified. The linearity range was determined, which lies for nitrofurans between 0.05–2 nmol.

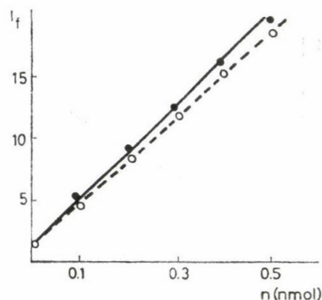


Fig. 4. Dependence of induction factor (I_f) on amount of nitrovin (n) ——— : in the absence of liver homogenate, ------ :in the presence of liver homogenate

The threshold values of detection for the given method are in the range 9×10^{-7}–8×10^{-5} mol dm^{-3} and of the sensitivities between 0.4–40 nmol^{-1}. We can compare these analytical parameters with those obtained by classical analytical methods. E.g. the threshold value of detection by colorimetric determination of nitrofurans (BORGATTI et al., 1970) is 10^{-7}–10^{-6} mol dm^{-3} and these are comparable values with those obtained by our approach.

Possibility of the determination of mutagenic compounds directly in true samples of foods was found. The NVAA in wine and nitrovin in chicken liver were chosen to serve as model mixtures. The threshold values of detection are sufficiently low in both cases (2 mg dm^{-3} for NFAA and 0.3 mg kg^{-1} for nitrovin) to determine these mutagens in true samples, e.g. in the imported wines or in the products of the poultry industry as well as in other foods directly, without pre-separation. Such determination is fully correct, when the sample contains a single mutagen without any interfering substances.

When the sample contains several mutagens the determination of the individual constituents is more questionable. Then the separation of the mutagen is recommended. Modification of the method enabling the determination of more than one compound is now in progress. Moreover, we are further modifying the method to increase the sensitivity and to lower the threshold value of detection.

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Acta Alimentaria, Vol. 16 (3), pp. 239–248 (1987)

MATHEMATICAL MODELLING IN FOOD ENGINEERING RESEARCH

B. HALLSTRÖM and C. TRÄGÅRDH

Division of Food Engineering, Lund University,
P. O. Box 124 221 00 Lund. Sweden

(Received: 17 March 1986; accepted: 10 July 1986)

The Food Engineering discipline was established at Lund University in Sweden in 1971. In the activities of the Division heat momentum and mass transfer problems, especially when applied to processing of food materials, calls for frequent use of mathematical models and accordingly a lot of work has been devoted to modelling technique. This interest was also demonstrated in an international symposium on "Mathematical Modelling in Food Processing" arranged in 1977 at Örenäs by the Division.

In this paper an attempt is made to describe different types of models of special interest in food process engineering. Further, examples are given of research projects at the Division in which modelling has been made use of. These problems mainly concern heat treatment of food materials, liquid as well as solid. References are made to the corresponding publications.

It is emphasized in the paper that a model is not better than the basic information it is fed with. It is therefore important to encourage all kind of basic research giving more details on process phenomenon, physical and "quality" properties as well as reaction kinetics.

Keywords: Mathematical modelling, heat momentum, mass transfer, food process engineering

A model is a very useful and compact way to summarize the knowledge about a phenomenon or a process. It is also a convenient way to communicate and teach regarding these problems.

There are several ways to classify models. One is to distinguish between static and dynamic models. A dynamic model is characterized through mathematical equations containing a differential equation where a dependent variable is a derivative of time of first or higher orders. They give possibilities to see how a system behaves in a time responding situation. A static model is characterized through mathematical equations where the dependent variable is a direct function of the independent variables for example through an algebraic expression or a derivative of an independent variable other than time.

Another way of classification is to distinguish between models based on physical principles and those based on experimental results.

It is important to recognize that a model never describes the full truth, it is only a way to simulate or describe a phenomenon with more or less accuracy. Especially when it comes to food-related problems, our physical and physico-chemical knowledge will never be so detailed that we can base our

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models on a theoretical background. Here we always have to rely on experimental results.

Models relevant to food process engineering problems contain both equations describing the physical processes and equations describing the kinetic reactions for the property variation in the foodstuff. The description of the processes originates from the chemical engineering discipline and is an equation or a number of equations based on transfer of momentum, heat and mass. These equations answer the question which external variation in e.g. temperature, water content, pH each element of the foodstuff is exposed to during the process as a function of process parameters.

Models are useful in several situations in food engineering and food technology research.

– Food properties are very complex and vary often considerably with origin, treatment, storing and so on. In order to use engineering properties of foodstuffs in scientific calculations a model of the responses to the variations is needed. Examples of such models are available in the literature e.g. water activity and water absorption isotherms, thermal and electrical conductivity, mass and momentum diffusivity. These models cited in the literature are sometimes useful but very often they have to be modified in order to fit your own foodstuff defined by experimental results.

– Time–temperature relationship in different heat treatment operations to be used in order to understand, design, operate, control and automatize and sometimes also optimize equipment, a processing line or a full plant.

– Also food quality properties are difficult to predict and here modelling is used. Reaction kinetics for these changes during processing may be described by models.

At the Division of Food Engineering of the Lund University, Sweden, modelling has been used as a tool in many projects during the years. Some examples are given below.

1. Models for physical processes

1.1. Models based on experimental results and dimensional analysis

All these models have in common that they describe the transfer process as a function of process parameters. They could have the form of pure statistical correlations or relationships between significant dimensionless groups. Even though the relationships obtained through dimensional analysis have a theoretical background and their exponents could have a physical interpretation they all could be called data fitting models. They thus summarize in a very compact form experimental results but are rather restricted as a tool for analysing and interpretation of experimental results. When using them

for process design it is very important to recognize their limitations and not try to extrapolate them beyond the original experimental conditions.

Well-known examples are in the case of stationary heat transfer.

$$\mathrm{Nu} = \mathrm{A}\ \mathrm{Re}^{b1}\ \mathrm{Pr}^{b2}$$

and in the case of unsteady heat transfer

$$\frac{T_s - T_t}{T_s - T_{t=0}} = f\left(\frac{x}{L'}\ \mathrm{Fo}, \mathrm{Bi}\right)$$

where T_s is the surrounding media temperature.

1.2. Models based on physical principles

As mentioned these relate to equations describing the conservation or transfer of heat, mass and momentum classically used in the sciences of physics and mechanics. The basic simplified equations for many of these models read in the one-dimensional case for a rectangular cartesian coordinate system: for momentum:

$$\varrho\left(\frac{\partial u}{\partial t} + u\frac{\partial u}{\partial x}\right) = -\frac{\partial p}{\partial x} + \frac{\partial}{\partial x}\left(\eta\frac{u}{x}\right) + S$$

for heat:

$$\frac{\partial T}{\partial t} + u\frac{\partial C_A}{\partial x} = \frac{\partial}{\partial x}\left(a\frac{\partial T}{\partial x}\right) + S$$

for mass:

$$\frac{\partial C_A}{\partial t} + u\frac{\partial C_A}{\partial x} = \frac{\partial}{\partial x}\left(D_{AB}\frac{\partial C_A}{\partial x}\right) + R_A$$

2. Models for physical properties and reaction kinetics of foods

This is the most difficult part of modelling in food process engineering as foods are varying in chemical and physical structure depending on previous treatment or earlier biological conditions. Foodstuffs also have a very complex chemical composition and physical structure, etc. This means that it is very difficult to predict in an absolute manner the course of predesigned treatment. It also means that it is often very difficult to evaluate experimental results.

2.1. Models based on experimental results

Due to the above mentioned difficulties it has also been difficult to mathematically formulate models having a theoretical background. Thus

large part of the models used in food process engineering are based on statistical correlation (regression analyses) or combined with analogies with similar phenomena.

The *D–Z* method describing the inactivation of microorganisms is a typical example of how the observed behavior of this phenomenon resulted in an established procedure for calculating lethality effect.

The coefficients involved are then experimentally determined and the above mentioned difficulties are relevant for their determination. Thus the *D*-value at a specified temperature is the time in minutes for the observed linear logarithmic reduction rate to 1/10 of the original value and *Z*-value is the observed change in *D*-value as the temperature varies.

The *D*-value corresponding to 121.1 °C (250 °F) was chosen as a reference and is in heat preservation (sterilization) problem known as the *F*-value. This *F*-value concerns, as mentioned above, thermal killing of microorganisms. Later, also quality properties have been simulated by the use of equivalent models and we have got *C*-values describing for instance the degradation of a certain property or reduction of the vitamin content at 100 °C.

Other examples are:

– thermal conductivity of liquid foods is a function of temperature

$$\lambda = A_0 + A_1 T + A T^2$$

– viscosity as a function of temperature

$$\eta = \eta_0 e^{-A/T}$$

2.2. Models based on physical principles

The most well-known example is taken from the thermodynamics of chemical reaction kinetics where the molecular collision theory and activated-complex theory form the basis. Thus

$$A \rightarrow B$$

$$-\frac{dC_A}{dt} = k\, C_A^n$$

$$k = k_0\, e^{-E_a/RT}$$

The latter is known as the Arrhénius equation. These equations have however been used in food reaction kinetics not only for single chemical reactions but also for complex property changes like inactivation of microorganisms, changes of sensory properties, destruction of vitamins, etc.

3. Examples from the research work of the Division of Food Engineering, Lund

3.1. Heat treatment of solid food

Thermal processing of solid foodstuffs has been studied at the division for a period of more than ten years. This work has mainly concerned meat products and bread, and the processes involved are cooking (roasting), deep fat frying, baking and thawing. Some of the results are summarized below.

Physical properties of the products have been studied. Thermal properties of meat products (thermal conductivity and specific heat) are reported on by SÖRENFORS and DAGERSKOG (1978), similar for bread by JOHNSSON and SKJÖLDEBRAND (1984). Water binding and water holding properties for meat products have been analysed by MOTARJEMI and SKJÖLDEBRAND (1982).

Several studies on heat transfer problems have also been executed. For external heat transfer in the processes mentioned above, also mass transfer plays an important role. Water evaporates from the surface layer of the product and the water evaporated contributes to the transfer of heat. These problems have been discussed by SKJÖLDEBRAND and HALLSTRÖM (1983). Heat transfer coefficients for the different processes have been summarized by HALLSTRÖM (1980).

Heat transfer studies inside the product also have been conducted. SÖRENFORS (1978) studied and simulated temperature profiles during deep fat frying while SKJÖLDEBRAND (1979) performed a similar program for air blast heating of meat products. The latter studies also involved crust formation, i.e. the surface drying of the product mentioned above. These studies analyse the heat and mass transfer in the surface layer as well as in the evaporation zone. Similar work concerning bread baking is going on.

The time—temperature relationships for each volume element in the food product during the heat treatment have been combined with reaction kinetics for different "quality properties". SKJÖLDBRAND and co-workers (1983) in this way simulated thiamin degradation during roasting of a meat product (Fig. 1). This work was followed up by HOLTZ and SKJÖLDEBRAND (1986).

3.2. Heat sterilization of liquid food in continuous heat exchangers

In addition to the previously presented models, flow models can also be used and combined with the others.

These may also be classified in a similar manner.

The most simple flow model is the plug-flow model often accounted for in food process engineering. According to the definition all fluid elements travel at a velocity giving them identical residential times in the system. This is a

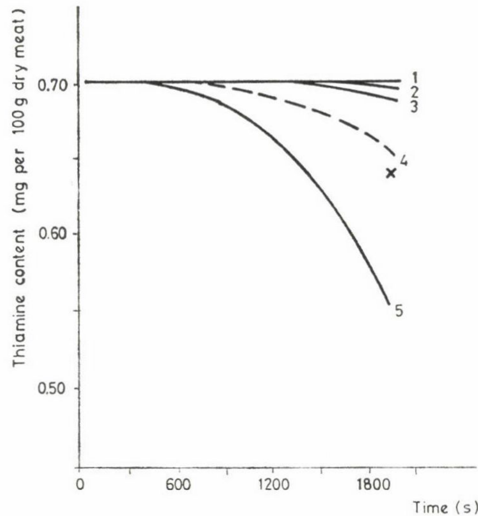

Fig. 1. Denaturation of thiamine content during baking of ground meat in a convection oven. Air temperature 150 °C, air velocity 9 m s^{-1}. Full lines indicate calculated values at different distances from the surface; the dotted line is the volume average. The $\bar{x}$ is experimentally determined average value. 1: centre; 2: 15 mm; 3: 10 mm; 4: mean value; 5: surface

non-physical flow system and could then be combined with empirical equations for heat transfer, physical properties and reaction kinetics to a system for the evaluation or prediction of performance characteristics. Correspondingly, the most simple model for the temperature profile is where only an axial variation is considered as determined by the process characteristics. This approach was used by HALLSTRÖM and DEJMEK (1977) and HALLSTRÖM (1977).

As this approach is physically unrealistic other models were evaluated. The goal is to achieve a model which is physically resembling the real process. Potentially it should then have better possibility to give more general and informative results. The first attempt in this direction was made by PAULSSON and TRÄGÅRDH (1984). A combined model was developed taking into account turbulent mixing effect both regarding food components and heat.

As a stochastic approach was used to simulate the travelling of the fluid "particles" through the system also an extension of the residence time distribution concept to thermal time distribution was possible. The model shown in Fig. 2 thus considers velocity and temperature gradients in both axial and radial directions and the chaotic turbulent flow pattern is simulated through a random choice of residence time in each compartment and pathway between the compartments. Three types of reaction rate kinetic models for product quality changes were tested, the *D–Z*-model as a representative for data fitting models and the Arrhenius and Casolari equations as representatives for physical principle models. They do differ in predicted process results despite that

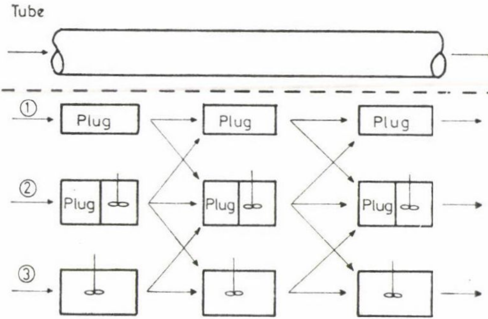

Fig. 2. A combined flow model for a tube; region 1 with plug flow reactors; region 2 with both plug flow reactors and perfect mixers; region 3 with perfect mixers in series. The flow pathways are symbolized by the arrows

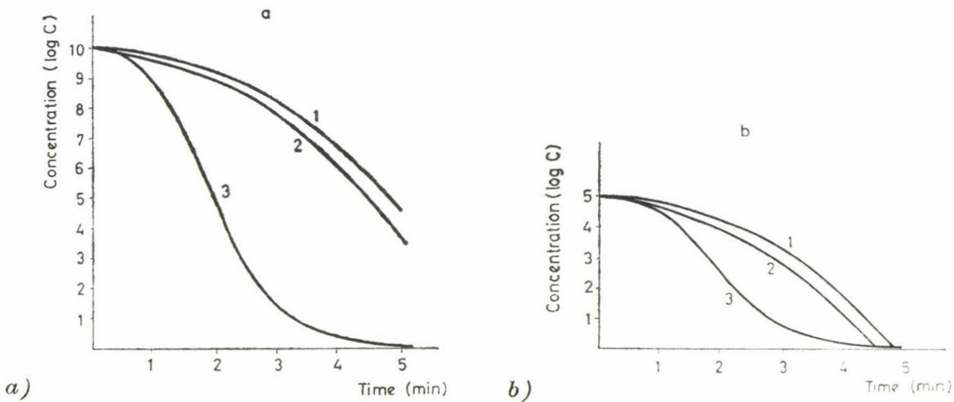

Fig. 3. Thermal inactivation of *Bacillus stearothermophilus* in water. Predicted figures using different activation models (1) the *D-Z* method (2) the Arrhenius type of equation and (3) the method of Casolari for two initial concentrations of micro-organism (a) 10^{10} per cm^3 and (b) 10^5 per cm^3. (TRÄGÅRDH & PAULSSON, 1986)

experimental data used to determine the required model coefficients were the same as in Fig. 3.

Further steps were then taken by ELLBORG and TRÄGÅRDH (1985) to approach the physical realities of the turbulent motion of the food liquid. The model developed here uses directly the equations for turbulent motion and other turbulence models to describe the individual pathways of the fluid particles. The temperature history these "particles" are exposed to is obtained through the corresponding equation for turbulent transport of enthalpy.

The integration of the time—temperature history of a sufficiently large number of particles give the time—temperature distribution. The contribution of the instantaneous velocity vector is obtained through a stochastic procedure and through a random walk model and the pathway is obtained.

Thus the trajectories are given by

$$X = \int_0^t u \; dt$$

where $u = \bar{u} + u'$.

The mean velocity vector $\bar{u}$ is given by the Reynold's equation for turbulent motion. The fluctuation velocity vector u' is simulated randomly from a Gaussian distribution function with zero mean value and a standard deviation of $\sqrt{0.667\,k}$. The k is the turbulent kinetic energy. The local integration time modelled as $0.20\,k/\varepsilon$. The ε is the dissipation rate of the turbulent kinetic energy. The transport equation for k and ε are computed simultaneously with the equations of motion and enthalpy.

The results are in close agreement with those obtained by the model of Paulsson and Trägårdh but they have a general validity which is of interest as it then can rely on the development in the theory of turbulence and computational fluid dynamics.

4. Conclusions

Mathematical modelling is of increasing importance in food process engineering. It is the base for design, operation, control and optimization of equipment, processes and plants. By means of today's computer resources it is possible in a simple way and at a low cost to build and to handle also rather

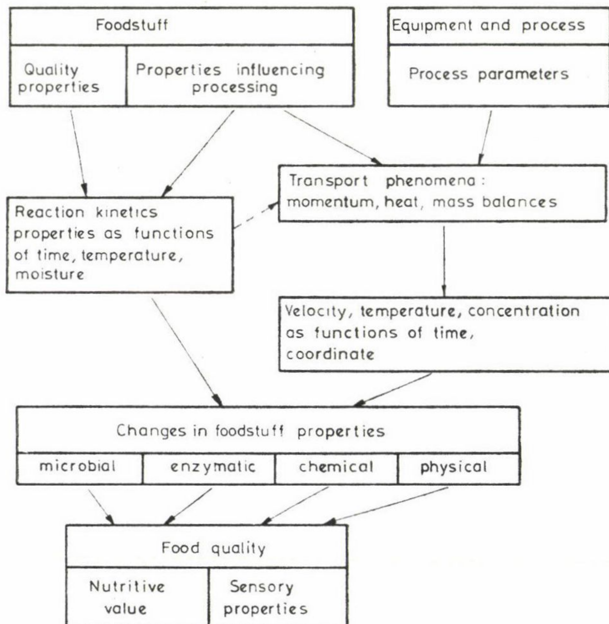

Fig. 4. Diagram showing relationships in food process modelling

complicated models. In food processing there are therefore great possibilities for this development in order to improve the quality of the final product and reduce waste and costs. But it has to be remembered that the model and the process it is based on is never better than the original data. In order to get better models and to keep up with the development in modelling work and in data processing, better knowledge or engineering properties, fundamentals of processes and reaction kinetics is needed. This calls for extended cooperation between all disciplines concerned with food problems. This is to some degree illustrated by Fig. 4 (from BRUIN et al., 1984).

Symbols

a	thermal diffusivity
A	constant
b	exponent
x	coordinate
X	position
L	length
t	time
u	velocity
p	pressure
S	momentum or enthalpy source
T	temperature
C_A	concentration of component A
D	diffusivity
R_A	reaction rate
E_a	activation energy
R	gas constant
Q	heat source
k	reaction rate coefficient/turbulent kinetic energy
n	reaction order exponent
ϱ	density
η	viscosity
λ	thermal conductivity
ε	turbulent kinetic energy dissipation rate

Dimensionless groups

Nu	Nusselt number
Re	Reynolds number
Pr	Prandtl number
Fo	Fourier number
Bi	Biot number

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THE EFFECT OF OXYGEN ABSORPTION RATE (AERATION RATE) ON THE METHIONINE CONTENT, PROTEIN CONTENT AND GROWTH RATE OF SOME METHIONINE RICH MUTANTS OF *CANDIDA GUILLIERMONDII* 812

A. HALÁSZ, A. ALI MUAYAD and B. MÁTRAI

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

(Received: 6 May 1982; revision received: 25 June 1986; accepted: 9 July 1986)

Strains of yeast selected from successive UV light treatments of *Candida guilliermondii* 812 were used. Selection was carried out according to the methionine, protein and nucleic acid content. All fermentations were carried out under similar environmental conditions using synthetic media supplemented with 2% glucose and 0.5% yeast extract. The only altered cultivation condition was the aeration rates. Different fermentors were used with aeration rates between 60–1250 $dm^{-3}h^{-1}dm^{-3}$, corresponding to oxygen absorption rates of 20–208 mmol O_2 dm^3h^{-1}.

The study showed that the aeration rate does effect the methionine content of the yeast biomass, in addition to its known effect on the growth rate. Protein content did not show any significant change.

Keywords: *Candida guilliermondii*, aeration rate, methionine protein content, growth rate

As methionine is one of the limiting amino acids, and as proteins fortified with free amino acids being defficient did not prove effective like those present in bound form in the protein, therefore it was felt necessary to determine the optimum conditions under which a methionine rich single cell protein could be produced.

During a previous work of the authors (MUAYAD et al., 1983) on producing, isolating and studying yeast mutants rich in methionine an observation was constantly made. Colonies were preliminarily isolated according to their growth on sulphur rich media. Then they were grown on solid media, shake cultures and later in batch fermentors. The only variable was the aeration rate. It was observed that the methionine content decreases when the aeration rate increases.

Although extensive data have been accumulated on the subject, the question of whether significant environmentally induced variations are possible in the amino acid composition of microbial still has not been answered properly. The rate of balanced growth of microorganisms in a batch culture can be varied by changing one or more of the fermentation parameters. When the composition of the medium is changed, the amount of RNA per unit bio-

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

mass increases with the growth rate whereas protein content remains relatively constant (ROSSET & MONIER, 1966). Based on similar observations, SCHAECHTER and co-workers (1958) calculated that in growing organisms the rate of protein synthesis per ribosome particle is constant and independent of the growth rate. In contrast with the above ROSSET and MONIER (1966) found increase in the efficiency of ribosomes in protein synthesis with increasing growth rate.

The effect of cultivation temperature on the RNA and protein content of microorganisms has been studied by several authors. BROWN and ROSE (1969) found that RNA and protein vary slightly per unit of biomass in batch cultures cultivated between 15–30 °C. SCHAECHTER and co-workers (1958) have suggested that temperature alters the rate constant for growth but not the amount or nature of the reactants that control growth. ALROY and TANNENBAUM (1973) cultivated *Candida utilis* in a glucose-limited chemostat culture at various pH levels, temperatures, dilution rates and nitrogen sources. They found that the ratio of total nucleic acid to protein increased with increase in dilution rate at constant temperature and decreased with increase in temperature at constant dilution rate. They also examined the effect of the same factors on the amino acid profiles of the cell residue. They concluded that the correlation covers increased levels of serine and glutamic acid with increased dilution rate when *Candida utilis* was examined, while in *E. aerogenes*, increased levels of glutamic acid correlates with increased dilution rate. They thought that changes in other amino acids like methionine could be only conceivably explained by varying degrees of destruction during acid hydrolysis.

The other variable which could play a role in affecting the growth and composition of microorganism is aeration. The presence of oxygen is known to bring about fundamental changes in the metabolism of a number of microorganisms. For some of them it is essential for growth, while for others it is toxic even in small amounts, but the mechanisms of the response to oxygen are still poorly understood. At present, there is no experience available on how amino acid metabolism is influenced by changes in fermentation parameters. The purpose of this study is to give experimental data on the effect of the aeration rate on the cell biomass composition and growth of *Candida guilliermondii* 812.

1. Materials and methods

1.1. Yeast strains examined

Candida guillermondii 812

Candida guillermondii 812-5/4, treated twice with UV light (240 and 60 sec).

Candida guilliermondii 812–11, treated twice with UV light (240 and 300 sec).

Candida guilliermondii 812–5/3, treated twice with UV light (150 and 300 sec).
Candida guilliermondii 812–300, treated three times with UV light (210, 180 and 300 sec).
Candida guilliermondii 812–12, treated twice with UV light (240 and 270 sec).

UV treatment was performed on strains cultured for 24 h. Cell concentration was determined by a haemacytometer. 0.1 cm^3 quantities of dilutions containing 20^2, 10^3, 10^4, 10^5 and 10^6 cells per cm^3 were spread over of prepoured solidified yeast extract media in Petri-dishes. The inoculated Petri-dishes were exposed to UV light from a Tungsram, Germicide lamp of 15 W at 30 cm distance. After irradiation the Petri-dishes were immediately transferred to a dark place and incubated at 30 °C for 72 h. Stable mutants with favourable growth characteristics were exposed to another series of UV treatment. Five strains were isolated after successive treatments of mutants.

1.2. Determination of protein content

Kjel–Foss automatic type instrument was used using 0.5 g dried yeast biomass.

1.3. Determination of methionine content

Thirty mg dried yeast biomass was digested by 6 cm^3 6 *N* hydrochloric acid for 24 h at 105 °C. Acid was evaporated in vacuum at 40 °C for 24 h, then methionine was determined according to BARTON–WRIGHT (1977) by using *Leuconostoc mesenteroides* P60 auxotroph mutant, as a test organism.

1.4. Preparation of yeast culture inoculum

Twentyfour hour inoculum was prepared from aseptically centrifuged shake culture grown on synthetic media with 2% glucose and 0.5% yeast extract, respectively.

1.5. Determination of oxygen absorption rate

Determination was carried out according to COOPER and MILLER (1944).

1.6. Fermentation

All fermentations were repeated three times in duplicate under the same conditions and the means of the results are presented.

1.6.1. Column fermentor. Six cm diameter double wall glass columns of 400 cm^3 capacity were used. Filtered air was introduced from the bottom of the column through a glass filter (G4).

Temperature was regulated by connecting the columns to a constant temperature circulating water bath. The pH was adjusted by the addition of 2.5% NaOH solution and maintained at 4.5. Aeration rates of 250, 500, 750, 1000, 1250 cm^3 h^{-1} dm^{-3} medium, corresponding to 28.1, 44.0, 58.0, 71.5, 84.2 mmol O_2 dm^{-3} h^{-1}, respectively, were chosen.

1.6.2. Biofer type fermentor. A 7 dm^3 capacity fermentor was used with automatic control of temperature, pH and foam. The equipment was also fitted to record CO_2(%) in outlet air, dissolved oxygen, pH, temperature and oxydation-reduction potential of the medium. Aeration rates of 28.5, 42.8, 57.1 dm^3 h^{-1} dm^{-3} medium were used corresponding to (70, 150, 200 mm O_2 dm^{-3} h^{-1}).

1.6.3. New Brunswick type fermentor. This type is of 5 dm^3 fermentation medium capacity. Fittings to measure pH and dissolved oxygen were provided. Aeration rates of 12, 48 and 60 dm^3 h^{-1} dm^{-3} were used and the pH was adjusted manually by adding 2.3% ammonia solution.

The fermentor was fitted with three blademixers of different sizes. Different oxygen absorption rates were obtained by changing the blades or the aeration rate, thus oxygen transfer rates of 60, 90, 150 and 208 mmol O_2 dm^{-3} h^{-1} were obtained.

1.6.4. CHEMAP laboratory type fermentor. This fermentor had a capacity of up to 20 dm^3 media, but only 7 dm^3 media were maintained through out the fermentation period. pH and temperature were regulated automatically. This fermentor also was designed and equipped for continuous cultivation. Ninety and 220 mmol O_2 dm^{-3} h^{-1} oxygen transfer rates were used.

2. Results

2.1. Column fermentor

2.1.1. Comparison of the five selected strains. Inocula from the selected trains were prepared. Fermentation was carried out using synthetic media at 32 °C, pH 4.5 and 1000 dm^3 h^{-1} dm^{-3} aeration rate. Each strain was cultivated in at least three columns, the average values are represented by the growth curves shown in Fig. 1 and Table 1. Methionine and protein contents were also determined. The results showed that strain 11 had the highest specific growth rate with a good methionine and protein content, while strain 5/4 was the next best. Therefore they were selected for further studies.

2.1.2. The effect of aeration rate on strain 11. Aeration rates of 250, 500, 750, 1000 and 1250 dm^3 h^{-1} dm^{-3} media were applied. The results showed

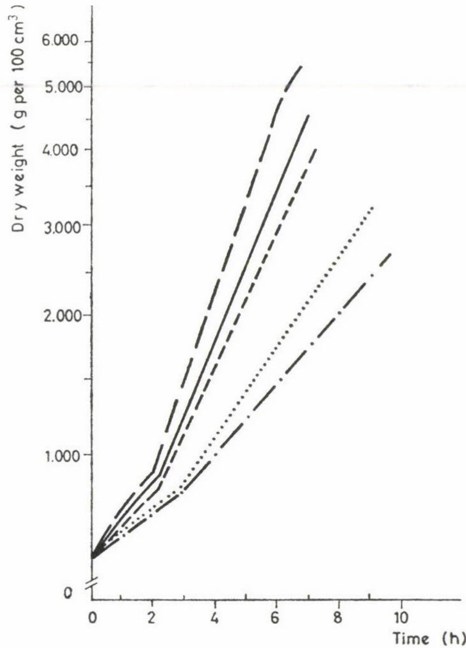


Fig. 1. Comparison of UV treated strains on growth rate and methionine content. - - - : strain 11; ——— : strain 5/4; - - - - : strain 5/3; . . . . . . : strain 12; —·—·—· : strain 300

Table 1

Comparison of UV treated strains on growth rate and methionine content

Mark of strain	Growth rate (h^{-1})	Generation time (h)	Protein content on dry weight (%)	Methionine content on dry weight (%)
11	0.36	1.92	46	0.8
5/4	0.30	2.30	43	0.7
5/3	0.26	2.60	42	0.79
12	0.19	3.60	45	0.82
300	0.11	6.30	46	0.71

clearly (Figs. 2, 3, 4 and Table 2), that the aeration rate, at 1250 $dm^3\ h^{-1}\ dm^{-3}$ gave the highest growth rate, with a generation time of 1,38 h, but methionine content decreased. The highest methionine content was obtained at an aeration rate of 750 $dm^3\ h^{-1}\ dm^{-3}$ but the specific growth rate was lower. Therefore it could be said that the specific growth rate corresponded directly with increased aeration rate while protein content was not affected.

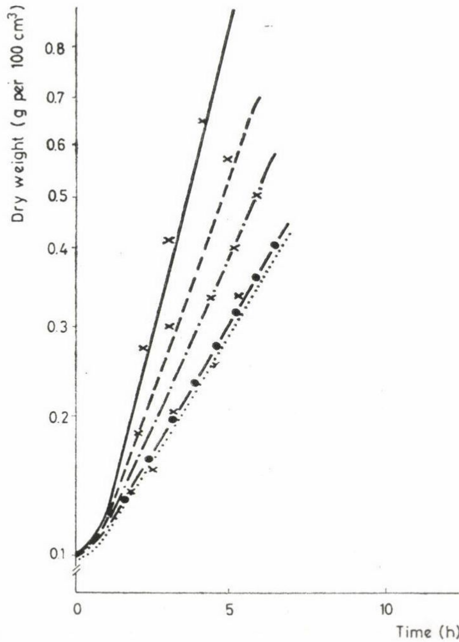
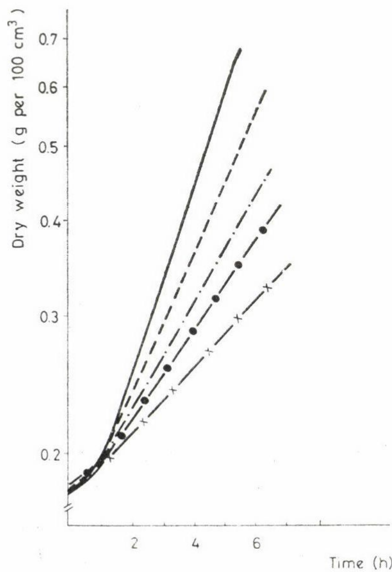


Fig. 2. Effect of aeration intensity on growth of strain 11 cultivated in column fermentor. ———: 1250 $dm^3 h^{-1} dm^{-3}$ (500 $dm^3 h^{-1}$); – – – –: 1000 $dm^3 h^{-1} dm^{-3}$ (400 $dm^3 h^{-1}$); –·–·–·–: 750 $dm^3 h^{-1} dm^{-3}$ (300 $dm^3 h^{-1}$); —— · ——: 500 $dm^3 h^{-1} dm^{-3}$ (200 $dm^3 h^{-1}$); ....: 250 $dm^3 h^{-1} dm^{-3}$ (100 $dm^3 h^{-1}$). Figures in brackets mean oxygen transfer



3. Fig. Effect of aeration intensity on growth of strain 5/4 cultivated in column fermentor. For legends see Fig. 2

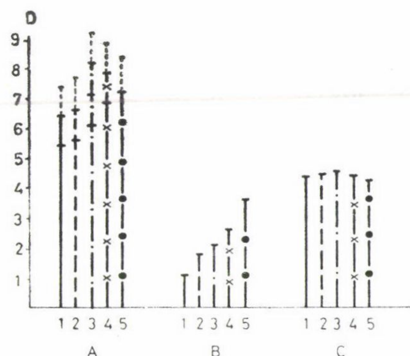


Fig. 4. Alteration of main characteristics of mutant strain 5/4 at different oxygen absorption rates cultivation in column fermentor. 1: 28.1 mmol O_2 dm^{-3} h^{-1}; 2: 44 mmol O_2 dm^{-3} h^{-1}; 3: 58 mmol O_2 dm^{-3} h^{-1}; 4: 71.5 mmol O_2 dm^{-3} h^{-1}; 5: 84.2 mmol O_2 dm^{-3} h^{-1}; A: = methionine content (%); B: = growth rate (h^{-1}) C: = protein content (%); D: = methionine content, % on dry weight $\times$ 10^{-1}, growth rate K (h^{-1}) $\times$ 10^{-1}; protein content, % on dry weight $\times$ 10

Table 2

Effect of aeration intensity on growth of strain 5/4 cultivated in column fermentor

Aeration (dm^3h^{-1})	Aeration ($dm^3h^{-1}dm^{-3}$)	Sulfite number (mmol $O_2dm^{-3}h^{-1}$)	Generation time (h)	Growth rate (h^{-1})	Protein content on dry weight (%)	Methionine content on dry weight (%)
500	1250	84.2	1.90	0.36	43.5	0.73
400	1000	71.5	2.10	0.26	43.8	0.79
300	750	58.5	3.30	0.21	45.2	0.82
200	500	44.0	3.85	0.18	44.8	0.66
100	250	28.1	6.30	0.11	43.1	0.64

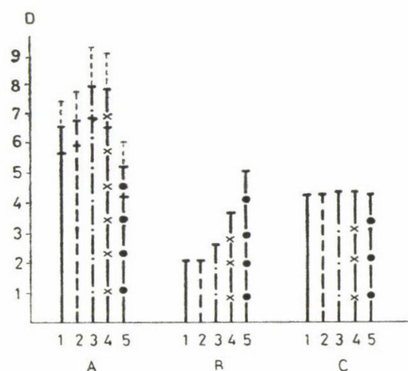


Fig. 5. Alteration of main characteristics of mutant strain 11 at different oxygen absorption rates cultivation in column fermentor. For legends see Fig. 4

2.1.3. Effect of aeration rate on strain 5/4. This strain gave similar results to those of strain 11 (Figs. 5, 6 and 7). The highest growth rate was not affected and methionine content showed the highest level at 750 $dm^3\ h^{-1}\ dm^{-3}$. At lower aeration rates neither growth rate nor methionine content were satisfactory.

	5	4	3	2
1	*	*	*	0
2	*	*	*	
3	**	0		
4	0			

Fig. 6. Changes in methionine content at different oxygen levels in mutant strain 5/4. Significance test. 1: 28.1 mmol $dm^{-3}\ h^{-1}$; 2: 44 mmol $dm^{-3}\ h^{-1}$3: 58 mmol $dm^{-3}\ h^{-1}$; 4: 71.5 mmol $dm^{-3}\ h^{-1}$; 5: 84.2 mmol $dm^{-3}\ h^{-1}$; **: $P \geq 99\%$; *: $P \geq 95\%$; 0: no significant difference

	5	4	3	2
1	*	*	*	0
2	*	*	*	
3	**	0		
4	*			

Fig. 7. Changes in methionine content at different oxygen levels in mutant strain 11. Significance test. For legends see Fig. 6

2.2. Fermentations in New Brunswick fermentor

These experiments were carried out in the Department of Agricultural Chemistry, Technical University, Budapest. Strains 11 and 5/4 were investigated.

Strain 11 showed also stable protein content at all rates of aeration while methionine value decreased with decreasing aeration rates.

At an aeration rate of 60 $dm^3\ h^{-1}\ dm^{-3}$ the specific growth rate was 0.57 h^{-1} which decreased correspondingly to reach a value of 0.185 h^{-1} at 12 $dm^3\ h^{-1}\ dm^{-3}$. Methionine content changed also positively with increasing aeration rates (Figs. 8 and 11 and Table 3).

Strain 5/4 was also grown in this fermentor under similar conditions. The results showed again (Figs. 9, 10 and Table 4) that the highest growth rate was obtained at the highest aeration rate used (i.e. 60 $dm^3\ h^{-1}\ dm^{-3}$). Methionine content showed the highest value also at this aeration rate while protein content did not change significantly.

Table 3

Effect of aeration on growth of 5/4 mutant strain in New Brunswick laboratory fermentor

Aeration ($dm^3h^{-1}dm^{-3}$)	Growth rate (h^{-1})	tg (h)	Protein content on dry weight (%)	Methionine content on dry weight (%)	Sulfite number ($mmolO_2dm^{-3}h^{-1}$)
60	0.31	2.23	53.7	1.08	208
48	0.22	3.15	53.8	0.97	150
12	0.16	4.4	53.9	0.81	60

Table 4

Effect of aeration on growth of mutant strain 11 in New Brunswick laboratory fermentor

Aeration ($dm^3\ h^{-1}$)	Aeration ($dm^3h^{-1}dm^{-3}$)	Growth rate (h^{-1})	tg (h)	Protein content on dry weight (%)	Methionine content on dry weight (%)	Sulfite number ($mmolO_2dm^{-3}h^{-1}$)
300	60	0.57	1.29	54.5	1.06	208
240	48	0.34	2.15	54.2	0.86	130
60	12	0.25	3.05	54.6	0.84	90
60	12	0.17	4.05	54.7	0.84	60

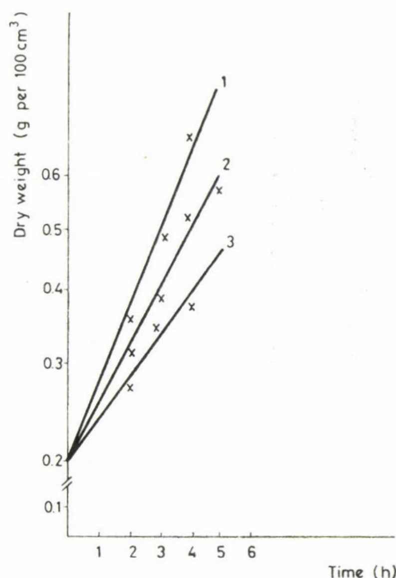


Fig. 8. Effect of aeration on growth of 5/4 mutant strain in New Brunswick laboratory fermentor. 1: 60 $dm^3\ h^{-1}\ dm^{-3}$, r = 0.9754; 2: 48 $dm^3\ h^{-1}\ dm^{-3}$, r = 0.9897; 3: 42 $dm^3\ h^{-1}\ dm^{-3}$, r = 4.5; Fermentation parameters: pH = 4.5; T = 30 °C; r.p.m. = 750

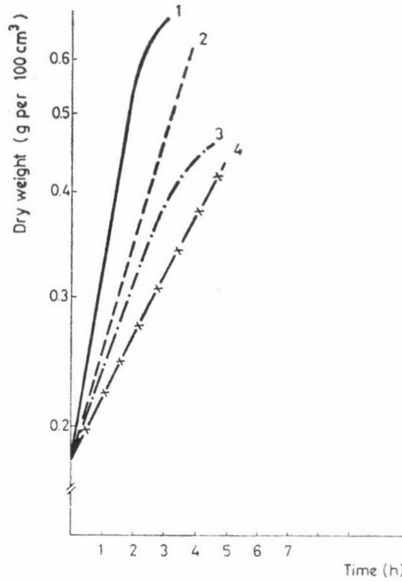


Fig. 9. Effect of aeration on growth of mutant strain 11 in New Brunswick laboratory fermentor. 1: 60 $dm^3\ h^{-1}\ dm^{-3}$; 2: 48 $dm^3\ h^{-1}\ dm^{-3}$; 3: 12 $dm^3\ h^{-1}\ dm^{-3}$; 4: 12 $dm^3\ h^{-1}\ dm^{-3}$

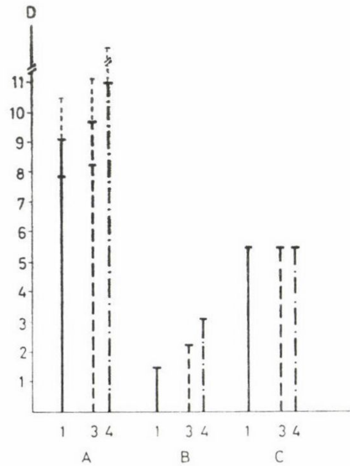


Fig. 10. Main characteristics of mutant strain 5/4 as a function of the oxygen supply. 1: 60 mmol $O_2\ dm^{-3}\ h^{-1}$; 3: 90 mmol $O_2\ dm^{-3}\ h^{-1}$; 4: 150 mmol $O_2\ dm^{-3}\ h^{-1}$; A: methionine content %; B: growth rate (h^{-1}); C: protein content %; D: in the case of A + 10^{-1}, B × 10^{-1}, C × 10

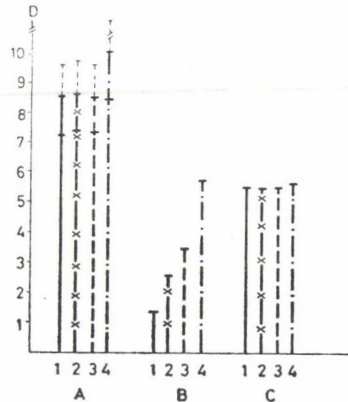


Fig. 11. Main characteristics of mutant strain 11 as a function of the oxygen supply in New Brunswick laboratory fermentor. 1: 60 mmol O_2 dm^{-3} h^{-1}; 2: 90 mmol O_2 dm^{-3} h^{-1}; 3: 150 mmol O_2 dm^{-3} h^{-1}; 4: 208 mmol O_2 dm^{-3} h^{-1}; A: methionine content %; B: growth rate (h^{-1}); C: protein content %; D: in the case of A $\times$ 10^{-1}, B $\times$ 10^{-1}, C $\times$ 10

2.3. *Fermentation in BIOFER type fermentor*

2.3.1. Effect of aeration level on strain 5/4. This strain was cultivated under environmental conditions similar to those of the parent strain *Candida guilliermondii* 812.

No change was observed in the protein content at different levels of aeration, but methionine content was affected.

It showed the lowest level (0.41%) at an aeration rate of 57 dm^3 h^{-1} dm^{-3}, while at a lower aeration rate (43 dm^3 h^{-1} dm^{-3}) it increased to 0.68% and at 28.5 dm^3 h^{-1} dm^{-3} aeration rate it was 0.65% (Fig. 12).

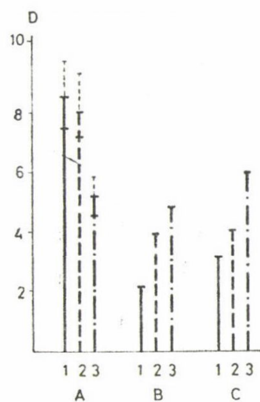


Fig. 12. Main characteristics of mutant strain 11 as a function of the oxygen supply in BIOFER fermentor. 1: 28.1 mmol O_2 dm^{-3} h^{-1}; 2: 49.2 mmol O_2 dm^{-3} h^{-1}; 3: 57.3 mmol O_2 dm^{-3} h^{-1}; A: methionine content %; B: growth rate (h^{-1}); C: protein content %; D: in the case of A $\times$ 10^{-1}, B $\times$ 10^{-1}, C $\times$ 10

2.3.2. Effect of aeration level on the strain 11. This strain was also cultivated under the same condition as it is given in 2.3.1. Protein again kept a stable level of 50 ± 2% at different aeration levels. Methionine content was 0.52% at the highest aeration rate of 57 $dm^3 h^{-1} dm^{-3}$. It increased to 0.80% when aeration was decreased to 43 $dm^3 h^{-1} dm^{-3}$ and it was 0.85% at 28.5 $dm^3 h^{-1} dm^{-3}$ aeration rate.

The specific growth rate was the highest, 0.51 h^{-1} at 57 $dm^3 h^{-1} dm^{-3}$, this dropped to 0.4 h^{-1} at 43 $dm^3 h^{-1} dm^{-3}$ aeration rate. And at 28.5 $dm^3 h^{-1} dm^{-3}$ the specific growth rate dropped to 0.32 h^{-1} (Fig. 13).

2.4. *Fermentation in CHEMAP type fermentor*

2.4.1. Effect of aeration on strain 11. Seven dm^3 culture media, the pH adjusted to 4.5, temperature at 30 °C, mixing rate 1500 r.p.m., aeration rates 28.5 and 57 $dm^3 h^{-1} dm^{-3}$ were used. These aeration rates corresponded to 90 and 220 mmol O_2 $dm^{-3} h^{-1}$.

Result showed that growth rate was highly affected by the aeration level. The specific growth rate at 28.5 $dm^3 h^{-1} dm^{-3}$ was 0.31 h^{-1} while at 57 $dm^3 h^{-1} dm^{-3}$ it was 0.52 h^{-1}.

Protein content was not affected by the aeration levels and was 48 and 48.5% of the dry mass. Methionine content showed a low value of 0.52% at the high aeration rate of 57 $dm^3 h^{-1} dm^{-3}$ (Fig. 13).

The parent strain *Candida guilliermondii* 812 and strain 5/4 were also grown in this type of fermentor. The results are shown on Figs. 14, 15.

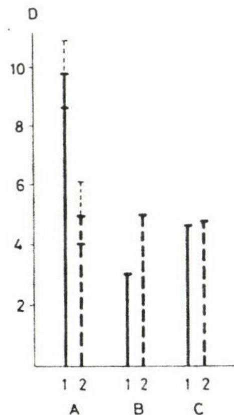


Fig. 13. Main characteristics of mutant strain 11 as a function of the oxygen supply in CHEMAP fermentor. 1: 90 mmol O_2 $dm^{-3} h^{-1}$; 2: 220 mmol O_2 $dm^{-1} h^{-1}$; A: methionine content %; B: growth rate (h^{-1}); C: protein content %; D: in the case of A × 10^{-1}, B × 10^{-1}, C × 10

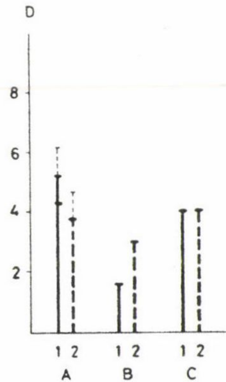


Fig. 14. Main characteristics of parent strain *C. guilliermondii* 812 as a function of the oxygen supply in CHEMAP fermentor. 1: 90 mmol O_2 dm^{-3} h^{-1}; 2: 220 mmol O_2 $dm^{-3}h^{-1}$; A: methionine content %; B: growth rate (h^{-1}); C: protein content %; D: in the case of $A \times 10^{-1}$, $B \times 10^{-1}$, $C \times 10$

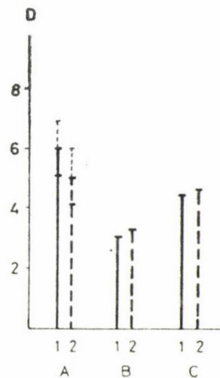


Fig. 15. Main characteristics of mutant strain 5/4 as a function of the oxygen supply in CHEMAP fermentor. 1: 90 mmol O_2 dm^{-3} h^{-1}; 2: 220 mmol O_2 dm^{-3} h^{-1}; A: methionine content % on dry weight; B: growth rate (h^{-1}); C: protein content % on dry weight; D: in the case of $A \times 10^{-1}$, $B \times 10^{-1}$, $C \times 10$

3. Discussion

The results showed clearly that aeration rate had a great effect on the specific growth rate of yeast strains examined. It is also obvious that not only the amout of air passed trough the media is important, but the oxigen absorption rate, vigorousity of mixing which determines the air bubbles surface aera available) and the volume and depth of the fermentor; therefore different fermentors showed different oxygen absorption rates at similar aeration levels. The importance of the oxygen absorption rate was clear on the specific growth rate when a high rate of aeration (1250 dm^3 h^{-1} dm^{-3}) was used, but still lower specific growth rates were obtained compared to lower

aeration levels of 60 dm^3 h^{-1} dm^{-3}. This is easily explained by knowing the oxygen absorption rate. This agreed with the results obtained by CHAPMAN (1966).

The protein content was found to be stable at different levels of aeration except one case, in which the parent strain *Candida guilliermondii* 812 cultivated in the BIOFER fermentor. Only in this case, higher aeration rate produced constantly yeast biomass of lower protein content compared to that at lower levels. Different fermentors showed different protein content even when the oxygen absorption rates were similar in both cases.

Methionine content was found to be affected by the aeration rate and the oxygen absorption rate. Different fermentors showed different results. The New Brunswick type fermentor showed higher values of methionine content at higher aeration levels and high oxygen absorption rate values.

While in BIOFER and CHEMAP fermentors the methionine content was very low at higher aeration rates. It could be suggested that up to certain limits, aeration does not effect the methionine content, when this limit is exceeded, methionine content starts dropping. This could lead to the conclusion that low aeration levels with high oxygen transfer rates would result in a yeast biomass with its highest methionine content.

*

The authors wish to thank the members of the Agricultural Chemistry Department of Technical University, Budapest, for their assistance in accomplishing a part of this work.

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Acta Alimentaria, Vol. 16 (3), pp. 263—270 (1987)

FACTORS AFFECTING THE QUALITY OF SMOKE-DRIED MEATS IN NIGERIA

D. O. ALONGE

Department of Veterinary Public Health and Preventive Medicine, University of Ibadan, Ibadan. Nigeria

(Received: 3 May 1985; revision received: 15 July 1986; accepted: 17 July 1986)

The smoking of meats in Nigeria is a preservative method in which salted or unsalted meat is set over a low burning wood fire in various type of ovens. The glow and smoking temperatures are high, reaching 160 °C and 180 °C, respectively. Smoking lasts 3–15 days leading to a dark smoked meat product. The temperatures and the intensity of smoking affect the meat product quality through the levels of dryness, phenol and benzo(a)pyrene contents. Smoke-dried meat products have low flavour. Most of the smoke-dried meats are considered unwholesome with an average benzo(a)pyrene level of 41.03 mg per kg but will remain ordinarily shelfstable at the moisture content of 41.65 g per 100 g and available water (a_w = 0.824).

Keywords: meat preservation, shelf-stability, smoking, wholesomeness

In all probability, the human population will drastically increase worldwide in the next decades and at the same time foodstuffs and especially meat will become more scarce and precious, because even by using advanced methods in breeding, feeding and production of livestock, enough meat will not be available for many people who would like to eat it (WORLD CONFERENCE ON ANIMAL PRODUCTION, 1933). Therefore, it will become even more necessary than today to prevent spoilage of meat. In this regard, the use of sprays for carcase meat, smoking and other simple methods and dissemination of simple technologies for the preparation of safe meat products storable without refrigeration will be valuable contributions (LEISTNER, 1983).

In the developing countries, including Nigeria, the shortage of meat is not only due to a scarcity of animals, but also often due to spoilage. The introduction of European style meat products to developing countries causes difficulties as such products need elaborate equipments for local storage (LEISTNER, 1984). Some of the locally produced meat products are the "Kundi" (Yoruba) and the "Banda" (Hausa) which are smoke-dried meats. Kundi is produced by smoking fresh beef, camel or horse meat in the Northern parts of Nigeria and storable without refrigeration. Fish is also smoked in Nigeria. The traditional smoking method is widely used throughout the country and involves exposing the fish directly to buring wood which leaves heavy smoke deposits on the product. Such smoked products in modern Nigeria have become a delicacy and are a valued component of the menu (AFOLABI et al., 1983).

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

Smoking is defined as "the process of allowing smoke produced freshly from natural wood, twigs, heather of the fruits of trees, sometimes with added herbs or spices to act on the surface of meat and meat products". Transfer of smoke to meat takes place by means of a number of physical processes — absorption, adhesion, condensation and diffusion (KLETTNER, 1979). Smoke precipitation on moist surfaces contains ten times more phenols than precipitated on dry surfaces (POLYMENIDIS, 1977). One of the methods of producing smoke is when wooden logs or sawdust is burning. Temperatures in this case are at and above 400 °C (AFOLABI et al., 1983).

The action of smoke on meats includes conferring of flavour, texture and colour. Modern smoking methods are important mainly for flavouring and not preservation (HARDY & MCGILL, 1979). The surface colour of well smoked meat products should be light golden yellow to dark brown and this colour is due to the reactions between the carbonyl group in smoke and the amino acids in the meat. The more carbonyls, the higher the intensity of the colour (TILGNER, 1977). Finally the heating effect of smoking evaporates the free water thereby reducing both the water content and water activity (a_w) and increasing shelf life.

Apart from the above effects, smoke has some undesirable effects on smoked products. The production and deposition of some polycyclic aromatic hydrocarbons in meat depend on the temperature of pyrolysis of wood (POTTHAST, 1980).

The benzo(a)pyrene content in the smoke produced by a smoke generator ranged between 5–20 mg per 100 g wood as temperatures increased from 400 to 1000 °C (TÓTH & BLAAS, 1972). The total phenol content of smoke rises as from 330 °C to about 600 °C and drops again as the temperature increases above 700 °C (KNOWLES et al., 1975). Most of the polycyclic aromatic hydrocarbons especially benzo(a)pyrene in smoked meat and fish have been epidemiologically linked with primary liver and stomach cancers in the consumers. Such evidences have come from Iceland (BAILEY & DUNGAL, 1958); USSR (DALGAT, 1974); India (KUMAR & RAMACHANDRAN, 1973) and Nigeria (EMEROLE et al., 1982).

In this work, the methods of smoke-drying meats in Nigeria were investigated and "Kundi" produced by the main commercial method in Nigeria, the oven smoking method, was analysed. The effects of the smoking temperatures on the water content, phenol and benzo(a)pyrene levels and the relevance of such levels to the quality of the final product are highlighted.

1. Materials and methods

Across Nigeria, the major meat processing areas of Sokoto, Kano, Bauchi, Borno, Gongola, Oyo and Cross–River States were visited. The various methods of smoking meat products were investigated and recorded. The types of ovens and smoking methods in use were noted (Tables 1 and 2). To be able to measure all the parameters incidental to meat smoking, a halfdrum oven was acquired and used for smoking in the laboratory. Fresh beef was bought and cut into pieces, common salt (NaCl) was sprinkled on the meat labelled with rust-free metal skewers and accurate weights were recorded. The meat pieces were placed on the smoking wire grill and smoked for about 3 days until they were considered well smoked. The final weights were recorded. The pieces were packed in polythene bags and kept in a carton for further analysis.

Table 1

Meat smoking methods in Nigeria

Method	Meat product	Procedure
Oven smoking[a]	"Kundi"	Salted or unsalted meat smoked over any of the described grill ovens (Table 2). Pork was first parboiled with salt before smoking
Charring and drying	"Banda"	Meat cut into pieces of about 150 to 200 mm spread on dry grass, grass is set on fire until charred, products left in the sun and wind for 4–15 days or more
Kiln smoking	"Tinko"	Smoke house is pre-heated. Meat pieces set on trays and left in the kiln till dry. Kiln may require more heating
Clay pot drying	"Randa"	A big clay pot is buried in the ground. Wood or charcoal fire is made inside. Clay plates are set on top of the fire timber. Meat pieces are packed on the plates and the pot closed and covered overnight with a mat
Roasting	"Balangu and "Kilichi"	Meat slices, 4–6 cm thick, spiced with groundnut cake, pepper or oil, roast over or around fire until done. Kilichi is the same, but slices thinner, 0.5–1.0 cm thick
Suya production	"Tsire"	Small pieces of spiced meat skewed and set round fire for roasting and drying
Mud cake	Dry meat balls	Minced meat balls are covered with smooth clay and fired for 4–5 days and stored. Clay cover broken when required, Keeps for 6–12 months if clay cover is not broken. Stored in fire place
Kebab roast	"Asun"	Fresh goat meat roasted over fire. Served with salt, pepper and onion. Keeps only for a few hours to a day

[a] The analyses described in the paper refer to this method and meat product

Table 2

Meat smoking/drying ovens in Nigeria

Type	Components
Half-drum oven	200 litre oil drum cut and covered with a metal grill with fuel space cut below
Whole drum oven	200 litre oil drum with rows of metal grill inside and fuel space below
Open wood oven	Fire resisted wood and ropes used as platform or grill over fire
Stone oven	3 big stones arranged partially with a wire mesh on top
Smoking basket	Wire basket hung over fire place
Perforated clay pot oven	Perforated earthen pot hung over fire place
Smoke house (Kiln)	Kiln built of mud under a thatched or corrugated iron roof
Mud oven	3/4 circular, short, mud wall reinforced with wood or metal
Buried clay pot oven	Burnt clay pot buried in the ground. Has a clay lid
Head pan charcoal oven	Deep head-pan with a wire-mesh cover. Charcoal fire usually made inside pan

1.1. Determination of water content

The water contents were calculated (g per 100 g) on recorded fresh and dry weights and adjusted to the average of 75 g moisture percentage in beef (LENTHER, 1981) (Table 3).

1.2. Measurement of temperatures

A Therm model 2210–6 (Therm, GFR) digital electronic thermometer fitted with a high temperature NiCr—Ni thermocouple able to measure temperatures between −200 to + 1370 °C was used. Glow and smoking temperatures were taken by inserting the thermocouple probe first into the smoking chamber and then into the smoking fire and the accurate temperatures were recorded (Tables 4 and 5).

Table 3

Yield and moisture content of smoked meat samples[a]

Sample	Fresh weight (g)	Dry weight (g)	Yield (%)	Moisture content
1	30.0	17.4	58.00	56.9
2	30.0	17.0	56.67	55.9
3	30.0	16.3	54.33	53.9
4	50.0	30.0	60.00	59.3
5	50.0	28.7	57.40	56.4
6	50.0	38.6	57.20	56.3
7	35.6	20.7	58.20	57.0
8	40.0	24.6	61.50	59.3
9	43.7	27.5	62.93	60.3
10	50.0	25.9	51.80	51.7
11	40.0	19.7	49.25	49.2
12	40.0	19.7	49.45	49.9
13	61.4	33.7	54.89	56.5
14	58.7	30.6	52.13	52.0
15	60.0	31.6	52.67	52.5
16	62.3	32.8	52.65	52.5
17	50.0	26.7	53.40	54.2
18	50.0	31.2	62.40	59.9
19	50.0	24.4	48.80	48.8
20	50.0	28.9	57.40	56.4
Mean ($\bar{x}$)				54.8

[a] Moisture content of the dried sample is calculated by the formula 75–(100–yield) × (yield)$^{-1}$ × 100 where 75 is the equilibrated moisture content of the fresh meat (LENTNER, 1981).

Table 4

Water activity (a_w) of smoked meats

Number of samples	a_w range	Mean
20	0.737–0.897	0.824

Table 5

Smoking temperatures of Kundi (°C)

Batch size	Temperature range (°C)	Mean
10	160–185	170.3

1.3. Determination of water activity

An a_w-value meter (model 5803 made by Lufft, FRG) was used for the water activity (a_w)measurements. The meter was first calibrated using saturated solutions of NaCl and $NaNO_2$. A standard curve was prepared.

To measure the a_w of the meat samples, 5 g of the sample was finely cut or minced. The sample was packed into the meter cup which was air tightly fixed onto the meter. The meter was then placed into an incubator, set at 23 °C. Meter readings were taken every 30 minutes for 3 h or until the last three readings were equal. The meter readings show the ERH value. Using the standard curves, the a_w values of the actual samples were read from the curves (Table 6).

Table 6

Glow temperatures of smoking ovens

Batch size	Temperature range (°C)	Mean
10	1053–1160	1130.3

1.4. Determination of total phenols

The method of POTTHAST (1977) was used. Smoke-dried meat samples were treated in a flask with liquid nitrogen and then minced using a mortar and pestle and then a kitchen type Moulinex (France) grinder. The phenols were distilled and spectrophotometrically measured at 635 nm. A standard curve for guaiacol was prepared. The total amount of phenols in relation to guaiacol mg per kg was calculated from a guaiacol standard curve based on the spectrophotometer reading (Table 7).

Table 7

Total phenols in smoked meats

Number of samples	Range of total phenols (mg per kg)	Mean
20	5.0–13.7	8.81

1.5. Determination of the level of benzo(a)pyrene

The method of POTTHAST and EIGNER (1975) with some modification was used. Benzo(a)pyrene (BaP) was extracted from minced meat in a chromatographiccolumn with propylene carbonate concentrated in cyclohexane, separated on thin layer chromatography acetylated cellulose plate and finallydetermined in a spectrophotofluorometer set at an excitation wavelength of 332 nm and an emission wavelength of 408 nm as recommended by TÓTH (1970) (Table 8).

Table 8

The level of benzo(a)pyrene in smoked meats

Number of samples	BaP range	Mean
20	20.85–66.91	41.03

ppb = mg per kg

2. Conclusion

Oven meat smoking in Nigeria is mainly carried out together with heating over smouldering hardwood fire in the different ovens listed in Table 2. To the traditional meat processor, it is enough to smoke-dry meats adequately to consumer acceptability. The level of "well smoked" is highly empirical. This work enables to judge oven smoking which is the main commercial method in Nigeria and the meat products by numerical data and qualitative tests. Such data include the temperatures of smoking and of the glowing fire, the moisture content of the smoked meats, the types and the levels of some smoke compounds transferred to the meats.

The controlled cold curing and hot smoking procedures practised in the technologically advanced countries are not advisable for adoption in Nigeria. The reason is that we are in the tropics, with high ambient temperatures and equally high humidities, spoilage is likely to overtake the cure and ruin the meat. Also, because of the humidity, there will be limited reduction of moisture content during meat drying unless sufficient heat is applied. This explains the need for such high glow and smoking temperatures. One way to improve oven smoking is to move the ovens into smoke-houses, the inside of which are relatively less humid than the outside where the oven is exposed to ordinary tropical climatic conditions.

The high smoking temperatures are beneficial as the meat is broiled and all endogenous infectious agents like beef tapeworm *(Cysticerus bovis)*, Trichinella, fungi and bacteria are killed. The slight salting during preparation gave the product a 0.5% NaCl concentration. The resultant low water activity (0.737–0.897) and the reduction of the water content to a mean of 54.8 % promote the products' shelf-stability as spoilage bacteria and fungi are controlled. Most meat spoilage fungi will not grow below $a_w = 0.61$ (RÖDEL & LEISTNER, 1982).

It is firmly established that if smoking is carried out at glow temperatures below 450 °C less, undesirable smoke components like benzo(a)pyrene and more taste enhancers—phenols—will be produced (POTTHAST, 1980). The smoke flavour of these meat products is very low because of the low levels of the phenols. The fact that the relative rate of production of carcinogenic polycyclic

aromatic hydrocarbons increases with temperature also favours the recommendation of lower glow and smoking temperatures.

The Nigerian smoked meats may be considered unwholesome because of the presence of high levels of the PAHs, especially benzo(a)pyrene (0.85–66.91 ppb mean 41.03 mg per kg). According to WALKER (1977), in countries where benzo(a)pyrene limits are set in foods, the acceptable limits are nil to 1 ppb in smoked meats. A mean of 41.03 ppb in Nigerian smoked meat renders such smoked meats risky to the consumer over a long period of consumption as benzo(a)pyrene is known carcinogen.

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Abstracts

of papers presented at the

VIth CONFERENCE ON FOOD SCIENCE

ORGANIZED BY

THE JOINT COMPLEX COMMITTEE ON FOOD SCIENCE OF THE HUNGARIAN ACADEMY OF SCIENCES AND THE MINISTRY OF AGRICULTURE AND FOOD, THE HUNGARIAN SCIENTIFIC SOCIETY FOR FOOD INDUSTRY AND THE CENTRAL FOOD RESEARCH INSTITUTE

Budapest
29–30 May 1986

IMPORTANCE OF UP-TO-DATE NUTRITION IN RELATION TO NATIONAL HEALTH

GY. BÍRÓ

National Institute of Nutrition, H-1097 Budapest, Gyáli út 3/a. Hungary

The task of up-to-date nutrition is the optimal qualitative and quantitative satisfaction of the energy and nutrient requirement and thereby the maintenance of health and the enrichment of the biological reserves.

Diseases which can be prevented by balanced up-to-date nutrition:

Preventable diseases	*Nutritional factor*
Protein-energy deficiency	Animal and plant proteins
Vitamin deficiency	Vitamins, provitamins
Deficiency of minerals	Macro and micro elements
Iodine deficiency (goitre)	Adequate energy
Dental caries (partly)	Proper ratio of nutrients
Diseases of the heart and the vascular system	Total fat; ratio of fatty acids
	Cholesterol
	Trace elements
	Dietary fibres
Diseases related to obesity	Adequate energy
Heart and vascular system	Proper ratio of nutrients
	Total fat; proportion of fatty acids
High blood pressure	Crystalline carbohydrates
Respiratory system	Common salt
Liver and bile ducts	Dietary fibres

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Locomotive organs	
Kidneys	
Endocrine glands	
Metabolism	
Diabetes (2nd type)	
Tumors	
Skin	
Disorders of the large intestine	Dietary fibre
Intestinal cancer	

On the basis of data obtained in large scale research programs, involving the observation of numerous strata of the population, diseases of the vascular system can be prevented by accounting for the following nutritional requirements:

- well balanced energy input, prevention of obesity, consumption of foodstuffs of lower energy density, lower sugar and higher complex carbohydrate consumption;
- the total fat content of the consumed energy should not exceed 20–30%, within which saturated fatty acids must not exceed 10%, poly-unsaturated fatty acids generally 5–6%, maximally 10% and a reduced cholesterol level;
- more dietary fibre, mostly pectin;
- alcohol consumption below 5 g per day in order to prevent high blood pressure.

On the basis of the above WHO experts recommend the consumption of more fruit and vegetable and cereals of high fibre content, fish, chicken, lean meat, defatted milk products, moderate egg and reduced salt. They recommend to agriculture and the meat and milk processing industries the production of goods congruent with the above principles. They stress the necessity of the declaration on the label the basic properties (consumption) of the food in terms comprehensible to the consumer.

For the obese the hazard of diabetes is about three-fold, of high blood pressure about two and a halffold in comparison with people of average body mass. Among the hazard factors of coronary disease high blood pressure takes the second place and serum cholesterol level the third (behind age in the first place). Both may be influenced by nutrition.

At present exact data on diseases and hazard factors in relation to nutrition in Hungary are not available. A representative research program in progress, comprising at a 2 thousandth level the adult population of the country above 14 years, is hoped to provide these data. In the course of preparing this

study the nutritional habits of 423 men and 465 women from 4 counties, were observed. The observations permit the following conclusions:

- energy intake, particularly on holidays, exceeds the necessary level;
- protein intake exceeds requirement by 20–30 g per day;
- fat consumption exceeds requirement by 22–47 g per day;
- complex carbohydrates are below the required level;
- the thiamine, riboflavin, ascorbic acid consumption is satisfactory in general, however, riboflavin consumption of women is marginal (observations were carried out in the autumn);
- calcium intake is 40% lower than the desirable, iron intake of women is about 50% of the requirement, while in men it is satisfactory. Potassium intake meets the requirement.

This type of nutrition leads to substantial obesity. Twenty percent overweight or even higher in the group studied shows the following distribution according to age:

Men	14 – 34 years 15%	35 – 60 years 38%	above 60 36%
Women	14 – 34 years 18%	35 – 55 years 38%	above 55 57%

Hazard factors of heart and vascular system diseases are in the following correlation with anthropometric data:

	Men			Women		
	14–34 years	35–60 years	above 60 years	14–34 years	35–55 years	above 55 years
Optimum body mass						
–systolic blood pressure	—	**	**	—	**	—
–diastolic blood pressure	—	—	—	***	**	—
Ponderal index						
–systolic blood pressure	***	***	***	***	***	***
–diastolic blood pressure	***	***	***	***	***	***
Optimal body mass						
–HDL cholesterol	—	—	—	—**	—**	—
Body fat %						
–serum cholesterol	**	**	—	—	**	—
–serum triglyceride	—	***	—	**	—	—

**: P < 0.05; ***: P < 0.001; —: correlation non-significant, or sign of correlation

The consequence as to general health: there is a close correlation between nutrition on one hand and hazard of heart and vascular diseases on the other. That means that the most important causes of death can be influenced by

nutrition and it is possible to achieve a substantial advance in the state of health of the population.

To improve the nutrition of the population the cooperation of the whole verticum of food production is necessary. The interest of the parties concerned has to be aroused. A large range of products can be improved by correcting its composition (e.g. less fat, salt and sugar) and the interest aroused by appropriate price policy.

BIOLOGICAL VALUE OF NEW BASIC FOOD MATERIALS

É. Gelencsér,[a] J. Gyebróczki,[b] T. Kárpáti[b] and K. Doborján[b]

[a] *Central Food Research Institute, H-1022 Budapest, Hermann Ottó út 15. Hungary*
[b] *Scientific and Production Association for Protein Technology, H-1536 Budapest, Pf. 340. Hungary*

Research results related to economical protein production and up-to-date nutrition have stimulating effect upon the utilization of plant proteins in human nutrition. Research work is mainly aimed at maintaining and increasing the biological value of these proteins and their mixtures.

The biological value of a number of soya products, prepared by different techniques (isolate: SI; textured protein: ST; flakes: SF) was investigated. The new product developed at the Scientific and Production Association for the Protein Technology were used for complementing certain plant products (potato flakes: F1; ground rice: F2; corn meal: F3; buckwheat meal: F4; powdered chestnut: F5) and for flour blends of direct purpose, PF. The effect of SF on protein of animal origin was also studied (milk protein concentrate: C1; powdered white of egg: C2, and blood plasma: C3) in order to obtain cheaper protein concentrates (PC).

The in vitro biological value was characterized by the Chemical Score (CS) based on the essential amino acid composition and by changes in the composition.

The in vivo biological value was measured by feeding tests of male rats in growth. The Net Protein Utilization value (NPU) and the Net Protein Ratio (NPR, g per 16 g N) were determined by direct measurement of change in body mass and body nitrogen per unit protein consumption. The test period lasted 10 days subsequent to a four-day adaption period.

The soya products manufactured by different techniques are of high biological value: NPU value of ST is 89%, of SF 84% and of SI 70%; NPR of ST 3.9, of SF 4.0 and of SI 3.0; CS of ST 83, of SF 57 and of SI 78. The plant protein products tested had a lower protein content (7.0–9.0 g per 100 g) and their protein content was utilized by the living organism depending on their origin (NPU 36 to 100%; NPR 2.5–4.6; CS 46–91).

The protein products of animal origin (C1, C2, C3) had a high protein content (56–82 g per 100 g) and high biological characteristics (NPU: 70–93%; NPR: 2.9–5.2; CS 69–100). The cheaper protein concentrates (PC1, PC2, PC3) had a protein content of 53–60 g per 100 g and their biological value was nearly as high as that of the products of animal origin (NPU: 61–76% NPR: 3.2–4.5; CS 62–100). In the knowledge of the in vitro nutrient indices of protein mixtures the in vivo biological value can be approximated.

The flour blends for direct purpose are recommended for a wide use in food products based on flour or meat.

SANITARY INSPECTION OF NEW VITAMIN ENRICHED AND DIETETIC FOOD PRODUCTS

Ö. Gaál, T. Novotny, P. Czuczy and É. Mezei

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

The compulsory procedure to be applied in relation to new and dietetic food products is laid down in Act IV, 1976.

In judging new basic food materials beside components favourable from the nutritional aspect the possible presence of toxic or utilization inhibiting substances has to be investigated, too.

In the case of vitamin enriched products the expediency of enrichment and the availability of vitamin has to be judged. The utilization of vitamins A, B_1, B_2, nicotinamide, ascorbic acid and vitamin D is permitted.

The carbohydrate content of products for diabetics has to comply to specification. Many problems arise in relation to the use of sugar-replacing substances (fructose and some sugar alcohols) and of synthetic sweeteners. In the first phases of metabolism of the sugar substitutes insulin is not necessary. The permitted amount is a maximum of 30 g per day and this has to be accounted for in the daily allowance of carbohydrates. Their consumption is advantageous because they are less detrimental to teeth than sugar.

In view of the high salt consumption in general and its relation to hypertonia even healthy people are advised to consume foods with low salt concentration. Since the limit value of 120 mg Na per 100 g as proposed in the Codex Alimentarius cannot always be observed it would be expedient to set up the category of foods of reduced salt content (maximum 300 mg Na per 100 g food).

The single way of keeping celiac disease under control seems to be the strict observance of gluten free diet which depends very much on sufficient experience in production, thus it is highly advised to control production.

FOODS FOR DIABETICS IN HUNGARY

J. Fövényi

Municipal Péterfy S. Hospital, Department of Medicine B.
1076 Budapest, Péterfy Sándor u. 8–14. Hungary

Food requirements for diabetics comply on the whole with general requirements on up-to-date and healthy nutrition. There are, however, two requirements which are specific to diabetics: they need low-energy food and must not consume saccharose containing food.

The supply of healthy and up-to-date food is generally satisfactory. There are some problems in relation to even distribution, particularly in small communities, which have to be solved.

The range of foods prepared without saccharose approximates the average choice available in Europe and substantially exceeds that in the socialist states. In this relation, too, the distribution between town and country is uneven.

As regards the choice the present situation is best with soft drinks of low energy content. However, an increase of quantity and a wider range is desirable. The selection of fruit juices, jams and stewed fruits needs enlargement and their quality can be improved. The range of products of the confectionery industry requires enlargement, too. Sweet milk products for diabetics prepared with artificial sweeteners are completely missing from the market.

The concept, that apart from the dietetic sweet products, all foods for diabetics can find their place in slimming diets or in diets designed to prevent obesity needs propagation and thereby the demand for them can be increased and production raised by about an order of magnitude (from 300 000 to 5 000 000).

EVALUATION OF THE BIOLOGICALLY ACTIVE RESIDUES IN FOODS

G. Biró, Gy. Lombai and É. Dobrádi

Department of Food Hygiene, University of Veterinary Sciences, Budapest,
H-1078 Budapest, Landler Jenő u. 2. Hungary

Analysis of biologically active residues in foods is getting more and more important. From the point of view of nutrition hygiene the residues present in foods are of the same significance to the consumer as the viable agents. Numerous international recommendations and specifications treat the problem of residues. In 1982 the concept of residue has been extensively specified by the Meat Hygiene Committee of the Board of FAO Codex Alimentarius.

FAO/WHO recommendations and USDA specifications contain exact limit values permitted for pesticides, antibiotics, sulfonamides, heavy metals.

International research and the improvement of analytical methods provide new results as a consequence of which new and strict restrictions have to be applied. Thus, for instance, chloramphenicol has been prohibited in the USA and in the FRG for use in the diet of milking cows and egg laying poultry.

The task is to reduce to the minimum the hazard of using chemical compounds. This is possible only by the strictest observation of specifications on the use and application of these substances. However, it is known, that about two-third of the undesirable chemical compounds gets in the human organism by way of food. Investigations carried out in Hungary have shown that chlorinated hydrocarbons have been found only in a few wild mammals and birds. Analyses of organic phosphoric acid esters and efforts to detect micotoxins in beef cattle and meat products have born negative results. Investigations to detect toxic trace elements have shown favourable results, too. Analyses of pork and beef muscles have shown, however, a tendency of growth in relation to the cadmium content.

To improve conditions in Hungary the following requirements have to be met:

- international recommendations and specifications in relation to residues have to be accounted for;
- practical methods of sufficient sensitivity have to be used;
- exact knowledge and strict observance of waiting times for reasons of health of medicines, preventive and nutritive substances;
- continuous control of residual substances.

Research, investigation and control are all aimed at meeting the requirements which are very important in view of home consumption and export of foods.

ABSORPTION AND DEFECATION AS AFFECTED BY THE VISCOSITY OF MILK PRODUCTS

M. Fingler, Z. Nemessányi, Gy. Mozsik, Á. Kovács, Gy. Farkas and S. Szakály

Central Radioisotope Laboratory, Health Management and Organization Unit, Medical University, H-7643 Pécs, Szigeti út 12. Hungary
Hungarian Dairy Research Institute, H-7614 Pécs, Tüzér u. 15. Hungary

Mucilaginous lactic bacteria can be used to improve the critical characteristics of acid milk products (viscosity, water binding capacity, consistency) and thereby extending their storage life and improving their sensory quality, on the one hand and to reduce the fat content in proportion with increased viscosity (without reduction of quality) and thereby reduce their energy content, on the other hand.

It was assumed that by increasing the viscosity the absorption of the different nutrients and the rate of bowel discharge can be influenced. To clarify these effects clinical investigations were carried out.

Absorption tests were carried out with 20 patients whose damaged glucose tolerance was detected in the course of induced hyperglucaemia but other routine laboratory test parameters were not indicative of absorption disorders. Yoghurt prepared with traditional and *Str. thermophilus* strains (Viscolact$_1$ culture) was used to compare their effect on glucose and D-xylose absorption.

Yoghurt prepared with Viscolact$_1$ culture was found to significantly inhibit and prolong glucose absorption in patients of reduced glucose tolerance and at the same time significantly reduced D-xylose urine extraction.

To establish the rate of bowel discharge 10 healthy persons were treated by radioactive tracer technique. In these studies, too, the effects of Viscolact$_1$ culture and traditional yoghurt culture were compared. Yoghurt of higher viscosity was found to cause a more rapid discharge of bowels in healthy people.

However, the investigations did not give conclusive information as to the mechanism of inhibition of absorption or the change in bowel discharge. Further experiments are needed to clarify these problems.

ROLE OF INDIVIDUAL NUTRITIONAL FACTORS IN LIPID PEROXIDATION IN THE LIVING ORGANISM

E. Dworschák[a], Gy. Biró[a], Gy. Pados[b], M. Horváth[a] and Á. Zsinka[c]

[a] *National Institute of Food Hygiene and Nutrition, H-1097 Budapest, Gyáli út 3/a. Hungary*
[b] Department 4 Internal Medicine, Municipal Hospital „Tétényi út", H-1115 Budapest, Tétényi út 12—16. Hungary
[c] Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary

The role of various factors of nutrition in the lipid oxidation phenomena in the living organism, particularly that of the quantity and quality of dietary fat, were discussed. In order to clarify the correlation between fat metabolism disorders and lipid peroxidation the serum parameters (serum urea, triglyceride, total cholesterol, HDL_{chol}, HDL_{2chol}, HDL_{3chol} content and malon aldehyde (MDA) metabolite used as index of lipid peroxidation were analysed under clinical control, in 70 obese patients (26 men, 44 women), who volunteered for slimming cure. The average total cholesterol content of the obese patients was found to exceed, the HDL_{chol} content to just reach the normal value. Of the different correlation coefficients a negative correlation was found between MDA and HDL_{chol} and among the subfractions that between MDA and HDL_{3chol} was even closer. Thus, according to the results obesity and the athero-

genic changes in fat metabolism indices accompany the increase of lipid peroxidation.

It is assumed that lipid peroxidation has a detrimental effect on the surface membranes of the chilomicrons and thereby reduce HDL synthesis.

Significant correlations between serum parameters

1st member	2nd member	n	r	P
MDA	TG	21	0.42	= 5%
MDA	HDL_{chol}	64	−0.42	≤ 0.1%
MDA	HDL_{2chol}	64	−0.23	= 5%
MDA	HDL_{3chol}	64	−0.52	≤ 0.1%

ROLE OF WINE IN NUTRITION BIOLOGY

S. FERENCZI

Department of Oenology, University of Horticulture, H-1118 Budapest, Ménesi út 45. Hungary

In recent years the number of researches aimed at clarifying the role of wine and its single components, its advantageous and disadvantageous properties, has substantially increased. A selection of the most significant results is presented here.

The grave consequences of continued over-indulgence in ethyl-alcohol consumption are well-known. However, wine cannot be considered a simple alcoholic beverage, because the polyphenol components indigenous to wine, mainly leucoanthocyanines and phenolic acids (caffeic acid, chlorogenic acid, ferulic acid, etc.) reduce the toxic effect of alcohol, the amount of transformation products and even the cholesterol level of blood and liver.

Wine has an advantageous effect on digestion and helps to keep the pH value of gastric juices at 2.5 level by its low pH and high buffer capacity. Cations are liberated (K, Ca, Mg) after oxidation in the tissues from the bound organic acids of wine and thus it provides the organism with important minerals and helps in maintaining the alkalinity of blood.

The vitamin content of wine is relatively low, however, it is very rich in substances of vitamin P activity, which are polyphenols, mainly leucoanthocyanines and catechins. Wine can meet the whole vitamin P requirement of the body.

The antimicrobial effect of wine against various bacteria (coli, typhoid fever, cholera, etc.) has been known for long. This antiseptic effect is also due to the polyphenol content of wine mainly to the colouring substances, in red wine

the anthocyanines and in white one the flavonoids. The pathogenic bacteria are killed already in a 5–10-fold dilution of certain red wines.

According to the latest results wine has an antiviral effect, as well. This is effected by polymerized polyphenol compounds (oenotannin). With increasing the extent of polymerization of the molecules of flavon structure increases their affinity to protein and thereby their antiviral effect.

These results prove that moderate wine consumption (300–500 cm^3 per day during meals) permits the functioning of its favourable biological and hygienic properties.

A cultured moderate wine consumption, as an eating habit, can not be considered as intemperance, rather as a possible means of fighting it.

THE IMPORTANCE OF UP-TO-DATE NUTRITION OF FOODS OF LOW ENERGY CONTENT AND RICH IN DIETARY FIBRE

J. Rigó

National Institute of Dietetics, H-1085 Budapest, Makarenko u 24. Hungary

The nutrition of the Hungarian population underwent an important change in the last fifty years. Beside the average daily intake of body building proteins (106 g per capita per day in 1985) significantly increased the consumption of energy-rich food. The fat consumption with 103 g per capita per day is substantially higher than desirable. Twenty-five percent of the daily 386 g carbohydrate consumption is native sugar which is considered a food giving plain energy. Alcohol consumption increased in the last 25 years nearly four-fold. There is nearly four-fold increase also in the consumption of the non-alcoholic beverages, which with their 8–12% sugar content provide a superfluous luxury of energy in the form of about 6 kg sugar per capita per year. At the same time substantially decreased the cereal and potato consumption. In 1985 the yearly per capita consumption of these two kinds of food was 108 kg and 58 kg, respectively. These dietary habits lead to the development of obesity and a number of so called civilisation diseases.

Up-to-date nutrition is promoted by food of low energy content (poor in fat and sugar) and at the same time rich in dietary fibre and in minerals, particularly in Mg and K. Because of the cooperation of the health authorities and the food industry there are already some favourable results. The production of about 200 tons of wheat bran per year and about 100 tons per year of wheat germ, of bread rich in dietary fibre, of soft drinks of low energy content, of milk and meat products poor in fat is an achievement and their increasing popularity is encouraging.

CELLULASE ACTIVITY OF THE HUMAN GASTROINTESTINAL TRACT

M. Szakács,[a] A. Halász,[a] J. Sawinsky[b] and A. Kovács[b]

[a] *Central Food Research Institute, H-1022 Budapest Herman Ottó út 15. Hungary*
[b] *Balassa Municipal Hospital, H-1088 Budapest, Vas u. 17. Hungary*

The objective was to determine the cellulase activity of the gastrointestinal tract and the effect of cellulose supplementation of a nutritionally complete diet related to cellulose digestion in human adults with clinical disorders. Because of public interest and concern relative to adequacy of dietary fiber in diets, fiber supplementation of certain processed foods might be advisable. Various forms of cellulose are currently being added to commercially marketed products. No information is available on the cellulase activity of the gastrointestinal tract in human adults.

Six adult men and 7 women hospitalized with slight clinical disorders served as subjects. All the subjects, age of 50–77, were given ordinary, low fiber and high fiber (3×50 g of wheat bran) diets. The 12-day study was divided into a 3-day adjustment period and three randomly arranged 3-day experimental periods. The day after each period samples were taken of the stomach and small intestine content. The cellulose digestion and the cellulase activities (FPA: filter paper activity; C_1: cotton hydrolyzing activity; C_x: carboxymethyl cellulose hydrolyzing activity) of the samples were determined by the reductometric method of Mandels and coworkers (Adv. Chem. Ser., *95*, 393., 1969). Moreover, the activities of the stomach and small intestine contents were also assayed after cellulolytic or tryptic pretreatment (Celluclast, NOVO, Copenhagen; 0.2% and 1%, resp.: 50 C°, 3h; Trypsin, Serva, Heidelberg; 0.2% and 1%, resp.: 35 C°, 3h; resp.). Blood sugar content was also determined.

The results of the cellulolytic activity measurements in the sample are shown in the Table. The values are given in International Unit per cm^3: 1 IU= = released glucose equivalent, mg from 100 mg of cellulose in 24 h at pH 4,8 and 50 C°.

There were no digestion disorders when dietary fiber was administered to the patients.

No correlations have been found between the age or sex of patients and cellulase activity, however, higher values were found for male patients. Higher blood sugar contents indicate also that dietary fiber is more effectively digested by male subjects.

The enzyme activity was increased by the addition of dietary fiber.

The quantity of sugars released from dietary fiber can be significant in case of diabetes mellitus.

Cellulolytic activity of stomach and small intestine content samples

		Activity (IU cm^{-3})															
		Sugar content (mg 0.5 cm^{-3})		FPA		C_1		C_x		NOVO Celluclast pretreatment				Trypsin pretreatment			
										0.2%		1%		0.2%		1%	
		S	I	S	I	S	I	S	I	S	I	S	I	S	I	S	I
Females Before high fiber diet	1	0.17	0.23	0	0	0	0	0	0	0	0	0.13	0.25	0	0	0	0
	2	0.24	0.24	0.03	0.03	0	0	0	0	0.14	0.21	0.31	0.42	0	0	0.08	0
	3	0.10	0.10	0	3	0.04	0.12	0	0	0.08	0.13	0.31	0.32	0.05	0.04	0.06	0.07
	4	0.05	0.01	0	0.02	0	0.14	0	0	0.13	0.24	0.35	0.41	0.11	0.02	0.09	0.04
	5	0.10	0.13	0.01	0	0.04	0.06	0	0.02	0.02	0.11	0.26	0.37	0.01	0	0.02	0
	6	0.21	0.23	0	0	0.22	0.30	0	0	0	0	0.03	0	0	0	0	0
	7	0.25	0.20	0.09	0.05	0.70	0.10	0	0.09	0.39	0.18	0.36	0.39	0	0	0.05	0.02
Females After high fiber diet	1	0.04	0.02	0	0.01	0	0	0	0	0.16	0.16	0,45	0.57	0	0	0	0
	2	0.23	0.21	0.01	0	0	0.06	0.06	0	0.22	0.26	0.29	0.31	0	0	0	0
	3	0.21	0.19	0.02	0	0	0.02	0.03	0	0.22	0.24	0.60	0.64	0.09	0	0.08	0
	4	0.05	0.06	0	0	0	0	0.02	0	0.04	0.17	0.24	0.27	0	0.05	0.02	0
	5	0.15	0.13	0.02	0.06	0.10	0.46	0	0.02	0.05	0.15	0.23	0.53	0.02	0	0.03	0.07
	6	0.30	0.41	0.12	0.08	0.30	0.37	0.03	0.01	0.06	0.08	0.12	0.21	0.04	0.04	0.02	0
	7	0.45	0.49	0.10	0.22	0.78	0.15	0.22	0.30	0.45	0.57	0.65	0.80	0.05	0	0.14	0
Males Before high fiber diet	1	0.23	1.15	0	0	0	0	0.04	0.07	0.38	0.66	0.45	0.40	0	0	0	0
	2	0.07	0.08	0	0	0.02	0	0	0.01	0.07	0.09	0.28	0.31	0.05	0.05	0.02	0
	3	0.10	0.16	0	0	0	0	0.02	0.10	0.10	0.28	0.30	0.32	0	0.05	0	0.12
	4	0.06	0.07	0.02	0.02	0	0	0	0	0.15	0.13	0.27	0.21	0.06	0.05	0.13	0.10
	5	0.18	0.26	0.07	0	0.04	0	0	0	0.05	0.10	0.30	0.34	0.09	0.08	0.03	0
	6	0.12	0.25	0	0.15	0	0.04	0.06	0.17	0.05	0.14	0.10	0.28	0.01	0.10	0.02	0.13
Males After high fiber diet	1	1.00	0.39	0.71	0.22	2.08	0.58	0.75	0.81	0.05	0.09	0.80	0.65	0	0	0	0
	2	2.59	1.86	0.65	0.32	2.58	2.78	0.11	0.27	0.15	0.20	0.42	0.35	0.10	0.05	0.05	0
	3	0.11	0.14	0.07	0.15	0.02	0.12	0.13	0.34	0.08	0.22	0.80	0.45	0.08	0.15	0.02	0.12
	4	0.19	0.16	0.01	0.10	0.15	0.20	0.12	0.15	0.05	0.10	0.24	0.41	0.15	0.15	0.20	0.15
	5	0.13	0.23	0.02	0.08	0.05	0.10	0.05	0.08	0.03	0.04	0.12	0.21	0.15	0.23	0.17	0.01
	6	0.07	0.04	0.03	0.07	0	0.08	0.17	0.25	0.03	0.02	0.21	0.31	0.05	0.20	0.06	0.28

S: Stomach sample, I: Small intestine sample

EFFECT OF WHEAT GERM ADDITION ON THE FATTY ACID COMPOSITION OF FLOUR PRODUCTS

É. Török,[a] K. Sirokmán[a] and M. Vicze[b]

[a] *Central Laboratory, College of the Food Industries, H-6701 Szeged, Marx tér 7. Hungary*
[b] *Budapest Sweets Industry Research Laboratory, H-1097 Budapest, Vágóhíd u. 20. Hungary*

Wheat germ is a very valuable food item therefore it would be very useful to consume it not only directly but also added to various food products. To study the composition of such products and their keeping quality shortcake biscuits were made with added wheat germ.

The fatty acid composition of wheat germ is very characteristic. Its oil content consists of more than 80% of unsaturated fatty acids. The oil used in the experiments contained 12.4% oleic acid, 52.5% linoleic acid and 17% linolenic acid. The wheat germ formed 28% of the flour content. The effect of wheat germ on the fatty acid composition was established under predetermined storage conditions.

During the 40 days' test period samples were taken every 10 days and these were analysed by gas chromatography, sensory and microbiological tests and other chemical tests and compared to control samples.

The proportion of unsaturated fatty acids to saturated ones is higher in the biscuits baked with wheat germ than in the control samples (3.4 and 2.6, respectively) and this difference remained unchanged during storage in spite of the slight reduction in both samples.

The high proportion of unsaturated fatty acids is mainly due to the higher amount of poly-unsaturated linolenic acid (16.6% in the wheat germ containing biscuit and 12.7% in the control). During storage a slight decrease occurs in the amount of linolenic acid but it becomes lower than the value of the control only after the expiration of the guaranteed time (30 days).

The biscuits prepared with wheat germ had a pleasant taste and due to their high essential fatty acid content were of high nutritional value and these favourable properties were maintained during the guaranteed time.

ECONOMIC EFFECTS OF FOOD ENRICHMENT WITH VITAMINS

I. Tomka

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

With increasing consumption of processed and refined foods less and less vitamin, mineral and fibre becomes available to the human organism. The increasing insufficiency of vital nutrients and the fact that improvement in this field cannot be achieved by increasing the quantity of food led to the realization that at least a part of the wasted nutrients has to be restored to the food.

In Hungary, first in 1958 was granted permission to add vitamin to foods, however, the proportion of enriched foods is still very low in comparison to the total range of foods.

Of the vitamins permitted by the Food Act for use (vitamin A, B_1, B_2, C, D and nicotinamide) vitamin C is most frequently used. However, the range of foods is very narrow and the amount permitted is low and needs revision.

The amounts of vitamin used for enrichment when converted to 100 g final product are very different. It would be expedient, similarly to the declaration of the energy content, to declare the vitamin content on the package of the product in relation to unit mass.

The costs of vitamin enrichment were investigated in the products where it was possible to separate them. (Three kinds of syrup, 1 kind of dragée, 2 kinds of processed cheese, 16 fruit nectars, 6 kinds of instant drink powder). The products manufactured on the basis of a license are mostly prepared from raw materials imported and contain already the necessary vitamins therefore the costs cannot be calculated (2 margarins, Deit drinks).

In the cases studied enrichment with vitamins increased the costs of materials by 0.4–6.2%. Related to the marked price the increase amounted to 0.5–2.9%. Thus, the guaranteed higher vitamin content did not cause a notable increase of cost or price.

In some of the products, e.g. rose-hips syrup, the higher nutritive value is not recognized in the price. The consumer could choose the biologically more valuable product at the same price would he be properly informed.

Thus, in most cases the consumer does not choose the product because of its higher biological value (e.g. all the margarines available on the market are vitamin enriched). The choice is rather a matter of quality or habit. This permits of influencing consumption unconsciously in a more wholesome direction.

Beside the consumption of natural fresh sources of vitamins it is considered desirable to promote by appropriate advertising the consumption of vitamin enriched foods and improve thereby the balanced vitamin intake of the population.

NEW POSSIBILITIES OF TRACE ELEMENT RESEARCH AND THEIR IMPORTANCE IN FOOD SCIENCE

I. Pais and P. Fodor

Department of Chemistry, University of Horticulture, H-1114 Budapest, Villányi út 29. Hungary

In the last two decades the interest in trace elements increased substantially. It was established that the components of foods were formed by enzymatic processes under the contribution of trace elements. The biochemical processes occurring during processing and storage of food are also controlled by trace elements. At the same time foods contain the trace elements required to the healthy functioning of the human organism. If the amount of trace elements consumed in less than the minimum requirement of more than the maximum, diseases are caused.

These findings gain significance in view of environmental pollution in consequence of which some elements get into the human body which were earlier not present in the biosphere and thus very little is known of their biological effect. Chemisation showing a growing tendency is a further factor in increasing the importance of micro element research.

Highly sensitive and rapid automated methods are needed to detect trace elements in the food. The ICAP-9000 argon plasma spectrometer appears to be a suitable instrument. In the last two years 1.6 million data were obtained with this instrument at the Chemistry Department, University of Horticulture, Budapest. The evaluation of these data will enable the researchers to establish the present situation in Hungary and make suggestions to solve the deficiency or toxicity problems.

COMPLEMENTING OF AMINO ACIDS AND FATTY ACIDS IN BABY-FOODS

A. Terts,[a] M. Nagy[a] and I. Smied[b]

[a] *Research Institute of the Canning Industries, H-1097 Budapest, Földvári út 4. Hungary*
[b] *Department of Biochemistry and Food Technology, Technical University, Budapest, H-1111 Budapest, Műegyetem rkp. 3. Hungary*

The complementing of two product families with 8 different additives (Purina–Soya, sunflower seed protein, UP-75, TVP, egg powder, blood plasma, vital gluten, FTTE soya flakes) was investigated by computerized modelling. On the basis of this work it is possible to shift out the additives the utilization of which seems advisable on industrial scale.

Results obtained with sunflower seed protein and UF-75 were very favourable while the utilization of egg powder and vital gluten seemed also feasible.Table 1 contains the percentage complementing additive which improved substantially the quality of proteins characterized by the Chemical Score.

Table 1

Recommended amounts of complementing additives

Baby food	Complementing additives (%)							
	Purina soya	Sunflower seed protein	UF-75	TVP	Egg powder	Blood plasma	Vital gluten	FTTE soya flakes
Carrot puree	1	2	2	2	2	1	2	2
Carrot puree with milk	—	4	2	—	5	—	4	—
Carrot puree with beef	—	5	5	—	5	—	4	—
Carrot puree with chicken	—	5	5	—	5	—	4	—
Pea puree	5	5	4	>5	5	—	4	5
Pea puree with beef	—	5	5	—	5	—	4	—
Pea puree with pork liver	—	5	—	—	>5	—	4	—
Pea puree with chicken	—	5	5	—	>5	—	4	>5

By use in practice of the suggested additives it will be possible to judge whether they show the same results in the concentration as applied in computerized modelling. A further task to be judged whether the combination of the additives will improve results. Another problem arises, too: is complementation with the additives economical (e.g. in the case of meat). While the first problem can be resolved in the present industrial structure, that of the second would infer the thorough transformation of structure.

Results of the fatty acid analysis of baby foods were compared with correspondent data of breast-milk (Table 2). It can be concluded that in the baby food not enriched, the oleic acid is substantially less, the linoleic acid more than in mother's milk, the baby food enriched with chicken meat is of about the same composition as mother's milk. Thus chicken meat is suitable to improve the fatty acid composition of baby foods.

Table 2

Fatty acid composition as percentage of total fatty acid in breast-milk and baby foods made in Kecskemét (Hungary)

Fatty acid		Breast-milk[a]	Baby foods[b]				
			Milk	Chicken	Beef	Liver	Natural
Oleic acid	C 18 : 1	37.3	25.3	37.0	24.9	22.0	12.5
Palmitic acid	C 16 : 0	26.6	17.6	28.0	15.6	10.2	7.2
Linoleic acid	C 18 : 2	10.5	33.1	9.3	33.5	50.2	73.8
Stearic acid	C 18 : 0	8.3	8.2	8.7	10.6	7.3	4.0
Miristic acid	C 14 : 0	7.9	5.9	1.2	1.8	—	0.1
Lauric acid	C 12 : 0	4.7	1.5	0.1	—	—	—
Palmitoleic acid	C 16 : 1	3.4	2.3	5.8	2.7	1.6	—
Capric acid	C 10 : 0	0.8	1.1	0.1	—	—	—
Linolenic acid	C 18 : 3	trace	1.5	1.4	1.5	2.1	0.5
Others		0.5	3.5	4.2	5.8	3.5	1.0
	C 20 : 1		—	3.2	3.6	3.1	0.9

[a] Data of Comm. Nutr. Amer. Acad. of Pediatrics (1971)

[b] Results obtained at the Research Institute of the Canning Industries

POSSIBLE UTILIZATION OF MILK PRODUCTS IN DIETS FREE FROM LACTOSE

I. Kisérdi[a], M. Máté[b] and G. Földházi[c]

[a] *Veterinary and Food Control Centre, H-1095 Budapest, Mester u. 81. Hungary*
[b] *János Hospital, H-1125, Budapest, Diós árok út 1. Hungary*
[c] *Department of Food Chemistry, University of Horticulture, H-1118 Budapest, Somlói út 14–16. Hungary*

In consequence of their composition and biological value milk products play an important role in up-to-date nutrition. In the case, however, of certain metabolic diseases their consumption has to be limited.

The aim of the present experiments was to detect galactose and lactose in butter, cheese and some new milk products and determine the quantity of galactose. The galactose content of the samples investigated had not been determinated earlier.

Two methods were applied to detect galactose and lactose: thin-layer chromatography and Guthrie's test. Thin-layer chromatography was carried out according to Humbel and Collart with protein-free samples extracted with water.

In the Guthrie's test a mutant *E. coli* strain is used from which galactose-1-phosphate uridylyltransferase is missing. If the galactose content of the sample placed on the nutrient medium is high the growth of bacteria is inhibited. Thus, a light inhibited zone is formed around the sample disc and this is proportional to the galactose content. With the use of standards the test gives semi-quantitative results.

The samples tested were divided into three groups according to their galactose content. The first group contains 6 kinds of cheese (Frankfurti, Karaván, Tura, Balaton, Köményes and Séd brie) all of them free of galactose. These kinds of cheese can be used in the diet of galactosemic children above 3–4 years and with older children in other lactosefree diets, as well.

The second group of food items has a low galactose content. This group a dietetic baby food (Sportrobi), butter, a processed cheese (Hóvirág) and a smoked ewe cheese (Parenyica) belong to. Sportrobi contains 10 mg galactose in a 15 g per 100 cm^3 solution, the other items 30 mg per 100 g product. These products can be given to galactosemic children above 6 years and to older children in other lactose-free diets.

Food items belonging to the third group are of high galactose content. Two kinds of cheese (Anikó and Mese) must not be given to galactosemic children and are not recommended in other diets either because of their 80 mg and 50 mg per 100 g product galactose content, respectively. Mildibé dietetic baby food (50 mg galactose in a 15 per 100 cm^3 solution) and milk flavoured with vanilla (2500 mg galactose per 100 g product) are not permitted in galactosemic diet and not recommended in other diets either.

ANALYSIS OF MEAT AND MEAT PRODUCTS WITH SUPER SCAN INSTRUMENT

L. Körmendy, K. Rékási and Gy. Mihályi

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

The Super Scan rapid analyser was used to determine the protein, water and fat content of 31 raw meat samples (pork and beef) and 60 cured meat products and the results were compared with those obtained by traditional methods.

Results were evaluated by Deming's regression formulas and the following conclusions were drawn:

– The instrument is suitable for the direct determination of fat and protein in raw meat, while it cannot be used for moisture content; although the signals shown on the instrument have given linear correlation with the reference method, they had to be corrected, because the relationship did not correspond to $y = x$. The value of the residual standard deviation $(s'\varepsilon)$ for fat after correction, however,was high (1.63), for the moisture content of pork as well (1.48); the linear relationship between Super Scan and laboratory results on the fat and protein content of pork and beef did not show significant differences. With the moisture content, however the difference between the two regression equations (pork and beef) was highly significant.

– Results are highly affected by the quality of protein. When analyzing the protein from the neck tendon, the Achilles tendon, pork rind raw meat, soya isolate Super Scan showed different results.

– In meat products, where the relation between moisture, fat and protein content is uncertain, the results obtained with the instrument showed a fairly good agreement: the protein values differed systematically from those obtained by traditional methods, but only slightly; the moisture content could not be determined.

– A definite advantage of the Super Scan instrument in relation to traditional methods is rapidity.

– When used in on-line control in the factory the method of sampling and the place requirement of the instrument has to be accounted for.

ROLE OF FOOD PRODUCT DEVELOPMENT AND RESEARCH IN BALANCED NUTRITION

K. PÓDER-ŐSY[a] and B. CZUKOR[b]

[a] *Department of Food Industry, Ministry of Agriculture and Food, H-1055 Budapest, Kossuth Lajos tér 11. Hungary*
[b] *Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

The development of the Hungarian food industry accelerated and became dynamic in the last four decades. It is a forward step that since 1967 agriculture and food industry are an integral whole under the direction of the Ministry of Food and Agriculture. Development of food products is the common task of different research and training institutions, of individual manufacturers and places of production. The number of establishments engaged in food industrial activity is 4746 of which 13 are research institutions.

In 1983 the Presidium of the Hungarian Academy of Sciences extended the scope of trends in food research by the requirements of up-to-date nutrition. This was based on an analysis of the average nutrition of the population. It was established that the general consumption can be characterized by high energy, fat, carbohydrate and sodium intake while the protein consumption is adequate (105 g per capita per day).

On comparing consumption in the period 1976–1980 with that between 1981 and 1984 it can be considered as an advance that total meat consumption increased by 7%, poultry consumption by 17% and milk and milk product consumption by 17% while flour consumption decreased by 4–5%. Another favourable symptom is the increase in fruit and vegetable juice consumption (in 1975 about 0.5, in 1982 4.0, in 1983 4.7, in 1984 5.3, in 1985 5.6 l per capita per year).

The negative symptoms of the same period were an increase in the total fat and sugar consumption (7% and 3.7% respectively), reduced potato and vegetable consumption (5.7% and 7.5%, respectively). Within the bread consumption white bread is still high (in 1983 97.3%).

In 1984 a review was made for Medical Sciences Section of the Hungarian Academy of Sciences on the research or development subjects of nutrition-biological importance. In the period examined (1975–1982) 106 subjects were found to be important. By the end of 1980 16.5% was finished, 44.3% was started in 1980 or after. Subjects of interest for product development, realized in practice were: production of wheat germ and bran for human consumption, milk protein concentrate, powdered, up-to-date baby foods, rape seed oil of reduced erucic acid content, corn germ oil, meat, milk, bakery, canned, frozen, confectionery products, soft drinks and beer of reduced energy content or dietetic, products of the poultry industry, products of increased fibre content. A subject requiring further study: development of the composition and technology of food of higher nutritive value.

In the meat, milk, canning, milling and vegetable oil industries investigated the proportion of new products in the period 1980–1985 was 16.8% (260 against 1550 items). The development in absolute value was most dynamic in the canning industry with 110 products and in relative value in the dairy industry (37.5%). The change and diversification was in the other branches, not investigated, also significant.

In forming the trends of development the expectations of higher authorities are decisive. The abrupt increase in the number of dietetic foods is partly due to the notice of the Ministry of Food and Agriculture in which the attention of all food producers was drawn to the necessity of diversification of dietetic foods.

Of foods granted permission to be manufactured in the period 1981–1985 72% was dietetic, 13% was "novelty", 4% was vitamin enriched and 11% was baby food.

On the whole this meant the up-dating the selection of foods. In the present period no sudden increase of consumption is expected, however, a change in the structure of nutrient intake is hoped for. In the interest of the consumers it is necessary to produce more foods valuable from the aspects of produce more foods valuable from the aspects of nutrition biology and meeting differentiated demands.

The national research program is among others aimed at attaining this objective. This objective fits into the WHO program: "Health for everybody in 2000". To achieve balanced nutrition the cooperation of research, training and health institutions and of food processing plants is necessary.

QUALITY SPECIFICATIONS OF FOOD ADDITIVES

I. Draskovics and E. Kunisch-Szabó

Veterinary and Food Control Centre, H-1095 Budapest, Mester u. 81. Hungary

In Hungary, too, the utilization of additives is regulated in general by the Food Act and specifically by the standard of the product in question.

The increasing severity of the quality requirements on food products raises the problem of quality of additives used in food products.

The toxicological safety is ensured by general specifications: additives have to meet the requirements of the valid Ministry of Health regulations.

The situation is more complicated in relation to quality in the sense of technology. Hungarian Standard 14 476 mentions 164 additives of which only 86 are listed in the specifications of different food products as "Substances permitted for use". As regards the quality requirements on these substances 27 of them are laid down in Hungarian Standards and in the case of 22 guid-

ing principles are described in the 6th edition of the Hungarian Pharmacopeia for the analyses. Further, there are 50–60 Hungarian Standards which treat the additives as raw material or as analytical reagent. Of the international specifications the most complete is the FAO list: "Specifications for the identity and purity of food additives". The aim is internationally acceptable unity of judgement.

UTILIZATION OF PLANT PROTEINS IN FOOD MANUFACTURE

B. Czukor, J. Petres, E. Horváth, Zs. Ormai-Cserhalmi,
Zs. Szabó and É. Senkálszky-Ákos

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

On the basis of results of experiments and in some cases on data found in the literature the possibility of utilizing plant proteins in the food industry is discussed. Beside the cereals, soya, sunflower seed and pea serve as sources of protein and may be utilized in food manufacture. Data obtained in chemical analysis, functional tests and in vivo experiments prove that plant proteins, in spite of their occasional deficiency in certain essential amino acids, in combination with one another and with protein of animal origin may form protein products of full value. They may be accompanied by fibre, minerals and trace elements, vitamins and unsaturated fatty acids useful as nutrients, without reaching the detrimental limit values of antinutritive substances and factors. Some of these proteins carry functional properties which improve the texture of the products. Their utility is supported in addition to experimental results by feasibility calculations.

ROLE OF STARCH DERIVATIVES IN UP-TO-DATE FOOD PRODUCTION

L. Ludvig, J. Kudron, Zs. Ledniczky and M. Madarász
*Research Institute of Alcohol Industry, H-1089 Budapest,
Diószeghy S. u. 8. Hungary*

Starch derivatives of different chemical, physical and rheological properties are important ingredients and additives of up-to-date food production in Hungary. Their consistency stabilizing and modifying quality enables their use for many different purposes (in the canning, refrigerating, confectionery and dairy industries).

At present modified starch derivatives are purchased mainly abroad, and therefore involve a substantial foreign exchange requirement.

The starch derivatives of foreign origin were exposed to chemical and rheological examination. From the other side, the technological requirements of the industries concerned were established.

Taking into account the analytical results of foreign products and the points of view of utilization starch derivatives were produced first at laboratory scale then at pilot plant scale. The starch products most important for the food industry are: starch phosphates, acid treated starches and oxidized starch.

Technology was developed for the production of Amilfrig-10 modified corn starch. The process involves heating with phosphate salts. This product is used by the canning and refrigerating industries as water binding, thickening and fat emulsifying agent.

A potato starch product Amylox-30 was produced by oxidation and heat treatment. This product is suitable for puddings made without boiling, because it solidifies with cold water and has a low water relasing capacity.

The starch derivative prepared by acid treatment is easy to dissolve in warm water and forms a gel of low viscosity. It can be used for enrichment of foods with soluble carbohydrate.

Other types of starch derivatives, such as acetates, citrates, adipates, used in the food industry, have also been studied. Because of the wide range of raw materials (corn, potato, wheat), a broad field of utilization (canning, refrigerating, confectionery and other industries) and a great variety of derivatives (oxides, phosphates, citrates, etc.) their investigation is highly divergent, too.

TECHNOLOGICAL FACTORS DETERMINING THE QUALITY OF GROUND PAPRIKA

GY. HORVÁTH

Veterinary and Food Control Station, Bács-Kiskun County, H-6001 Kecskemét, Széchényi krt. 29. Hungary

Since ground paprika is a blocked biological system, the processing technology has to be evaluated as a function of chemical changes and experiences have to be utilized relative to requirements on the quality of the final product.

The following chemical parameters have been studied:

– correlation between grinding technology on the one hand and pigment content and visual colour sensation, on the other;

– correlation between the stability of the pigment content and various parts of the fruit present in ground paprika;

– pigment stability as affected by its development.

The level of contamination considered as an indicating parameter of hygienic conditions, was also studied. With appropriate management, accounting for chemical changes, it is possible to avoid contamination and to meet market requirements.

UTILIZATION OF NEW MILLED PRODUCTS IN THE BAKING INDUSTRY

Á. Sigora

Research Development and Servicing Joint Enterprise for the Baking Industry, H-1117 Budapest, Dombóvári út 5–7. Hungary

In accordance with modern principles of nutrition science the Hungarian food industry diversified its products and introduced low energy foods, a wide range of dietetic foods, particularly those for diabetics, foods rich in fibre, etc. This development did not take place without contradictions. One of them was the banishment of carbohydrates from everyday food with the result of substantial reduction in bread consumption. Today it is known that bread is the most important source of carbohydrates and of dietary fibre.

The natural harmony of wheat composition fell a victim to the development of the milling industry. Now it is tried to return the lost valuable components to our food by new products of the milling industry: dietary wheat bran, Graham flour, coarse wheat meal, coarse meal of 4 cereals, wheat germ. Lately a series of new products are placed on the market: sunflower seeds, extruded soya flour, pea flour, brown rice, millet and buckwheat.

A new development is small packages of nonperishable goods with declaration of the components (carbohydrates, protein, fat and energy content). The dietary fibre content is determined by the Hellendorn–Mosonyi method and is declared, as well.

The composition of the basic materials of the baking industry was computerized and a program was made which enables the planning of the nutrient composition of the products and the control of the composition of products manufactured by the industry. Determination of the composition by this method is rapid and of high accuracy.

For the milling industry it would be of advantage to raise the extraction rate of the flours, however, flours of this kind have low baking value and cause a dark colour of the product. The deterioration of the baking quality could be compensated by additive but this has a cost increasing effect. The dark colour elicits aversion in the consumer. Therefore, it is necessary to introduce fibre without this effect of colour (by light coloured bran particles, corn bran, bran of durum wheat).

The extrusion technology has an influence on the nutrient utilization. An example of this is the extrusion of bran enriched corn. As an effect of this process about the half of the indigestible fibre content became soluble.

The aim of this work is the formation of a nutrition culture taking into account the principles of nutrition science and achieve the consumption of products of the baking industry which meet food habits, age, way of life of the consumer.

It is the task of interdisciplinary research to clarify the problems related to the prevention of diseases and the health of future generations.

APPLICATION OF THE PRINCIPLES OF UP-TO-DATE NUTRITION IN THE CONFECTIONERY INDUSTRY

J. Brazsák

Budapest Sweets Industry Research Laboratory, H-1097 Budapest, Vágóhíd u. 20. Hungary

The general opinion in relation to confectionery products is that they play an important role in the development of obesity and dental caries and of diseases connected with food habits, thus their consumption is disadvantageous or even unnecessary. Although these opinions hold good in relation to certain products they resist generalisation. Most of them contain also essential nutrients and help in complying with one of the basic rules of up-to-date nutrition, with diversity of food.

According to statistical data the consumption of sweets in Hungary is relatively low (11 kg per year per capita or 30 g per day per capita). In relation to average consumption that of the children is many-fold. This stresses the importance of modifying the composition of sweets in accord with modern health requirements.

Taking the analysis of the composition of the available products as a starting point the tasks awaiting the industry become immediately apparent. The proportion of "added" sugar, fat and white wheat flour has to be cut and by the addition of "natural" raw materials the proportion of essential nutrients and of dietary fibre has to be increased.

In spite of the fact that the realization of this apparently simple task is hindered by many factors (high price of raw materials of the industry, difficulties in purchasing, reduction of hedonic value, etc.) the research laboratory of the confectionery industry prepared the production of several products for which the bad opinions on sweets are not valid.

The new products making use of soya, cereals and milk products, dried fruit, sunflower seed cake, etc. are expressly rich in essential nutrients. The food evaluation system developed in the laboratory can be computerized and permits optimalization of nutrient composition of the new products.

CONCENTRATION AND QUALIFICATION PROBLEMS OF INDUSTRIAL APPLE AROMA CONDENSATES

E. MADARASSY-MERSICH, M. PETRÓ-TURZA, I. SZÁRFÖLDI-SZALMA,
K. FÜZESI-KARDOS and GY. TELEKY-VÁMOSSY

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

The aim of aroma research is the retainment, improvement or development of the flavour of a given food material during processing. The food industry in general and the Hungarian industry, as well utilizes increasing amounts of aroma preparations, mainly synthetic ones. These have the advantage of being cheaper than the natural aroma preparations of a higher stability of composition and a higher intensity.

In recent years, however, the demand for natural aroma preparations is on the increase, because of aversion for synthetic products. The possibility of manufacturing natural aroma extracts is given in Hungary, a wide range of fruits and vegetables is available. Of the fruits apple is grown on the largest scale.

In Hungary, some of the state estates produces apple aroma. The quality of these products, since they are dilute, aqueous solutions, is fluctuating, their keeping quality is limited, their storage necessitates large storage capacity. Thus, experiments were made, on behalf of the industry, to concentrate these dilute apple aroma preparations and establish their quality. The latter task meant the development of a reproducible, rapid analytical method the results of which could be correlated with the sensory value of the product. For separation of the components, identification of the most important ones and their quantitative determination methods of gas chromatography were developed after a convenient sample preparation. The sensory evaluation of the individual aroma substances was based on taste and odour tests.

Correlations were looked for between individual compounds or group of compounds and the effect of their proportions on the dilution threshold value, smell intensity and apple character.

A practically water-free extract of strong apple odour was obtained by an extraction method of concentration. The quality and quantity of the composition of this extract corresponded with that of the initial condensate, but needed substantially less storage volume, could be stored without diminution of quality for a longer period and its use extended over a larger field.

NUTRITIVE VALUE AND MARKETING EXPERIENCES IN RELATION TO MILK PRODUCTS OF REDUCED ENERGY CONTENT

Á. KOVÁCS, S. SZAKÁLY and GY. FARKAS

Hungarian Dairy Research Institute, H-7614 Pécs, Tüzér u. 15. Hungary

In order to enlarge the selection of fresh milk products in the desirable direction the dairy industry developed a number of milk products of reduced energy content, mainly of reduced fat content.

These, in relation to the traditional product of the same or similar function, are shown in the following table.

Product group	Product of reduced fat content	Nutritional advantage of the product
Liquid fresh milk product	Milk of 1.5% fat content Milk of 2.2% fat content	Fat reduced by 46% reduced fat globule diameter Fat content reduced by 20%
Sour milk products	Sour cream with viable flora Cottage cheese with sour cream	Fat content reduced by 20–60% Exclusive L(+) lactic acid content Better absorption properties
Butter products	Creamed butters	Fat content reduced by 50–60% Protein content five-fold Mainly corpuscular fat absorption, without enzymatic decomposition
Product made of by-products	Whey based blended drinks, puddings and ice-cream mixes	Fat completely removed; valuable milk protein, lactic acid, minerals, vitamin and bio substances.

PROTEIN PRODUCTS UTILIZED AS FOOD ADDITIVES

J. SOHÁR

National Institute of Nutrition, H-1097 Budapest, Gyáli út 3/a. Hungary

In recent times a great effort was made to develop protein products and to enlarge their field of utilization.

Of the new protein products of animal origin the blood and plasma powders, the UP milk protein concentrate and the sodium caseinate preparation deserve mentioning. The selection of plant proteins increased, too, with soya products, sunflower seed, pea, leaf protein concentrates.

Protein products are used in the food industry in three main fields: as additives, improving the nutritive value by increasing the protein content and/or complementing it as replacement of meat protein partly or completely.

In the three fields of application the toxicological safety of the protein products, the adequate nutritive value of the product prepared with the additive and the unambiguous information of the consumer are equally important.

The protein products are toxicologically safe if the raw material is properly selected, the production technology is appropriate and the quality and purity of the final product is regularly controlled.

As source of protein only raw materials suitable for human consumption, free from microbiological and chemical impurities and residues, can be used. The production technology should ensure, as completely as possible, the removal of the undesirable flavour substances, the antinutritive components naturally present in plants (protease inhibitors, phytin, factors of favism, isoflavons of oestrogenic effect, nucleic acids, etc.) and oligosaccharides causing flatulance. The technology must ensure the saving of the original nutritive value while excluding the formation of harmful amino acid derivatives (e.g. lysinoalanine, formed by oxidation of sulfur containing amino acids) and mutagenic substances (products of the Maillard reaction, nitrogen containing heterocyclic compounds).

If a new source of protein or a new technology is used the protein product must be analyzed, the biological value determined and its wholesomeness proven by toxicological and chemical tests as specified by the health authorities.

Protein products are used as food additives because they possess excellent fat and water binding capacity, they form stable emulsions and improve the structure of foods. They play an important role also in the colour of bakery products. Protein hydrolysates are used as flavouring substances and flavour improving agents.

Protein preparations can be used as structure improving agents only if they contain more than 70% protein, comply with the specifications as regards purity at a maximum of 20 g per kg in various meat, canned, bakery, refrigerated and confectionery products, in the form of concentrates and isolates. Protein hydrolysates can be used in accordance with industrial practice.

If a protein preparation is used as an additive for functional purpose it needs not be declared.

The manufacture of a food additive in Hungary is bound to permission of the Ministry of Health based on expert opinion.

The functional application of protein preparations must not be accompanied by an unjustified increase in water or fat content and must not serve to cover some unpleasant flavour.

MANUFACTURE OF WHITE BLOOD JELLY AND ITS USE IN MEAT PRODUCTS IN THE DEBRECEN CANNING FACTORY

B. PAPY

Debrecen Canning Factory, H-4002 Debrecen, Monostorpályi út 92. Hungary

The industrial scale processing of blood of animal origin for human consumption is a widely investigated subject of research in every country with a developed food industry. The utilization of blood and its fractions is impeded by its short shelf life and characteristic taste, colour and smell.

At present, blood plasma is used mainly for food manufacturing purposes, obtained by separation of whole blood previously treated with anti-coagulant reagent. Plasma has to be processed immediately upon separation. At present two methods of preservation are known: freezing and spray-drying. Both methods require high investment and the energy requirement of both processes is high, too.

In the Debrecen Canning Factory experiments were carried out to utilize fluid blood plasma in canned meat. The experiments were not successful because of the poor microbiological state and fluid consistency of the plasma.

The method developed at the University of Veterinary Sciences Wroclaw (Poland) was then applied. The principle of this method is that the coagulation mechanism of the blood treated traditionally with anti-coagulant was utilized to get a jelly.

The jelly obtained in Debrecen resembled the consistency and colour of cooked white of egg, was tasteless and odorless and was named white blood jelly. The advantage of the procedure is that it does not require further investment, is easily integrated in the manufacturing line and the product due to its good microbiological quality can be stored at 0 to 4 °C for 3 weeks.

The protein content of the white blood jelly is between 7.6 and 10.4%, depending on the original protein content of the blood plasma. In addition to the protein content the preparation contains 3.5–8.0 mg per kg iron. The yield of the process is 78%, but related to protein content the yield amounts to 96%.

During 1985 60 tons of white blood jelly were produced in Debrecen and this was used mainly in minced meat type products. Five percent of the meat as specified in the composition formula was replaced by blood jelly so that in the place of 1 kg meat and 1 dm^3 water 2 kg jelly was added. For 1986 the use of 100 tons was projected according to preliminary calculations.

Experience has shown the following advantages of the process: solid consistency, a safe storage period of 21 days at 0–4 °C, gaining a complete protein. No investment is required, it can be processed on the normal equipment. By the use of blood plasma the prime cost is reduced.

APPLICATION OF SOYA PRODUCTS IN LOW ENERGY DIETS

E. GREINER,[a] T. KÁRPÁTI[b] and P. HAUK[c]
[a] *National Institute of Nutrition, H- 1097 Budapest, Gyáli út 3/a. Hungary*
[b] *Scientific and Production Association for Protein Technology H-1122 Budapest, Goldmark Károly u. 24. Hungary*
[c] *Korvin Ottó Hospital, H-1071 Budapest, Gorkij fasor 9–11. Hungary*

The interest in soya products rapidly increases because of its high nutritional value. By proper technological treatment it can be freed from its antinutritive components. It has a cholesterol reducing effect and exerts a favourable influence on carbohydrate and fat metabolism. Thus, it can be used to prevent obesity, coronary and vascular diseases and in dietotherapy.

Taking into account that in Hungary obesity is becoming a widespread disease and it affects not only almost the half of the adult population, it spreads year by year among the children, too, diets of low energy content were worked out and tested on hospitalized children (in two children's hospital) and on adults (in two clinics for internal diseases). Since one of the requirements on low-energy diets is the consumption of adequate protein, the diets contained soya products. Foods prepared by the addition of soya were: meat products, soya flakes, soya isolate. Of the total protein content of the meat products about 40% was derived from soya and half of the fat was vegetable oil. The soya flakes were first tested with animals and the NPU and NPR indices were determined.

When giving the diet to children and adults a significant body mass reduction was achieved. The fat metabolism improved or normalized. Both age groups found the diet good and satisfying and consumed the foods enriched with soya readily. The foods tested complied with the medical biological requirements.

QUALITY OF THE FINAL PRODUCT AS AFFECTED BY THE SIZE OF WHEAT GRAIN

Á. MOSONYI
Research Institute of Milling Industry, H-1024 Budapest, Kisrókus u. 15/b. Hungary

It is proven by use as well as by research results discussed in the literature that wheat grain distribution according to size affects the quality and quantity of the extracted flour. It was shown by experiments that the rentability of milling flour from small-size wheat grain fraction is lower because of the milling characteristics and quality of flour gained. Since in recent years the wheat cultivars have been continuously changed in Hungary, it seemed necessary to study, beside the general structure and composition of the new

cultivars, the relationship between grain size and the complex properties of wheat, too.

Samples of known wheat varieties and of identified varieties put to commercial milling were classified into 3 groups according to grain size. The following characteristics were examined: distribution of fractions according to grain size, average grain mass, hardness of grain, ash, raw protein content, gluten content of the meal of the complete wheat grain and the indirect proteolytic activity. The quantity of flour extractable from individual fractions, ash, lightness instrumentally established, and the characteristics as determined by the farinograph, gluten properties, amylolytic activity of each fraction were studied.

The results of the investigations can be summed up as follows: grains of different size have different structure becoming apparent in greater hardness of grains of larger size. The wet gluten content of greater size grains is significantly higher. Greater size grain fractions yield higher amounts of flour and their water-binding capacity, as shown by the farinograph is higher, too. With increasing extraction rate, however, deteriorates the quality established with the farinograph. Due to preliminary cleaning the smaller-size fraction of commercially milled grain samples was lower and this became apparent in the results of tests, too.

Classification according to grain size is a question of rentability calculations.

FAT ABSORPTION OF SOME FOODS DURING FRYING

J. PERÉDI and A. BALOGH

Research Institute for Vegetable-Oil and Detergent Industry, H-1106 Budapest, Maglódi út 6. Hungary

The high cholesterol and triglyceride level of blood is a significant hazard factor of heart and vascular diseases. To prevent or treat these diseases it is expedient to bear in mind the following dietary points of view:

– fat consumption shall not exceed 30% of the total energy consumed;

– within the fat consumed the ratio of polyunsaturated to saturated fatty acids shall be about 1 (ratio P/S);

– the daily cholesterol intake must not exceed 300 mg.

Examination of statistical data of consumption in Hungary and those related to 22 food items and their estimated fatty acid composition and cholesterol content leads to the following conclusions:

– in Hungary the fat consumption amounts totally to 50.8 kg per year per capita and the 60% of that is made up by visible and 40% by hidden fat content;

– the total fat consumption is very high: 31.9% of the total energy consumption originates from fat (the energy consumption in itself is very high, too: 13 586 KJ per day per capita or 3245 Kcal per day per capita);

– of the total energy consumption 14–16% is made up by saturated fatty acids;

– of the total energy consumption 6–7% is made up by polyunsaturated fatty acids;

– in the consumed fat the P/S ratio is 0.39–0.49, that is relatively low;

– the daily per head consumption from cholesterol is 550 mg.

About 60% of the saturated fatty acids originates from lard, while about 40% of the polyunsaturated fatty acids from vegetable oil. Thus, vegetable oils have a polyunsaturated fatty acid increasing effect and in foods which originally contained fat, this effect is enhanced during frying.

On frying in vegetable oil (sunflower seed oil)

– with foods originally being practically free from fat 8–30% fat of P/S ratio 6 is consumed;

– with meats having a low fat content about 10–20% of the hidden fat melts into the frying oil while the absorbed oil increases the P/S ratio of the fat consumed to 1.3–2.7;

– with meats originally having a high fat content about 46–65% of the hidden fat melts into the frying oil while the absorbed oil increases the P/S ratio of the fat consumed to 0.5–1.6.

UTILIZATION OF SYNTHETIC SWEETENING AGENTS IN FOOD TECHNOLOGIES

Zs. Sallay, B. Czukor and A. Fövenyessy

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

Model experiments were carried out to find out how much of the synthetic sweetening agents may be used in the processing of natural raw materials to prepare diabetic foods and foods of low energy content. The experiments have shown that the whole amount of saccharose can not be replaced by a single synthetic sweetener, neither in model solutions nor in food products. To obtain a pleasant taste and good consistency a combination of sweeteners must be used. On the basis of experimental results it was established that a generally applicable synergistic sweetener combination does not exist, the composition has to be formulated in every case to fit the character of the product and the technology used. For some products the processing technology and commercialization has been devised and thus the selection of dietetic and up-to-date foods widened.

APPLICATION OF MICROELECTRONICS TO MACHINE PACKAGING OF QUICK-FROZEN FOODS

E. Déri-Kuncze

Hungarian Deep-Freezing Industry, H-1054 Budapest, Dorottya u. 1. Hungary

The spreading of microprocessors enabled the development of program-controlled packaging machines. The greatest field of application of microprocessors is in the control of pouch forming-filling and closing machines, feeding and checkweighing scales.

The machines packaging quick-frozen foods are microprocessor operated Programmable Logic Controls with a freely programmed unit. The 10 available program functions permit a great flexibility of packaging. It is possible for instance to fill a multi-component vegetable into a single pouch with three separately controlled scales and by a volometric filler. The scales can store 35 programs.

The mass fillers and the volumetric filler are regulated by the tendency of the deviations from the adjusted limits at the electronic checkweigher. The electronic scales with data display can be connected to optional peripheries, to counter or recorder providing the possibility of complex mathematical analysis of the data. The system meets the requirements of the international specifications of accuracy.

The electronically controlled packaging machines of the Hungarian refrigeration industry have been working continuously for over 1 year. This new technique is insensitive to the industrial environment, particularly to temperature changes. Thus the speed of the operation is high. The extended use of microelectronics is planned in the concepts of development. By the end of 1986 three packaging lines and twelve batching scales controlled by microelectronics will be in operation.

The use of microprocessors increases the speed of the packaging lines and thereby keeps the quality of the quick-frozen products.

ECONOMICAL EVALUATION OF NEW TRENDS IN FOOD PACKAGING

M. Szőke and K. Cserháti-Nagy

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

Development of food packaging is one of the most important tasks of the industry. In the last few years the most dynamic development was the introduction of cardboard boxes as packing material for retail milk, soft drinks and table wine.

Data in the literature show that the expense of multi-way glass bottle packaging exceeds by 12–65%, the plastic bottle by 10–57% the expenses of cardboard packaging, depending on the number of turns of the glass bottles and on the material of the plastic bottles, respectively. In addition, a substantial saving is expected in the expenses of transport, energy and wages as against the traditional expenses.

In Hungary the possibilities of saving can be checked only in part because the use of cardboards has been introduced about a year ago.

At present cardboard packaging is applied at three firms. Two of them uses TETRA BRIK and the third PKL COMBIBLOE type boxes. Comparative calculations seem to suggest that the packaging of the same product in cardboard is cheaper than in glass bottles. Depending on the distance expenses are balanced with 2 or 4 turns of bottles.

Further possibilities of saving in the field of commerce cannot be exactly assessed but can be expected because of lower refrigeration, storage and wage expenses.

It was studied, with the help of the Hungarian calculation method (price-cost-profit-coverage) how far the coverage point is affected by the expected increase in expenses upon the modernisation of the packaging of the product. The 20% and 40% increase in the cost of packaging amounting to 5–10% of prime cost increased the volume of coverage only by 2% or 7%, thus the removal of the point of coverage is not substantial.

In cases where the cost of production and the return from sale is not proportional to the product volume not only one, but two points of coverage may be formed. In the course of evaluation it is expedient to use the mean value of the two coverage points.

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ACTA

ALIMENTARIA

An International Journal of Food Science

Editor

J. HOLLÓ

Volume 16 **December 1987** **Number 4**

Akadémiai Kiadó
Budapest

D. Reidel Publishing Company
Dordrecht & Boston

ISSN 0139—3006

CODEN ACALDI

ACTA ALIMENTARIA

An International Journal of Food Science

Sponsored by the Joint Complex Committee on Food Science of the Hungarian Academy of Sciences and Ministry of Agriculture and Food.

Editorial office:

Central Food Research Institute, H-1525 Budapest, Herman Ottó út 15, Hungary

Acta Alimentaria is a quarterly journal in English, publishing original papers on food science. The main subjects covered are: physics, physical chemistry, chemistry, analysis, biology, microbiology, enzymology, engineering, instrumentation, automation and economics of foods and food production.

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P.O. Box 149, H-1389 Budapest 62, Hungary

For all other countries:

D. REIDEL PUBLISHING COMPANY
P.O. Box 17, 3300 AA Dordrecht, Holland

Publication programme, 1987: Volume 16 (4 issues).
Subscription prices per volume: Dfl. 266,—/$ 111.00 including postage.

Second class postage paid at New York, N. Y. USPS No. 756-270. U. S. Mailing Agent: Expediters of the Printed Word Ltd., 515 Madison Avenue (Suite 917), New York, N. Y. 10022, U. S. A.

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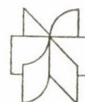

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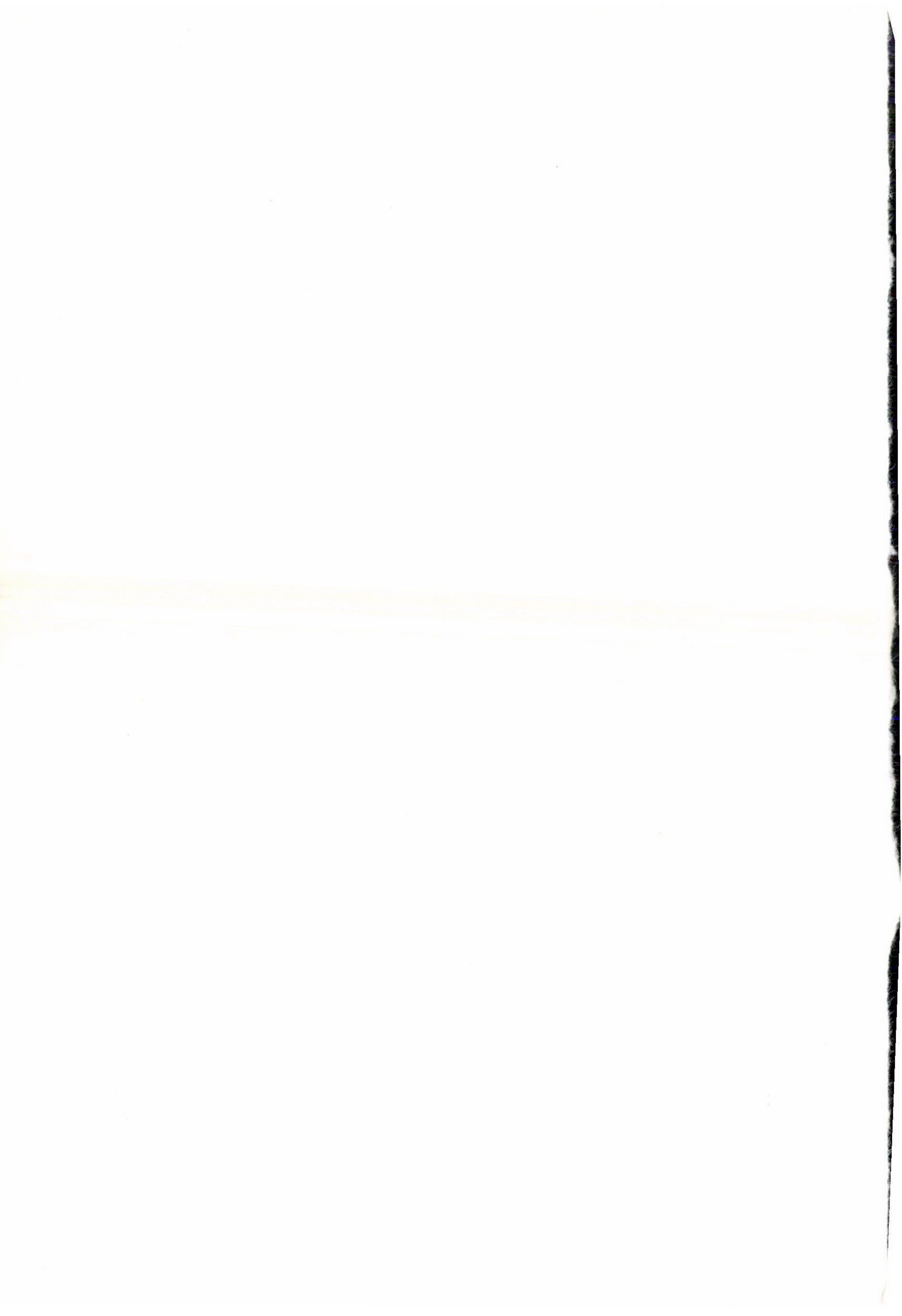

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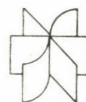

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Acta Alimentaria, Vol. 16 (4), pp. 305–315 (1987)

MECHANICAL DEWATERING OF SUGAR-BEET PULP: PROCESS CHARACTERISTICS AND MATHEMATICAL MODELLING

J. O. Arwidsson and C. Trägårdh

Division of Food Engineering, Lund University, P. O. Box 124, S-221 00 Lund. Sweden
(Received: 12 May 1986; accepted: 17 September 1986)

The mechanical dewatering of sugar-beet pulp can be described as two simultaneous processes; compaction of a solid matrix and permeation of the enclosed liquid. Both these processes depend on the chemico-physical properties of the pulp. Apart from the biological characteristics of the beets these properties are, to a large extent, influenced by the conditions in the sugar extraction process preceding the dewatering and any additives that may be used. The influence of extraction pH, extraction temperature and added calcium ions on the dewatering results is reported here. Positive results were obtained with a decreased pH, decreased temperature and the additional of calciumions.

A semi-empirical mathematical model describing the dewatering process is also presented and evaluated with respect to pulp characteristics. It is demonstrated also how the model could be used in the design and optimization of the process.

Keywords: sugar-beet, dewatering, mathematical modelling

The energy consumption in the drying of sugar-beet pulp is quite a significant fraction of the total energy used in a beet sugar factory (Baloh, 1980). As the mechanical dewatering has a specific energy demand of the order of 1% of that used in drying in a conventional hot air rotary drier, improved efficiency of the former process seems undoubtedly a way of reducing the total energy use in beet sugar production. Thus the goal of the project presented here was to find methods of improving the efficiency of this operation. Both qualitative and quantitative description of the process was desired, therefore, attempts were made to construct a mathematical model which could reproduce the results obtained experimentally.

1. Materials and methods

1.1. Sugar-beets

In order to ensure that the sugar-beets were of a consistent quality they were chosen from a small experimental lot of the Swedish Sugar Company outside Staffanstorp. They were all of the same ordinary shape and size and weighed 2.5 kg. For each experiment or series of experiments where the results of individual experiments were to be compared, the pulp was produced from one beet.

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1.2. Beet slicing

The beets were cut in a hand-operated slicer to a shape (V-shaped cross section) and size (thickness 5 mm and length 3–8 cm) similar to that encountered in industrial processing.

1.3. Sugar extraction

The sugar extraction was performed in batches in two stages. Five litres of distilled water, at 71 °C and pH 5.5, were mixed with 1 kg of sliced sugar-beet under agitation for 15 min. This procedure was repeated with another 5 l of fresh distilled water for 15 min.

In the literature it has been reported that process conditions such as time, temperature and pH during the extraction could influence the physical and chemical properties of the pulp in relation to the subsequent mechanical dewatering (VUKOV, 1977). Thus, in addition to the conditions mentioned above, extraction temperatures of 61 °C and 81 °C, and extraction pH values of 4.0 and 7.0 were used.

Additives, in the form of calcium and aluminium ions, have also been reported to improve the mechanical dewatering operation when added either during or after extraction (DEMAUX, 1983; GIORGI et al., 1983; LE BLANC, 1983; MATHISMOEN et al., 1981; SHORE et al., 1983). Here calcium ions, in the form of calcium phosphate, were added to the extraction liquid at two levels 80, and 120 mg Ca^{2+} per kg beet pulp.

1.4. Beet pulp expression

In order to evaluate the effects of varying the extraction parameters and to monitor the expression procedure a special compression unit was designed and built. Figure 1 shows the overall set-up which consists of a test cell where the compression chamber is located, a microcomputer (Luxor ABC 80) with a suitable in/out board for analogue signals and for regulating the valves of the hydraulic system controlling the movement of the piston in the compression chamber. The physical data, that are collected and stored on a floppy disc, are: total load on the piston, load caused by friction in the test cell, liquid pressure in the press cake, amount of water expressed and position of the piston. A more detailed diagram of the cell is shown in Fig. 2 and as it is mounted, here only single-sided drainage (at the bottom) is used. The diameter of the piston and the expression chamber is 0.05 m. The design is based on the idea of SCHWARTZBERG and co-workers (1977).

The expression procedure was as follows: 50 g of beet pulp were placed in the chamber and a constant compression rate of 5×10^{-5} m s^{-1} was applied

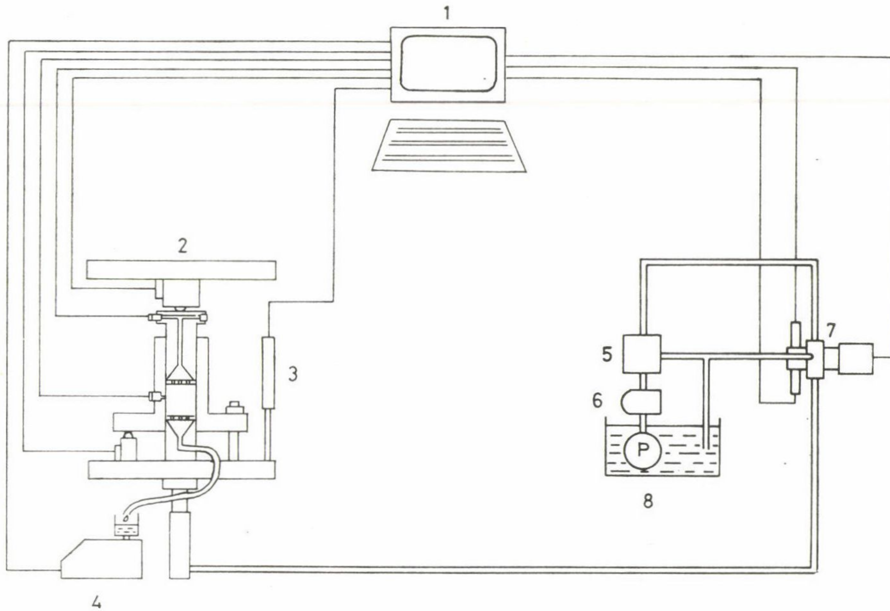

Fig. 1. General layout of the experimental set-up for pulp expression. 1: Microcomputer (Luxor ABC 80); 2: Test cell; 3: Displacement transducer; 4: Digital balance; 5: Manual pressure valve; 6: Filter; 7: Flow regulating and magnetic valves; 8: Oiltank with pump

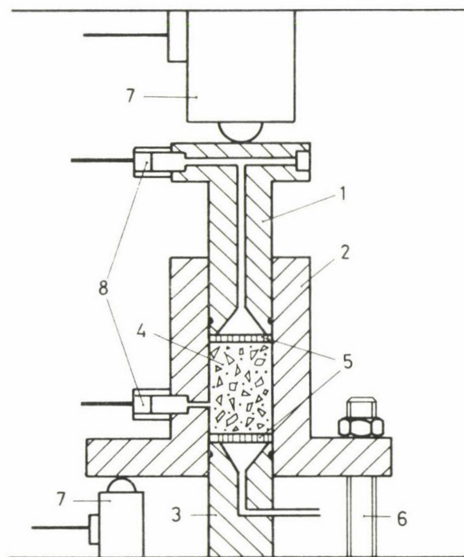

Fig. 2. Design of the compression unit. The diameter of the compression chamber is 0.05 m. 1: Piston with drainage pipe; 2: Cylinder with circular flange; 3: Bottom-plug with drainage pipe; 4: Sample chamber; 5: Perforated discs; 6: Support pillar; 7: Load cells; 8: Pressure transducers with adapter

until the compression load reached 2 MPa when the piston was stopped and held in that position for 120 s. After that time the piston applied a constant pressure of 2.3 MPa until a total compression time of 1500 s was reached. The temperature during all the compression experiments was 25 °C.

1.5. Chemical and physical analyses

1.5.1. Dry matter content. The solid samples were freeze-dried to constant weight. Liquid was first evaporated and the samples were then placed in an oven at 105 °C overnight.

1.5.2. Water-holding capacity. Smaller and simpler compression chambers with single-sided drainage were used; one for reference and one for the sample with modified extraction conditions. A pressure of 12.5 MPa was applied slowly and maintained for 3 h. The compressed material was analysed for its water content which is a measure of the water-holding capacity.

1.5.3. Content of pectic substances. A photometric method using 3-hydroxy-diphenyl as a reactive agent was used (DISCHE, 1947).

1.5.4. Elasticity. Before slicing the sugar-beet a cylindrical sample was punched out of it with a diameter of 8 mm and length of 55 mm. The sugar was extracted as in para. 1.3. to ensure the same treatment. Figure 3 shows the principle of the measurement technique. The load (50 g) was applied with the aid of the displacement transducer of the expression unit. The deflection length as a function of time was registered. The values obtained, after the initial fast deflection, were extrapolated to time zero and used to calculate the modulus of elasticity (modified after VUKOV, 1977). Note that the beet does not have true elastic properties. In this method most relaxation effects were avoided through the extrapolation procedure.

1.6. Mathematical model

In an earlier publication by TRÄGÅRDH and ARWIDSSON (1984) the compression and expression phenomena were described by a number of equations which together form the mathematical model. The basis for the model is a stress balance over the press cake under compression

$$\sigma_{tot} = G\,\sigma_s + \frac{1}{n}\,\sigma_l$$

and assuming that wall effects and body forces can be neglected

$$\frac{\partial \sigma_{tot}}{\partial x} = 0$$

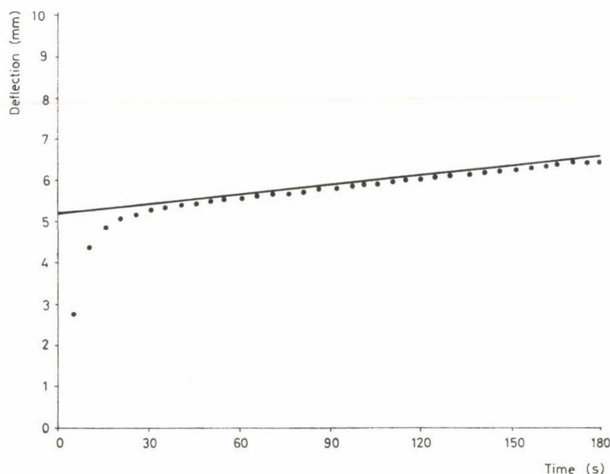

Fig. 3. Determination of modulus of elasticity, showing the use of the time-deflection relation in the extrapolation procedure

Two models are needed, the first to describe the liquid stress (which relates to the pressure drop created as the liquid flows through the porous solid), and the second to describe the stress created in the porous solid while being deformed.

The hydraulic flow resistance model is described by Darcy's law

$$-\frac{\partial \sigma_l/n}{\partial x} = k(u_l - u_s)$$

where

$$k = \frac{k(1+e)}{e^3}$$

(the Kozeny-Carman equation).

The model for the deformation of a porous viscoelastic body is

$$G\,\varepsilon_s = \int_{t'=0}^{t'=t} Y(t-t')\,d\varepsilon_s(t'),$$

where $Y(t) = A \exp(B/e)\,(1-(\exp(-t/D))$.

This in the mathematical model 5 process or rather beet-pulp characteristics, need to be determined.

2. Results and discussion

Before discussing the results presented in Tables 1, 2 and 3 some of the premises and assumptions will be discussed. The original idea was to obtain process characteristics (experimental parameters in the model) through a single dynamic experiment. The expression procedure presented earlier was planned accordingly. The reason was that the conventional experiments using static methods, could give erroneous results due to inhomogeneous compaction towards the drainage surface.

The data collected from the expression experiments and chemical analyses were compared with those computed using the model. The relative difference between measured and computed pressures, total and liquid, was the function to be minimized. The procedure adopted here was a Simplex-method where the coefficients were varied in a certain manner to find the minimum of the relative pressure difference function. However, this procedure failed despite much work to overcome the problems encountered. There are at least two reasons for this. The first is that the relative pressure difference function has so many local minima that a global one was impossible to obtain, and thus almost any set of coefficients could be obtained.

The second is that the model earlier presented, which should represent a model based on physical principles was too simple to describe all the phenomena encountered. It did not fulfill the necessary requirements. One reason for this is that the complex structure of the cake of V-shaped beet slices under compaction cannot be described by these simple equations. As an example we can take the liquid flow behaviour. Liquid flow can occur between the slices, in the vascular channels, through the cells. The rate-controlling mechanism is determined, for example, by the compaction ratio and time. The hydraulic resistances for these flows are described by different equations and thus varies throughout the expression procedure. The same is valid for the mechanical deformation resistance. Also the liquid contains particles of colloidal size originating from the cell interior, and these might accumulate at the drainage surface resulting in a tremendous increase in flow resistance.

Another observation made during the evaluation of the experimental results was that gas was present in the cake. This was concluded from the fact that the amount of expressed liquid did not correspond to the movement of the piston. During the relaxation phase when the position of the piston was fixed, the liquid pressure also followed the slowly decreasing total pressure and had a magnitude of more than 90% of the total pressure. It is worth noting that during all the experiments the liquid pressure was very close to the total pressure as soon as it had reached 0.3–0.4 MPa which shows that the liquid flow resistance at the press cake drainage surface controls the compression course after the initial compaction stage.

The result having to include all these deviations from the initial assumptions in the model was that more of a "data-fitting" approach had to be made at this stage. Work was started to find suitable model coefficients such that the model simulated the general trend of the experimental results. The first step was to reduce the number of variable coefficients by fixing some of them. The coefficients B, C and K were given the fixed values 15, 20 and 2, respectively, this was not entirely satisfactory as steps should also be taken to model the complex structure of the flow channels in the press cake under compaction.

The coefficient K was thus replaced by

$$K = K1/(1 + e)^{-10}$$

resulting in a reasonable reproduction of the extreme increase in flow resistance occurring when the void ratio decreased, as observed from the experimental data. This modification has a physical basis as it models a continuous closing of large channels where the flow resistance is low. The coefficients presented in Tables 1, 2 and 3 are those obtained by chosing the coefficients A and $K1$ so that the compared expression had a shape and magnitude close to the experimental one. Input data for the computer model were the initial void ratio and the flow rate of the expressed water as measured by the balance. When calculating the void ratio the water held by the solids was regarded as belonging to the stationary "solid" phase. The expressed amount of liquid and not the position of the piston was used due to the fact that gas was included in the cake, as mentioned earlier.

The deviation from the original intention to use a model based on physical principles makes it difficult to both quantitatively and qualitatively inrerpret the coefficients A and $K1$. Coefficient A should be a measure of the rigidity of the material but when comparing these values with the modulus of elasticity A increases while the modulus of elasticity decreases as a function of increasing temperature. The variation in A as a function of increasing pH and Ca ion concentration is within the experimental errors, so we can conclude that the varying these parameters does not significantly affect the value of A. As mentioned earlier in this case flow resistance is a complex phenomenon. In relation to high flow resistance two contributions are worth considering; flow resistance in the cell walls and flow resistance at the drainage surface. The latter contribution may be subdivided into two parts: first the normal resistance caused by a decrease in the porosity and secondly, a filtration effect caused by the entrappment of colloid particles suspended in the liquid to be expressed. Thus also in respect to the influence of extraction temperature we have interpreted the contradictory behaviour of the modulus of elasticity and coefficient A partly as a flow resistance phenomenon; the high value of elasticity modulus measured is a result of the fact that the low temperature

has not completely inactivated the cell membranes causing turgor pressure and thus also causing an active flow resistance across the cell walls when they are deformed.

The water-holding capacity is a measure of the maximal obtainable dewatering, and the press cake water content shows what was achieved under the present operating conditions. The difference is then mainly a function of compression resistance (mainly flow resistance), applied pressure and time (pressure and time history is the same for all experiments). Thus comparing the experimental values of press cake water content and water-holding capacity with the obtained coefficient K, a direct relationship between the flow resistance K and the dewatering results obtained within the series would be expected. This was however not always the case for reasons which will be seen later.

Table 1 shows how the dewatering capacity varies with extraction temperature.

Table 1

Chemical and physical data of expression experiments where the sugar extraction temperature was varied

Extraction temperature (°C)	L_1 (mm per g TS)	L_2 (mm per g TS)	Press cake water content (g water per g TS)	Content of pectic substances (g per g TS)	Water-holding capacity (g water per g TS)	Modulus of elasticity (mPa)	Coefficients in mathematical model A	$K1$
61	2.86	1.52	2.9	0.25	0.55	16.0	330	1.5×10^{-18}
71	3.84	2.30	4.4	0.21	0.48	2.9	760	24×10^{-18}
81	4.21	2.60	4.7	0.18	0.59	2.9	820	19×10^{-18}

TS: total solids

The variation of the two press cake height variables L_1 and L_2 and the water content in the press cake shows that the expression of water becomes more difficult with increasing temperature.

The content of pectic substances on the contrary, decreases with increasing extraction temperature. A greater amount of the pectic material is thus dissolved in the liquid phase. This fact might actually be one reason why beet material is more difficult to dewater when treated at a higher temperature. Some of the polysaccharide molecules in the liquid phase are probably too big to pass through the pores in the material, and are mechanically retained in the material. If the molecule size is about the same as the pore size, the pores will probably be blocked to a certain extent. Hence the fluid flow resistance increases.

In addition there is probably another effect whereby the solvated and retained pectic molecules and particles bind and hold water in their structure (hydration, etc.). The L_1, L_2 and water content values lead us to believe that the lowest temperature 61 °C, is the best while with respect to the water-holding capacity (WHC) 71 °C is the most favourable value.

Table 2

Chemical and physical data from expression experiments where the extraction pH was varied

Extraction pH	L_1 (mm per g TS)	L_2 (mm per g TS)	Press cake water content (g water per g TS)	Content of pectic substances (g per g TS)	Water-holding capacity (g water per g TS)	Modulus of elasticity (mPa)	Coefficients in mathematical model	
							A	$K1$
4.0	4.18	2.74	5.00	0.15	0.84	2.9	780	8.4×10^{-18}
5.5	3.90	2.73	0.17	0.17	0.75	2.6	700	9×10^{-18}
7.0	3.88	2.63	4.88	0.16	0.81	3.2	740	16×10^{-18}

Table 3

Chemical and physical data from expression experiments where calcium sulphate was added

Calcium addition (mg per kg pulp)	L_1 (mm per g TS)	L_2 (mm per g TS)	Press cake water content (g water per g TS)	Content of pectic substances (g per g TS)	Water-holding capacity (g water per g TS)	Modulus of elasticity (mPa)	Coefficients in mathematical model	
							A	$K1$
0	3.79	2.40	4.45	0.12	0.99	4.8	500	4×10^{-18}
80	3.40	2.10	3.74	0.14	0.96	5.0	490	0.9×10^{-18}
0	3.94	2.83	5.27	0.13	1.26	5.7	450	4×10^{-18}
120	4.10	2.69	4.77	0.15	1.10	5.2	500	8×10^{-18}

When measuring the WHC the pore size does not seem to influence the final result as much as in the conventional compression tests.

In WHC measurements we deal with a compression procedure in which we have an extremely high pressure during an extremely long time. As mentioned earlier, we assume that the flow resistance is increased by the bulky polysaccharide molecules although this effect is probably not the limiting factor for expression under these extreme conditions. The high pressure and long time overcome the fluid flow resistance and instead other forces (capillary, hydrative) determine the WHC. We have to be aware of some sources of error that accompany these water-binding and water-holding measurements: inhomogeneity in the biological material, and rewetting of the cake.

Looking at the pH variation (Table 2) we see, somewhat surprisingly, that a pH of 4.0 gives the highest L_1 and L_2 values (although the differences between the L_2 values are small and hardly significant). This indicates that pH 4.0 is possibly too low a value for good dewatering characteristics.

A pH value of 5.5 gives the highest water content values, but the lowest value for water-holding capacity, which is rather strange. A pH of 5.5 also gives the highest value of pectic substance content (although the differences here are very small and not significant). The tendencies in these measurements

cannot easily be explained. What we can say is that the differences between the measurements are often not significant. The inhomogeneity of the sample material might induce variations that override the pH-induced variations.

Table 3 shows the behaviour of the beet material after being treated with different amounts of calcium ions.

Comparing L_1, L_2 and the water content values we see that an addition of 80 mg Ca per kg beet pulp definitely lowers these values and thus makes the material more easily dewatered. The pectic substance content rises a little after Ca treatment, which is natural as one can imagine that the Ca^{2+} ions help to bind the polysaccharides more strongly to the solid material. This should then result in less bulky pectic molecules in the water phase and less plugging of the pores.

The WHC is lowered after treatment with Ca ions and this is also what we would have expected in accordance with the other measurements.

What is perhaps a little surprising is that an increase in the amount of Ca (to 120 mg per kg pulp) does not necessarily improve the dewatering characteristics of the material. When comparing the various measurements we see that an addition of 120 mg Ca per kg beet pulp gives the same or perhaps worse dewatering characteristics than 80 mg Ca. This must reflect the inhomogeneity of the material.

The mathematical model, together with derived coefficients, was also used to simulate the expression as a tool for process optimization. Figures 4 and 5 show two examples of process simulation. The intention here is to investigate how two parameters for process design influence the dewatering results. In these simulations the processing capacity in terms of total dry substance per unit drainage surface and per unit time was constant. The dewatering result is here expressed in terms of ratio dry substance content of beet pulp to that of press cake.

In Fig. 4 it is demonstrated how the model simulate effect of initial compression rate (until a preset pressure is reached which in this case is 1 MPa). The minimum reflects the problem with a high flow resistance at the drainage surface if the high pressure is reached fast (resulting in low void ratio here). The initial slope is a result of one of the premises namely constant time.

In Fig. 5 it is demonstrated how a high flow resistance at the drainage surface causes a poor efficiency when an increasing volume of liquid must be transported through this surface.

3. Conclusion

The work presented here demonstrates some results of research and development in an applied engineering discipline. First quantitative and qualitative results are given in the form of data, of how process variables and

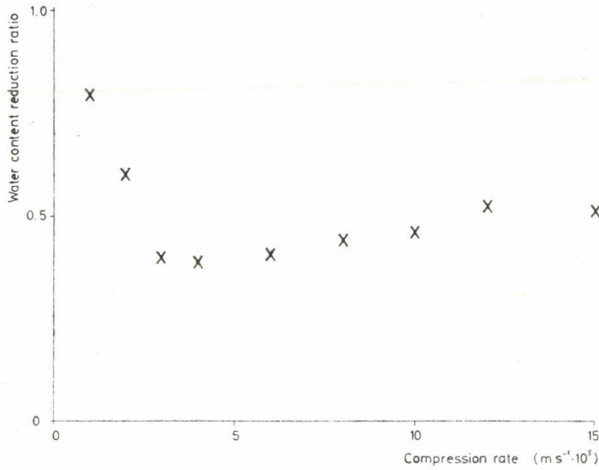

Fig. 4. Simulation of the dewatering process. Efficiency as a function of compression rate. The capacity in terms of load of dry substance per unit area and time is constant

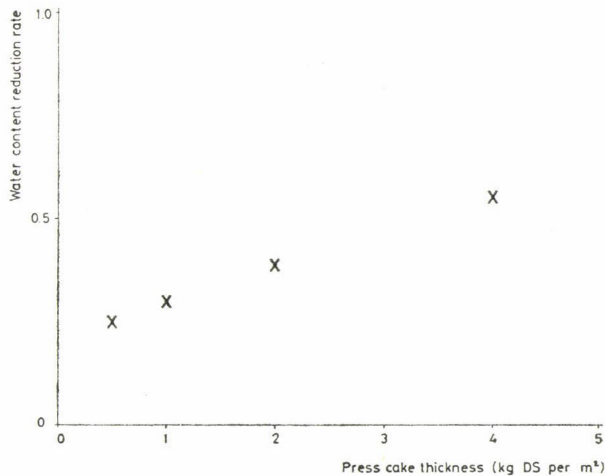

Fig. 5. Simulation of dewatering process. Efficiency as a function of press cake thickness. The capacity in terms of load of dry substance (DS) per unit area and time is constant

an additive in the process preceding the mechanical dewatering influence the course and result of this operation. Further it is shown in two examples how a mathematical model could be used in the design and optimization of equipment for this operation.

Note that certain values of the investigated process variables (pH and temperature of sugar diffusion) are of the range for what is optimal in beet sugar processing; a too high diffusion temperature causes a disintegration of

the beet slices while a low temperature does not fully inactivate the cell-membranes and a too low pH causes hydrolysis of the disaccharides. These "abnormal" variable values were chosen anyhow as it could be of interest to make small changes in the sugar diffusion practice if significant advantages were gained. An evaluation of such effects is outside the scope of this work.

The other results of this work are related to the various problems encountered. The first concerns the experimental procedure and experimental scale. Early in the work it was observed that there were large differences between the individual beets with respect to the results obtained and this lead to a discussion on a suitable experimental scale and procedure to overcome this problem. We do not know if the approach made here is the most appropriate. The second is concerned with the problem of finding a suitable mathematical model based on physical principles which was one of our goals. It is obvious that we have not fully succeeded in our ambitions.

Symbols

A, B, C, D	compression coefficients
e	void fraction (volume pores per volume solids)
G	modulus of elasticity
$K, K1$	flow resistance (permeability) coefficient
L_1	relative thickness of press cake at the commencement of relaxation
L_2	relative thickness of press cake at the end of the compression
n	porosity (volume pores per total volume)
x	length
t	time
u	velocity
Y	relaxation modulus
ε	linear strain
σ	stress
Subscripts	
l	liquid
s	solid

*

This work was supported by the Swedish National Board for Technical Development (STU) and the Swedish Sugar Company (SSA).

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Acta Alimentaria, Vol. 16 (4), pp. 319—330 (1987)

CHEMICAL COMPOSITION AND RHEOLOGICAL BEHAVIOUR OF STRAWBERRY JAMS

RELATION WITH FRUIT CONTENT

E. Costell, E. Carbonell and L. Durán

Instituto de Agroquimica y Tecnologia de Alimentos (C. S. I. C.), Jaime Roig 11., 46010 Valencia. Spain

(Received: 5 April 1986; revision received: 2 September 1986; accepted: 16 September 1986)

Relationships between fruit content in jams and both certain fruit components and some rheological parameters of the broken down gels were studied. Fifteen experimental strawberry jam samples were analyzed. Fruit content ranged from 28 to 61%. Correlation coefficients between fruit content and two chemical indices (Mg and N) and between fruit content and two rheological indices: yield stress (τ_0) and relation between two apparent viscosity values (η_1/η_{10}), selected by multiple stepwise linear regression, were similar ($r = 0.894$ and 0.841, respectively) and significant at P = 0.01%. Correlation coefficient between fruit content and both chemical and rheological indices (Mg, N, τ_0, flow index and consistency index) was higher ($r = 0.947$). These results show that the joint consideration of the variability of two chemical and three rheological indices may explain $\simeq$90% ($r^2 = 0.897$) of the variability in fruit content, which is clearly higher than the proportions explained by chemical indices (80%) or rheological indices (70%) separately.

Keywords: strawberry jams, composition, rheology

Estimation of fruit content in jam samples taken at the point of sale and subjected to examination and analysis has not been a satisfactory solution, even though considerable effort has been applied to this approach. Many publications have appeared giving analytical data of fruit used in the preserving industry (Goodall, 1969; Gherardi et al., 1980; 1983; Fuchs & Habitzky, 1981; Eksi, 1981; Skrede, 1983). In other papers, fruit content was estimated by comparing the values of several characteristics in tested jam samples against preselected chemical indices (Ryan & Dupont, 1973; Goodall & Scholey, 1975; Clerc et al., 1975; Fuchs & Ekström, 1984). Some work has also been done on different statistical treatments of analytical data (Christensen, 1972; Goodall & Scholey, 1975; Nehring et al., 1977).

The failure of commonly used methods of fruit content determination in jams is largely due to the occurrence of wide natural variations in composition. In the last years, the study of the relations that link rheological behaviour of liquid fruit products to the chemical, physical and structural characteristics (Mizrahi, 1979; Costell & Duran, 1983) tried to explain the changes undergone during manufacture (Duran & Costell, 1985) as well as the structural basis of some of their quality attributes. In a previous work

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(COSTELL et al., 1985) relationships between fruit content in apricot jams and the values of some rheological parameters of the broken down gels were studied. Results indicated the possibility of using some of these parameters as indices of the fruit content in jams. In this line, this paper deals with the usefulness of both chemical and rheological data as indices of the fruit content in strawbery jams.

1. Materials and methods

1.1. Samples

Jam samples were manufactured from three commercial strawberry pulps of different origin, packed in 5 kg cans, whose characteristics are summarized in Table 1. Pulps were passed through a screw pulper with a 3 mm diameter sieve before jam manufacture.

Table 1

Chemical characterization of strawberry pulps

Pulp	pH		Soluble solids (° Brix)		Total solids (%)	
	$\bar{x}$	±s	$\bar{x}$	± s	$\bar{x}$	±s
F.1	3.73	0.01	9.0	0.0	11.18	0.06
F.2	3.77	0.01	8.25	0.1	8.53	0.03
F.3	3.89	0.01	10.5	0.0	11.13	0.06

$\bar{x}$ = mean value
± s = standard error of 3 measurements

Fifteen jam samples (five from each pulp) were prepared in an open pan following the traditional procedure. Genu pectin citrus type D, slow set, 150° USA-SAG was used at a concentration of 0.5% over the weight of the rest of ingredients.

1.2. Chemical determinations

In the final jams the following determinations were carried out in duplicate:

— Soluble solids: °Brix was measured at 20 °C with an Abbe-Zeiss refractometer.

— pH with a Beckman digital potentiometer.

— Total solids: samples were thoroughly mixed with sand and dried in a vacuum oven at 70 °C to constant weight.

— Acidity: Diluted samples were titrated with 0.1 *N* NaOH up to pH = 8.20 ± 0.02. Results are given as percent anhydrous citric acid.

— Ash content: Samples were heated slowly until swellings stop; then they were ashed in a muffle furnace at 550 °C during twelve hours.

— Nitrogen content: By Kjeldahl's method (AOAC, 1984).

— Minerals (P, Ca, Mg, Na and K): Sample solutions prepared according to AOAC (1984) were dried on a water bath and ashed at 550 °C to obtain a white ash. This was dissolved in a 1 : 4 HCl–water solution and purified to eliminate SiO_2. Phosphorus was determined by the molybdenum blue micromethod (AOAC, 1984) using a Perkin-Elmer UV-visible spectrophotometer model Lambda 5 computer. The other minerals were determined with a Perkin-Elmer atomic absorption spectrophotometer model 703.

1.3. Rheological measurements

Gel structure of jams was broken down in a laboratory grinder during 30 s before measurement.

Rheological measurements were made in a concentric cylinder viscometer Rheomat–15 (Contraves A. G., Switzerland) with attached recorder. Samples were maintained at maximum speed value for 5 min in the viscometer cell before measurements. This was done at a temperature of 22.5 ± ± 0.5 °C. Cell MS/C was used. Torque was measured in two sub-samples of two different cans of each jam sample at a complete cycle of rotor speeds, first in descending order and then in ascending order.

Shear stress (τ) was calculated from the average of the two torque values, registered at each rotor speed, by using the expression of CONTRAVES (1966). Non-Newtonian shear rate (D) was calculated according to the KRIEGER and ELROD (1953) equation, as simplified by HAUGEN and TUNG (1976). Yield stress (τ_0) was derived as the square of the intercept for the regression line between the square root of the shear stress values and the square root of non-Newtonian shear rate values (COSTELL & DURÁN, 1979). Using the Herschel and Bulkley equation ($\tau = \tau_0 + KD^n$), n was the slope of the line obtained by plotting log $(\tau - \tau_0)$ versus log N, N being the rotor speed in r.p.m., K was the antilogarithm of the intercept at the ordinate axis when plotting log $(\tau - \tau_0)$ versus log D.

Calculation of all rheological parameters was carried out in a Wang PC computer using a specially designed program.

1.4. Statistical analysis

Standard error of the mean of three measurements for the pulp characteristics and of two measurements for the other variables was calculated.

Relationships between fruit content of jams and both chemical and rheological parameters were studied by stepwise multiple regression analysis. Significance of the equations coefficients was analysed by a t test at $\alpha = 0.05\%$ probability level.

2. Results and discussion

2.1. *Chemical composition of jams. Selection of chemical indices*

All prepared experimental jams showed sensory and commercial characteristics within legal limits except for the fruit content that, consequent with the objective of this study, varied from 28.1 to 61.0% (Table 2).

Table 2

Quality characteristics of strawberry jam samples

Sample	Fruit content (%)	Soluble solids (° Brix)		pH		Total solids (%)		Titratable acid (%)	
		$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s
F.1.1	61.0	67.0	0.0	3.27	0.01	69.33	0.08	0.905	0.005
F.1.2	51.0	64.0	0.0	3.29	0.00	65.68	0.04	0.750	0.000
F.1.3	44.1	64.5	0.0	3.28	0.00	66.24	0.03	0.567	0.004
F.1.4	37.4	65.5	0.0	3.27	0.01	67.65	0.08	0.578	0.000
F.1.5	30.4	66.5	0.0	3.24	0.01	68.53	0.25	0.480	0.003
F.2.1	56.4	62.0	0.3	3.27	0.00	63.31	0.01	1.321	0.000
F.2.2	51.1	63.9	0.1	3.28	0.00	65.01	0.01	1.224	0.003
F.2.3	43.9	63.9	0.1	3.30	0.00	65.18	0.03	1.063	0.000
F.2.4	35.4	61.8	0.2	3.29	0.00	63.06	0.01	0.886	0.004
F.2.5	30.1	65.7	0.0	3.30	0.00	67.87	0.04	0.764	0.003
F.3.1	57.9	64.6	0.1	3.42	0.01	66.03	0.01	1.070	0.000
F.3.2	52.3	66.6	0.1	3.46	0.01	68.34	0.18	0.948	0.000
F.3.3	46.2	68.6	0.2	3.50	0.01	70.31	0.01	0.836	0.003
F.3.4	39.4	70.3	0.2	3.52	0.01	72.32	0.04	0.725	0.000
F.3.5	28.1	62.5	0.3	3.49	0.01	64.09	0.23	0.548	0.003

$\bar{x}$ = mean value; ± s = standard error of the mean of 2 measurements
F_{1i}, F_{2i} and F_{3i}, strawberry jam samples manufactured from 3 different pulps (Table 1)

Table

Chemical composition

Sample	Ash (%)		Nitrogen content (%)		Phosphorus content (ppm)	
	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s
F.1.1	0.284	0.009	0.125	0.005	83.0	1.0
F.1.2	0.257	0.011	0.065	0.003	69.4	0.3
F.1.3	0.201	0.003	0.065	0.003	60.0	0.3
F.1.4	0.191	0.010	0.042	0.000	51.2	0.1
F.1.5	0.167	0.019	0.031	0.000	42.5	0.8
F.2.1	0.238	0.001	0.083	0.003	93.8	0.4
F.2.2	0.210	0.002	0.068	0.002	94.0	7.8
F.2.3	0.186	0.005	0.054	0.002	85.0	2.5
F.2.4	0.156	0.001	0.037	0.001	72.2	2.8
F.2.5	0.139	0.000	0.033	0.000	70.3	1.8
F.3.1	0.216	0.001	0.060	0.000	71.5	0.8
F.3.2	0.200	0.003	0.056	0.001	62.4	0.3
F.3.3	0.181	0.017	0.053	0.000	58.0	0.4
F.3.4	0.163	0.010	0.049	0.000	49.3	1.3
F.3.5	0.114	0.008	0.035	0.000	36.8	0.1

$\bar{x}$ = mean value; ± s = standard error of the mean of 2 measurements

Ash, nitrogen, phosphorus, calcium, magnesium, sodium and potassium were chosen as possible chemical indices of fruit content and the results obtained are shown in Table 3.

Selection of these indices was made on the basis of the following considerations: Sugars and acids are poor index components because they are commonly added ingredients of jams. Amino acid content may be altered in the process of jam making. On the contrary, inorganic elements are stable to processing and can easily be determined with great accuracy.

For jams manufactured from the same fruit lot, relationship between chemical parameters and fruit content was linear. Correlation coefficients for ash, phosphorus, magnesium and potassium were high ($r \geq 0.945$) and statistically significant ($P < 0.05\%$) (Table 4).

In the cases of sodium and calcium, correlation coefficients were lower ($0.758 < r < 0.997$ and $0.680 < r < 0.975$, respectively) and their statistical significance varied among lots. Besides this the ordinate intercepts (a values in Table 4) were abnormally high, which might be attributable to the easy contamination of the product by these elements during processing.

Relationships between chemical parameters — ash, phosphorus, magnesium, nitrogen and potassium contents — and fruit content are shown in Figs. 1 to 5.

Samples obtained from pulp F.2 (see Table 1) had higher P contents (Fig. 2), which may be attributed to the possible phosphate contamination of the sugar used in this lot. This unproved but possible contamination of jam ingredients hinders the usefulness of phosphorus as an index of fruit content.

3

of strawberry jam samples

Calcium content (ppm)		Magnesium content (ppm)		Sodium content (ppm)		Potassium content (ppm)	
$\bar{x}$	$\pm$ s	$\bar{x}$	$\pm$ s	$\bar{x}$	$\pm$ s	$\bar{x}$	$\pm$ s
121.4	3.9	94.7	2.0	187.6	1.8	694	47
107.6	2.6	85.2	0.3	170.7	4.8	543	1
92.2	1.8	75.6	0.3	165.8	8.1	504	8
77.9	0.4	65.3	0.6	130.4	9.5	459	4
77.4	2.2	53.3	1.3	129.4	7.0	330	1
119.4	3.9	73.4	1.3	136.3	3.0	864	32
102.7	0.5	66.0	0.0	135.4	0.6	717	7
93.8	1.6	57.3	0.0	125.7	3.0	698	25
102.6	5.0	50.0	0.3	128.0	9.9	465	15
97.0	3.7	43.3	0.6	128.6	3.3	431	1
144.4	2.2	78.0	0.0	200.7	6.0	735	26
153.3	9.8	70.7	0.0	190.7	7.3	603	30
142.2	2.2	64.3	2.3	175.8	1.8	592	52
130.2	9.0	56.7	5.8	166.1	8.2	465	56
100.3	1.9	41.2	8.8	139.3	6.0	280	24

F_{1i}, F_{2i} and F_{3i}, strawberry jam samples manufactured from 3 different pulps (Table 1)

Table 4

Relationship between chemical parameters (ash, N, P, Ca, Mg, Na and K) and fruit content in strawberry jam samples manufactured with three different lots of pulp (F_1, F_2 and F_3)

Component	F.1			F.2			F.3		
	a	*b*	*r*	*a*	*b*	*r*	*a*	*b*	*r*
Ash	0.032	0.0042	0.977**	0.027	0.0037	0.997**	0.022	0.0034	0.994**
Nitrogen	−0.08	0.0032	0.945*	−0.03	0.0020	0.991**	0.01	0.0009	0.980**
Phosphorus	1.80	1.33	0.999**	36.23	1.08	0.975**	4.90	1.13	0.997**
Calcium	20.67	1.67	0.975**	45.09	1.34	0.680 NS	61.19	1.63	0.917*
Magnesium	13.22	1.38	0.992**	9.63	1.11	0.997**	7.74	1.22	0.998**
Sodium	54.67	2.28	0.953*	105.75	0.58	0.758 NS	83.02	2.04	0.997**
Potassium	−0.97	11.31	0.981**	−117.88	17.36	0.974**	−118.57	14.60	0.986**

Regression parameters: a = intercept at ordinate axis; b = slope and r = correlation coefficient
* significant at $P \leq 0.05$ probability level; ** highly significant at $P \leq 0.01$ probability level; NS: non-significant

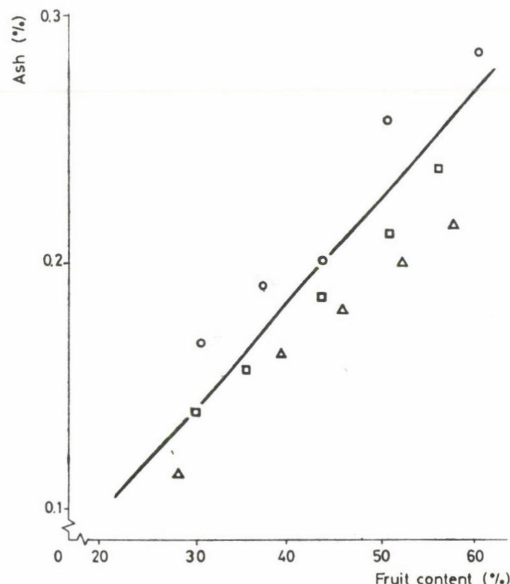

Fig. 1. Relationship between ash and fruit contents of strawberry jam samples manufactured from different pulps: F_1 (○), F_2 (□) and F_3 (△). Regression equation: $y = -0.015 + 0.047\,x$; correlation coefficient $(r) = 0.887$ $(n = 15;\ P \leq 0.01\%)$

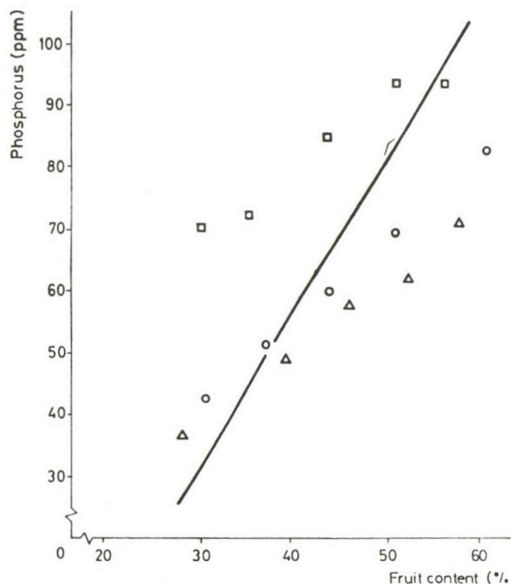

Fig. 2. Relationship between phosphorus and fruit contents of strawberry jam samples manufactured from different pulps: F_1 (○), F_2 (□) and F_3 (△). Regression equation: $y = -44.16 + 2.50\,x$; correlation coefficient $(r) = 0.661$ $(n = 15;\ P \leq 0.01\%)$

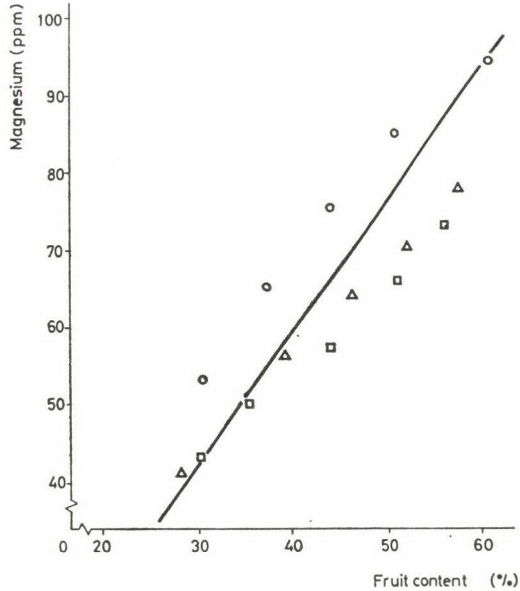

Fig. 3. Relationship between magnesium and fruit contents of strawberry jam samples manufactured from different pulps: F_1 (○), F_2 (□) and F_3 (△). Regression equation: $y = -6.28 + 1.61\,x$; correlation coefficient $(r) = 0.885$ ($n = 15$; $P \leq 0.01\%$)

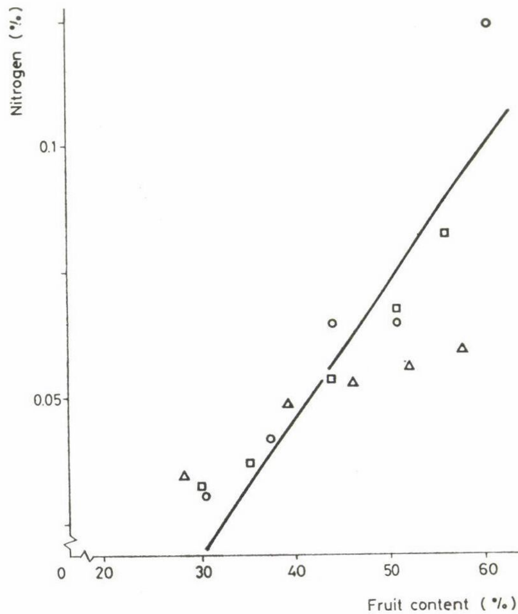

Fig. 4. Relationship between nitrogen and fruit contents of strawberry jam samples manufactured from different pulps: F_1 (○), F_2 (□) and F_3 (△). Regression equation: $y = -0.062 + 0.0027\,x$; correlation coefficient $(r) = 0.840$ ($n = 15$; $P \leq 0.01\%$)

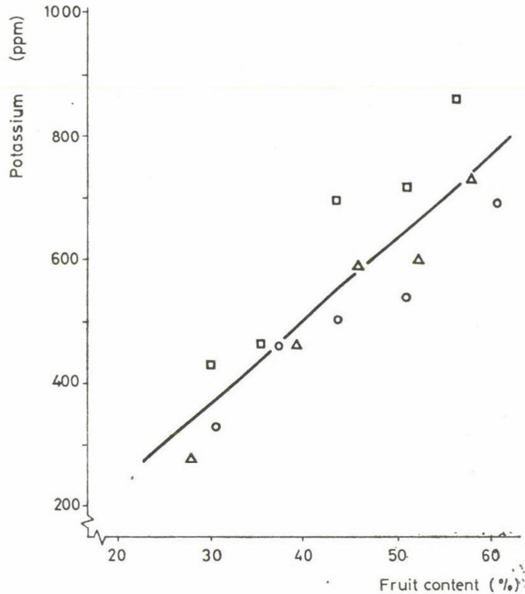

Fig. 5. Relationship between potassium and fruit contents of strawberry jam samples manufactured from different pulps: F_1 (○), F_2 (□) and F_3 (△). Regression equation: $y = -36.19 + 13.42\,x$, correlation coefficient $(r) = 0.880$ ($n = 15$; $P \leq 0.01\%$)

Potassium content variations with fruit content showed to be linear (Fig. 5) but the legal and common industrial practice of adding K salts as preservatives, invalidate this parameter as a useful index of fruit content.

According to these results and considerations, relation between fruit content and ash, magnesium and nitrogen contents was studied by multiple stepwise regression analysis. The obtained equation:

$$y = 9.18 + 0.40\,x_1 + 156.03\,x_2 \quad (r = 0.894)$$

where y = fruit content (%)

x_1 = Mg content (ppm)

x_2 = N content (%)

indicates that 80% of the variability of the fruit content in the analysed jams can be explained by the joint variability of the Mg and N contents ($r^2 = 0.799$).

2.2. *Characterization of flow. Selection of rheological indices*

Once the gel structure destroyed, the studied jams showed a plastic type flow: overcome the yield stress, they flowed as pseudoplastic. This behaviour adequately fits to the Herschel and Bulkley model: $\tau = \tau_0 + KD^n$ (COSTELL et al., 1986) the correlation coefficient being higher than 0.996 for

all samples. These may be thus characterized by the rheological parameters: yield stress (τ_0), flow index (n) and consistency index (K) (Table 5).

It was previously found by the authors (COSTELL et al., 1985; 1986) that for both apricot and strawberry jams, the decrease in apparent viscosity (η_{ap}) when increasing the shear rate (D) was of greater magnitude in jams with higher fruit content (Fig. 6).

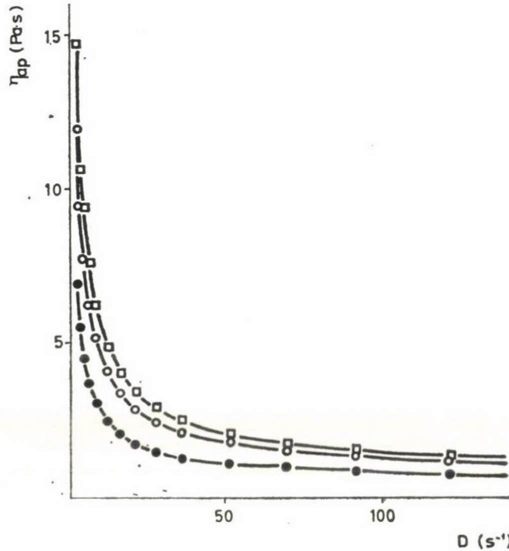

Fig. 6. Evolution of apparent viscosity (η_{ap}) values with increasing shear rates (D) for three strawberry jams with different fruit content from the same pulp: 56.4% (□), 43.8% (○) and 35.4% (●)

A new rheological index was then defined as the ratio of η_{ap} values at two rotor speeds (η_1/η_{10}) (Speed No.1 = 5.595 r.p.m. and speed No. 10 = 77.98 r.p.m.) (Table 5).

By applying a stepwise regression analysis to the relation between fruit content and the four rheological parameters, the following equation was obtained:

$$y = 7.68 + 8.46\, x_3 + 0.26\, x_4 \quad (r = 0.841)$$

where y = fruit content (%)

$x_3 = \eta_1/\eta_{10}$ ratio

$x_4 = \tau_0$ (Pa)

This equation shows that about 71% of the variability of fruit content may be explained by the joint variability of the ratio between apparent viscosities at two different shear rates and of the yield stress values ($r^2 =$ = 0.707).

Finally, by applying the same type of regression analysis, considering both the selected three chemical indices — ash, magnesium and nitrogen contents — and the four rheological ones — yield stress, flow index, consistency index and ratio of apparent viscosities — the expression obtained ($r = 0.947$):

$$y = 109.83 + 0.78\, x_1 + 133.72\, x_2 - 0.97\, x_4 - 158\, x_5 + 1.34\, x_6$$

where y = fruit content (%)
x_1 = Mg content (ppm)
x_2 = N content (%)
$x_4 = \tau_0$ (Pa)
$x_5 = n$
$x_6 = K$ (Pa s^n)

shows that the joint consideration of the variability of two chemical and three rheological indices may explain $\simeq$90% ($r^2 = 0.897$) of the variability in fruit content, which is clearly higher than the proportions explained by chemical indices (80%) or rheological indices (70%) separately.

Table 5

Rheological parameter values of strawberry jam samples

Samples	τ_0 (Pa)		K[a] (Pa · s^n)		n[a]		η_1/η_{10}[b]	
	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s
F.1.1	74.33	1.00	23.27	2.62	0.61	0.00	6.14	—
F.1.2	33.75	1.07	7.34	0.00	0.68	0.01	5.85	0.10
F.1.3	30.26	0.01	6.97	0.15	0.69	0.01	5.68	0.14
F.1.4	25.21	1.55	6.37	0.29	0.69	0.00	5.46	0.02
F.1.5	21.69	0.71	6.34	0.08	0.70	0.00	4.51	0.44
F.2.1	23.35	0.08	5.22	0.00	0.67	0.00	6.04	0.04
F.2.2	22.81	0.01	5.45	0.00	0.67	0.00	5.84	0.01
F.2.3	17.99	0.07	4.74	0.04	0.68	0.00	5.52	0.02
F.2.4	10.02	0.14	3.08	0.03	0.68	0.00	5.19	0.01
F.2.5	14.85	0.69	4.65	0.06	0.69	0.00	5.09	0.10
F.3.1	29.30	0.10	7.41	0.45	0.68	0.00	5.60	0.13
F.3.2	36.38	0.81	10.01	0.58	0.66	0.00	5.58	0.16
F.3.3	30.67	0.80	11.25	0.81	0.67	0.01	4.83	0.05
F.3.4	32.39	0.95	14.65	0.59	0.66	0.01	4.55	0.03
F.3.5	4.82	0.12	2.09	0.23	0.76	0.00	3.55	—

[a] Herschel and Bulkley equation parameters
[b] Ratio of apparent viscosity values at two rotor speeds
$\bar{x}$ = mean value; ± s = standard error of the mean of 2 measurements

*

The authors are indebted to the Comision Asesora de Investigación Cientifica y Técnica (program No. 237/87) for financial support.

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Acta Alimentaria, Vol. 16 (4), pp. 331–337 (1987)

ABSORPTION OF VITAMIN A DISSOLVED IN VARIOUS OILS AND FATTY ACIDS OF DIFFERENT CHAIN LENGTH IN RATS

A. Blaskovits, L. Gampe, M. Ruby and J. Borvendég

National Institute of Pharmacy, H-1051, Budapest, Zrínyi u. 3. Hungary
(Received: 12 June 1986; accepted: 12 November 1986)

Blood level and liver concentration of vitamin A dissolved in various oils and fatty acids of different chain length were studied given p.o. to sexually mature male CFY rats. It was found that the absorption and accumulation in the liver of vitamin A dissolved in various edible oils depended largely on the oil used.

A correlation was established between the fatty acid composition of the oils and accumulation of vitamin A in the liver.

Triglycerides formed from shorter chain fatty acids are easily absorbed, thus, vitamin A dissolved in them accumulates in greater amount in the liver. If vitamin A was dissolved in the fatty acids then the difference in the absorption influencing effect of the different chain length fatty acids diminished or even ceased. This observation suggests that the biological utilization of vitamin A depends largely on the digestibility of the oil used as solvent.

Keywords: vitamin A, absorption, liver

Absorption of many fat-soluble, biologically active substances is possible only in the presence of lipids. The chemical structure, digestibility and fatty acid composition of the lipids varies within a wide range. Great differences manifest themselves in their absorption.

Triglycerides of medium chain fatty acids (MCT) which can be absorbed in the ileum without reesterification and chylomicron formation through the v. portae (Greenberger et al., 1966) have been used for years in the symptomatic treatment of defective anabolism.

The enzymatic degradation of MCT containing oils in the intestine is very rapid and goes ahead in the absence of bile acids, too (Entressangles, 1964). They can be, however, absorbed without hydrolysis, that is without undergoing change, thus they can be successfully applied in the treatment of patients suffering from defective anabolism (Barna et al., 1978). A further dietetic advantage of oils containing MCT is — as shown in healthy children when given MCT oil or butter — that their serum fat parameters are less affected than on consuming butter (Barna et al., 1981).

Vitamin A is one of the fat soluble vitamins, thus its absorption and biological utilization is to a substantial extent affected by fats. More then 90% of the vitamin A stored in the body is to be found in the liver and only about 1–3% circulates in the blood (Raica et al., 1972). Vitamin A is present in the blood bound to proteins, not in a free state. The retinol binding protein is specific for vitamin A (RBP). The normal amount of RBP in the plasma is

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

40–50 μg cm^{-3} (SMITH & GOODMAN, 1979; SMITH & GOODMAN, 1971). The vitamin A binding protein is synthesized in the liver and catabolized in the kidney (MUTO et al., 1972).

The aim of the present study was to find out:

— the correlation between fat absorption on the one hand, and the vitamin A concentration in the liver, on the other;

— the effect of the chain length of fatty acids on the vitamin A concentration in the serum and in the liver.

1. Materials and methods

1.1. Materials

Sunflower seed, rape seed and coconut oil were kindly put at our disposal by the Laboratory of Kőbánya Vegetable Oil Factory, Budapest.

Capric acid ($C_{10:0}$), palmitic acid ($C_{16:0}$) and behenic acid ($C_{22:0}$) used in the experiments were obtained from the firm Sigma (USA).

The absorption experiments were carried out with vitamin A alcohol, manufactured by Fluka (Switzerland).

1.2. Methods

1.2.1. Vitamin A determination. To determine vitamin A in the blood the fluorometric method was used (BLASKOVITS et al., 1980; HANSEN & WARWICK, 1978). The liver vitamin A concentration was determined by the fluorometric method of THOMPSON and co-workers (1971). The measurements were carried out on Type Perkin-Elmer MPF 44B instrument, using wavelength 340 nm for excitation and wavelength 480 nm for emission. Amplification was 30 fold.

1.2.2. Effect of oils on vitamin A absorption. The effect of various oils on the absorption of vitamin A was tested on LATI CFY (Laboratory Amimals Institute, Gödöllő) male rats of 200–250 g body weight, kept under standard conditions. The test animals were divided into three groups, 6 animals each. The fourth group of the animals served for control.

In the first group the test animals were given 3 mg vitamin A dissolved in sunflower seed oil, in the second group the same amount in rape seed oil, while in the third in coconut oil. The volume of oil was 0.5 cm^3 and was introduced p.o. with a probe.

To check the absorption of vitamin A 100 μl blood was taken immediately prior to introducing vitamin A from the tail-vein of each animal, then after 1, 2, 3, 4, 6 and 8 h. The vitamin A content was determined by the fluorometric method as given in para. 1.2.1.

Separate experiments were carried out to establish vitamin A concentration in the liver. The animals were kept, prepared, grouped and probed as above. Sixteen hours after the introduction of vitamin A in the various oils both the treated and the control animals were bled to death and vitamin A was determined in their liver.

1.2.3. Vitamin A absorption in fatty acids. To study the absorption of vitamin A dissolved in various carbon chain length fatty acids an emulsion was prepared from capric acid ($C_{10:0}$), palmitic acid ($C_{16:0}$) and behenic acid ($C_{22:0}$) and 3 mg of vitamin A was dissolved and given to the animals by p.o. probe.

1.2.4. Preparation of the emulsion. Thirty mg retinol and 1000 mg fatty acid were dissolved in 2 cm^3 alcohol then 1000 mg Na-taurocholate were added in 38 cm^3 water. The precipitate thus obtained was treated for 5–10 min in an MSE ultrasonic desintegrator and the milk-like emulsion was immediately used.

The animals were prepared, grouped according to para. 1.2.2. with the difference of giving them instead of the oils 4 cm^3 emulsion each. The 100 μl blood taken from the tail-vein was tested immediately prior to introducing vitamin A and 1, 2, 3, 4 and 6 h after.

The concentration in the liver of vitamin A dissolved in emulsion was studied in separate experiments. The animals were grouped as described before. Sixteen hours after giving the animals 4 cm^3 emulsion each, they were bled to death and vitamin A was determined in the liver.

2. Results

The fatty acid composition of the oils used in the experiments is given in Table 1. The fatty acid composition of vegetable oils was measured by the Laboratory of Kőbánya Vegetable Oil Factory, Budapest.

Table 1

Fatty acid composition of vegetable oils
(As percentage of the total fatty acid content)

Fatty acid	Coconut oil	Sunflower seed oil	Rape seed oil
Caprylic acid ($C_{8:0}$)	3.3	—	—
Capric acid ($C_{10:0}$)	0.5	—	—
Lauric acid ($C_{12:0}$)	50.3	—	—
Myristic acid ($C_{14:0}$)	23.0	—	0.1
Palmitic acid ($C_{16:0}$)	11.3	6.0	3.4
Stearic acid ($C_{18:0}$)	2.3	4.2	1.1
Oleic acid ($C_{18:1}$)	7.8	20.9	16.3
Linolic acid ($C_{18:2}$)	—	68.0	16.3
Erucic acid ($C_{22:1}$)	—	—	44.5

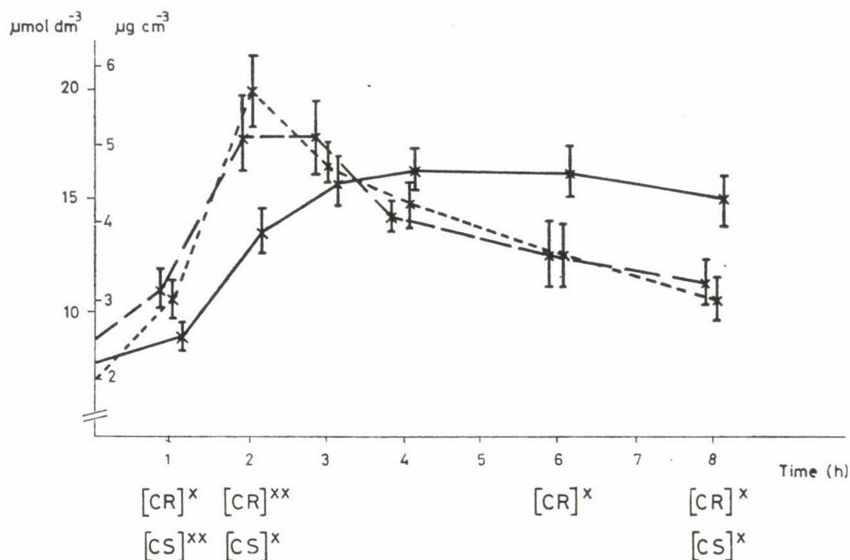

Fig. 1. Vitamin A level in the blood of rats upon the p.o. administration of 3 mg vitamin A dissolved in various oils. The vertical bars represent the standard deviations. x: mean value of 6 measurement * Significant at $P < 0.05\%$ probality level; **Highly significant at $P < 0.01\%$ probality level. ——— Coconut oil (C); — — — sunflower seed oil (S); ...... rape seed oil(R)

Results of measurements are illustrated in Figs. 1, 2 and 3.

Figure 1 shows the vitamin A level in the blood of rats given p.o. 3 mg vitamin A in coconut oil, sunflower seed oil and rape seed oil, respectively. As shown in the figure, the difference in the absorption of vitamin A dissolved in rape seed and sunflower seed oil was practically nil. Absorption of vitamin A dissolved in coconut oil was substantially lower after 1 h and particularly after 2 h. After 4, 6 and 8 h, however, the absorption in coconut oil was higher than in the other two oils.

Figure 2 shows the vitamin A level in the blood of rats given p.o. 3 mg vitamin A dissolved in the emulsion of behenic, capric and palmitic acids, respectively. As seen in the figure the absorption of vitamin A is extremely rapid, it reaches the maximum level within 1 h and subsequently gradually decreases.

Figure 3 shows the increase of the vitamin A concentration in the liver upon giving the rats 3 mg vitamin A dissolved in various oils and fatty acids. The vitamin A concentration in the liver increased in comparison to the control by 18%, when administered in rape seed oil (difference non-significant), by 48% in sunflower seed oil and by 82% in coconut oil. Vitamin A increased in the liver by about 45% when given in behenic acid emulsion and by about 80–90% when given in capric or palmitic acid emulsion as compared with the control. The figures show the mean values of results and the standard deviations.

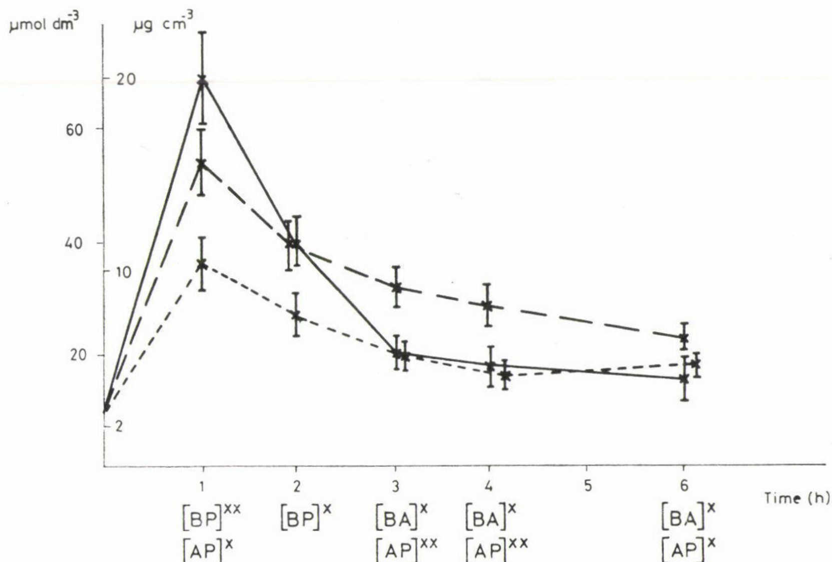

Fig. 2. Vitamin A level in the blood of rats upon p.o. administration of 3 mg vitamin A dissolved in various fatty acids. The vertical bars represent the standard deviations. x: mean value of 6 measurements; * Significant at $P < 0.05\%$ probality level; **Highly significant at $P < 0.01\%$ probality level. ——— Behenic acid (B); — — — capric acid (A); ...... palmitic acid (P)

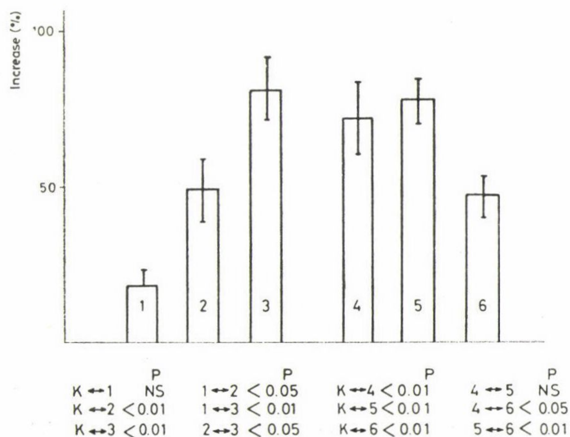

Fig. 3. Increase in the vitamin A content in the liver upon p.o. administration of 3 mg vitamin A dissolved in various oils and fatty acids. The vertical bars represent the standard deviations. x: mean value of 6 measurements. K: Control. 1: Rape seed oil; 2: sunflower seed oil; 3: coconut oil. 4: capric acid ($C_{10:0}$); 5: palmitic acid ($C_{16:0}$); 6: behenic acid ($C_{22:0}$)

3. Conclusion

The substantial difference in the absorption of vitamin A (as seen in Fig. 1) dissolved in coconut oil on the one hand and sunflower seed oil or rape seed oil on the other can be explained by the different absorption mechanism of coconut oil of high MCT content and the other two oils rich in long chain fatty acids. As it is well known long chain triglycerides get first through the endothelium into the circulation of the lymphatic system, while medium chain triglycerides get by way of the vena portae into the liver and are metabolised there. The enzymatic degradation of MCT containing oils is very rapid in the ileum (Greenberger et al., 1966; Entressangles, 1964). The soluble protein fractions in the liver bind the free fatty acids (Góth, 1976) thus, the medium chain fatty acids are separated out of the blood getting in the liver by way of the vena portae. The triglycerides then are transformed in the liver into carbondioxide and ketons while a part is transformed into long chain fatty acids.

However, 4, 6 or 8 h after the introduction of vitamin A the plasma level was higher upon the use of coconut oil than when the other two oils were used. This phenomenon could be explained by the circumstance that the fatty acid composition of coconut oil would permit theoretically its absorption in the digestive tract from the aqueous phase, too.

The peripheral blood level of vitamin A dissolved in various chain length fatty acids is illustrated in Fig. 2. As it can be seen, the absorption of vitamin A is extremely rapid and the maximum level is achieved in the first hour to diminish afterwards. It is surprising that the level of behenic acid is so high in the first hour. This can be probably explained by the circumstance that in the presence of behenic acid, strange to the organism, the accumulation of vitamin A in the liver is very slow in the initial phase.

The percentage increase of the vitamin A content of the liver in comparison with the control upon using various oils and emulsions of different chain length fatty acid as solvents of vitamin A, is shown in Fig. 3. The digestibility of the fats is shown to affect to a substancial extent the accumulation of vitamin A in the liver. The vitamin A content in the liver increased by 18% in comparison to the control, when rape seed oil was used (the difference non-significant), by 48% upon the use of sunflower seed oil and by 82% using coconut oil. The vitamin A content of the liver of the control animals was found to be 47.1 $\pm$ 3.8 μg per g. Results demostrate how much the oil selected as vehicle can influence the absorption of fat-soluble medicaments and vitamins. This is particularly important in view of defective anabolism where it is a help in the absorption of the medicament (Barna et al., 1978) and at the same time improves the supply of nutrients (calorie intake).

Using behenic acid emulsion as a solvent for vitamin A the vitamin content of the liver increased by 45% in comparison with the control. The

capric and palmitic acid emulsions increased the vitamin A content of the liver by about 80–90% in comparison with the control. The high vitamin A content in the liver as induced by the use of palmitic acid emulsion is probably due to the presence of palmitic acid promoting substantially the accumulation of vitamin A in the liver. Vitamin A is stored in the liver in the form of palmitic acid ester bound to fraction VLDL (GOODMAN, 1980).

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Acta Alimentaria, Vol. 16 (4), pp. 339–350 (1987)

SEPARATION AND IDENTIFICATION OF TOMATO FRUIT PIGMENTS BY TLC AND HPLC

H. G. DAOOD, P. A. BIACS, Á. HOSCHKE, M. HARKAY-VINKLER and F. HAJDU

Central Food Research Institute, H-1022, Budapest, Herman O. út 15. Hungary
(Received: 26 June 1986; accepted: 3 November 1986)

High-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) techniques were applied for separation of the pigments of tomato fruit. More than 10 pigments were separated and identified in ripe fruit of ventura cultivar of the tomato plant.

A new mobile phase for both TLC and HPLC system was developed and better separation for the major as well as the minor pigments was achieved. These procedures were found to be reproducible, rapid and not to cause remarkable damage to the separated pigments or to the adsorbents of the chromatographic systems.

The individual pigments of tomato fruit were identified according to their physicochemical and chromatographic properties (retention time in HPLC, R_f-value in TLC and visible-absorption spectra).

The fresh ripe fruit accumulated mostly lycopene, neurosporin, β-carotens and lutein, besides neoxanthin, xanthophylls, chlorophylls and prolycopene as minor pigments.

The new mobile phase of HPLC was also applied to the separation of red pepper pigments as a comparison sample.

Keywords: pigment, HPLC, TLC

A rapid, simple and highly efficient chromatographic method for the separation of pigments in foodstuffs with a minimum of oxidation and isomerization has long been needed.

Separation of different pigments of many fruits and vegetables was achieved by thin-layer and column chromatography using several types of adsorbents and mobile phases (VINKLER & KISZEL-RICHTER, 1972; JEN, 1974; BALOCH et al., 1977; BUCKLE & RAHMAN, 1979).

The new HPLC technique of high sensitivity and reproducibility is being used extensively in food analysis, including pigment composition and colorant content of many food products (BARANYAI et al., 1982; LEE et al., 1984; CALVEY & GOLDBERG, 1982; COX & MCCLURE, 1982).

Application of HPLC technique for qualitative and quantitative determination of provitamin A carotenes in some fruits and vegetables has been the subject of many research works in recent years (BUSHWAY & WILSON, 1982; HSIEH & KAREL, 1983).

Chromatographic separation of tomato fruit pigment has already been achieved by using column and thin-layer chromatography with some difficulties and problems. The results of many investigations were reviewed by HERRMANN (1979).

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

Investigation, by HPLC, of provitamin A and lycopene content of tomato fruit was carried out by ZAKARIA and co-workers (1979) without paying any attention to the other minor pigments. In a previous work HPLC with a mobile phase containing acetone–water 9 : 1 (v/v) was used for the qualitative determination of tomato fruit and red pepper pigments during the last stage of ripening (BIACS et al., 1985; DAOOD et al., 1986).

The purpose of the present investigation was to separate the different pigments of tomato fruit using TLC and HPLC techniques with new mobile phases of minimum harmful effect on the adsorbents and the pigments, during separation.

1. Materials and methods

1.1. Materials

Tomato fruit was obtained from local market and the experimental fields of the University of Horticulture, Budapest. Kieselgel 60 F_{254} pre-prepared TLC plates were from Merck, FRG. All organic solvents used were from Reanal, Hungary.

1.2. Methods

1.2.1. Extraction of pigments. The method of IKAN (1969) was applied with minor modifications. Four tomato fruits were cut into small pieces and deseeded. Ten gram samples, in duplicate, were taken, disintegrated with quartz sand in a mortar, and mixed with 30 cm^3 of methanol. The methanol fraction was separated by filtration through a piece of cotton in a small funnel. The residues (pigments) were put into a stoppered conical flask and the pigment extracted twice with 60 cm^3 of CCl_4–methanol 2 : 1 under continuous shaking for 30 min. The CCl_4 fraction was separated from the aqueous phase in a separatory funnel and dried over Na_2SO_4. The solvent was then evaporated to dryness in a vacuum rotary evaporator.

The pigments of red pepper powder were extracted by shaking 0.5 g of the powder with 100 cm^3 acetone in a mechanical shaker for 30 min and then filtered. Twenty cm^3 of the filtrate were used as sample and the solvent evaporated under vacuum in a rotary evaporator.

1.2.2. TLC separation. The dry pigments were redissolved in 3 cm^3 of 16% chloroform in acetone with an ultrasonic device. Three hundred μl of pigment solution was spotted on a 6 cm long baseline on the ready-made TLC plates. Pigment separation was carried out in darkness using two different mobile phases: A: hexane–benzene–acetone–acetic acid 80 : 10 : 5 : 5 (v/v); B: hexane–benzene–acetone 90 : 8 : 2 (v/v). The average of 3–4 R_f-values for each pigment was taken. Variation upon replications was negligible.

1.2.3. HPLC analysis. The dry matter of the extracted pigments was redissolved in 5–10 cm^3 of a 1 : 1 (v/v) mixture of chloroform and mobile phase, with an ultrasonic device. Twenty μl of the resulting solution was injected onto the column of a Liquochrom 2010 HPLC system with the following parameters:

Column: Chromsil C_{18} 10 μ, 250×4.6 mm

Detection: 438 nm

Flow rate: 1.5 cm^3 min^{-1}

Two mobile phases were used for the separation of the pigments. The first consisted of acetone–water 9 : 1 (v/v) (BIACS et al., 1985). The second one consisted of acetonitrile–isopropanol–water 200 : 288 : 13 (v/v). The acetonitrile must be redistilled prior to use.

The peak area (mm^2) of each component was calculated by means of a Shimadzu C-R3A Chromatopac integrator either connected to the HPLC system, or by using the following formula:

Peak area (mm^2) = height of peak (mm) × width of peak at half height (mm).

Concentration (%) of each component in the extracted pigment samples was determined using the average of four measurements.

1.2.4. Identification of the pigments. After separation of the pigments on TLC plate, the solvent was evaporated and the appropriate bands were scraped off the plate and eluted in the proper solvent, filtered and made up to an appropriate volume for spectrophotometric measurement. The spectrum of each pigment was recorded in a Specord M-40 spectrophotometer.

Identification was achieved by means of the retention time of available standards like lutein, β and ζ-carotene, the R_f-value of each pigment developed with a mobile phase previously used, and by the position of the absorption maximum of the individual pigment scraped off the plate and eluted by appropriate solvent. The values obtained were compared with those reported earlier (CURLE, 1961; BUCKLE & RAHMAN, 1979). Chlorophyll was identified by means of the absorption maximum at 411, 669 nm, according to FRASER and FRANKL (1985).

2. Results and discussion

The result of the TLC separation (Table 1) revealed that tomato fruit contains two groups of pigments differing in polarity. The first group includes: the apolar pigments as carotenes, neurosporene and lycopene. The separability of the members of this group increased with the decrease in the polarity of the mobile phase. It was found that in order to achieve a complete separation of apolar pigments, the proportion of benzene and acetone should be at the minimum. The first band was found to contain ζ-carotene, β-carotene and

neurosporene, however in either of the mobile phases used they could not be separated. Rechromatography in a less polar system was found to be necessary for the separation of the components of this band.

Table 1

Separation of tomato fruit pigments on Kieselgel 60 F_{254} using two mobile phases

Band colour	R_f-values		Components of the band
	A	B	
Orange-yellow	0.93	0.95	carotenes + neurosporene
Red	0.92	0.9	lycopene
Yellow	0.85	0.5	unidentified
Yellow	0.8	0.42	unidentified
Orange-pink	0.41	0.35	prolycopene
Pink-violet	0.28	0.15	violaxanthin
Pink-violet	0.23	0.1	unidentified
Green	0.20	—	pheophytine
Yellow	0.15	—	neoxanthin
Yellow	0.08	—	cis-mutatoxanthin
Yellow	0.06	—	lutein
Yellow	—	0.04	yellow xanthopylls
Yellow-green	—	0.00	chlorophyll derivatives

A: hexane–benzene–acetone–acetic acid 80 : 10 : 5 : 5 (v/v);
B: hexane–benzene–acetone 90 : 8 : 2 (v/v)

The second group consisted of polar pigments including chlorophyll derivatives, yellow and violet xanthophylls and pro-lycopene. The pigments of this group were individually separated with increasing R_f-values by using a more polar mobile phase. Among the pigments of this group pro-lycopene, two violet xanthophylls, pheophytin and lutein were identified. Neoxanthin migrated together with an unidentified yellow pigment, but by increasing the polarity of the mobile phase it could be separated. The presence of violet xanthophylls in tomato fruit is important, since they together with lycopene are responsible for the violet-red colour of the fruit of many tomato varieties. The existence of pro-lycopene and violet xanthophylls in ripe red tomato fruit has not been reported before (VÁGYUJFALVI & SZENTE, 1982; HERRMANN, 1979; CURLE, 1961). The above authors separated and identified most of the tomato fruit xanthophylls; but the colour of each component on the TLC plate was not mentioned.

The yellow bands appearing below lycopene on the plate may be due to various carotene isomers since they have a similar absorption maximum in the visible-spectrum.

2.1. Spectrophotometric identification

The visible-spectrum of each colour band separated on TLC plates gave additional information about the coloured substances in addition to its use for identification purposes. Table 2 shows the maximum absorption wavelength of each coloured substances separated by TLC. The visible spectrum of the first (orange yellow) band (Fig. 1a) indicated the presence of ζ-carotene in tomatoes even at the over ripe stage. The absorption maxima at 380, 409 and 435 nm are attributable to ζ-carotene (ULRICH & MACKINNEY 1968; BALOCH et al., 1977) which migrated together with β-carotene.

Table 2

Absorbance maxima of TLC-separated pigments of tomato fruit

Pigment	Colour on TLC plate	Absorbance maxima	Solvent[a]
ζ-Carotene	orange-yellow	376, 410, 436	chloroform–acetone 2 : 1
β-Carotene	yellow	430, 454, 478	chloroform–acetone 2 : 1
Neurosporin	yellow	416, 440, 466	petroleum ether
Lycopene	red	456, 482, 514	chloroform
Prolycopene	orange-pink	454, 483, 516	chloroform
Violaxanthin	violet	425, 456, 480	chloroform–acetone 2 : 1
Unidentified	violet	406, 424, 461	chloroform–acetone 2 : 1
Pheophytin	faint green	411, 666,	acetone
Neoxanthin	yellow	421, 448, 477	chloroform–acetone 2 : 1
Cis-mutatoxanthin	yellow	425, 461, 480	chloroform–acetone 2 : 1
Lutein	yellow	429, 455, 481	chloroform–acetone 2 : 1

Computerized Specord M-40 spectrophotometer was used for the determinations
[a] Solvent used for redissolving the pigment scraped off the TLC plate

The faint yellow band between lycopene and β-carotene is caused by neurosporene which has absorption maxima at 415, 438–440, and 466–468 nm, respectively. The appearance of absorption maxima at 412 and 436 with the shift from 454 nm of β-carotene maxima to 460 indicated the presence of *cis*-neurosporene which is reported to have these maximum absorption values (BALOCH et al., 1977).

The orange-pink coloured band was found to have absorption maxima similar to those of lycopene (416, 454, 483 nm) with a small shift. The only difference between them is the ratio of $A_{483} : A_{415}$ which appeared to be smaller in lycopene. The decrease in the ratio is one of the biochemical characteristics of poly-*cis* lycopene (prolycopene) (ULRICH & MACKINNEY, 1968). Tomato fruit contains some xanthophylls like lycoxanthins and lycophylls which have similar absorbance maxima as lycopene, but the concentration of these components in tomato fruit is too small (BRUCKNER, 1981) to be detectable by TLC analysis under the conditions applied.

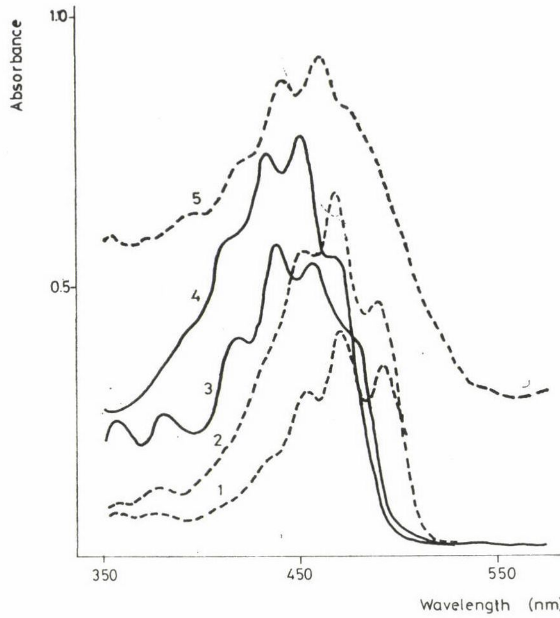

Fig. 1a. Visible spectra of tomato fruit pigments scraped off TLC plate and redissolved in appropriate solvent. 1: Prolycopene; 2: lycopene; 3: ζ-carotene + β-carotene; 4: β-carotene; 5: neurosporene

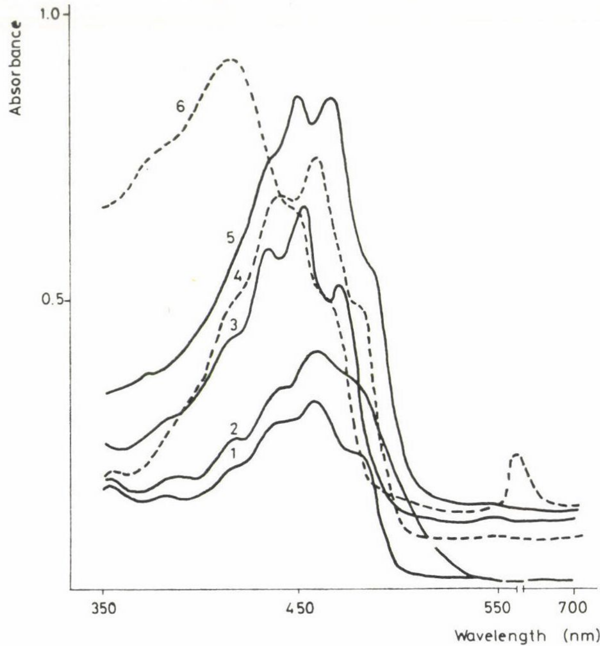

Fig. 1b. Visible spectra of some xanthophylls and chlorophyll of tomato fruit. 1: Violaxanthin; 2: unidentified violet pigment; 3: lutein; 4: *cis*-mutatoxanthin; 5: neoxanthin; 6: pneophytin

The absorbance maxima of the two violet pigments and their R_f-values revealed that they belong to the xanthophyll group of pigments (Fig. 1a). These pigments which have maximum absorbance at 461 nm and 456 nm (in petroleum ether) may belong to violaxanthin and neochromes, respectively (CURLE, 1961).

Chlorophyll derivatives were separated to some extent and identified to be mostly pheophytin A which migrates with an R_f-value of 0.22 in the polar solvent system and shows absorption maxima at 411 and 666 nm. The minor chlorophyll derivative which stayed on the start line in both mobile phases was chlorophyll B.

The yellow xanthophylls with low R_f-values were lutein, neoxanthin, and *cis*-mutatoxanthin. Separation and identification of these components requires further work.

It is important to mention that the appearance of additional absorption maxima in the spectra of some pigments is due to overlapping of pigments and to probable isomerization occurring during separation on a Silica layer.

2.2. HPLC-separation

Separation of both minor and major pigments of tomato fruit at different stages of ripening was achieved without any derivatization or saponification. It was found that dissolving of extracted pigments in the eluting solvent system is not satisfactory and unusual chromatograms were obtained (Fig. 2). An improvement was achieved by dissolving the pigments in the eluent containing 50–100% chloroform (Fig. 3). In order to detect both the major and the minor pigments, a concentrated sample should be used (Fig. 4), however in this case lycopene overloads the column.

On a reverse-phase HPLC column the more polar components like chlorophylls and xanthophyll are eluted first (ZAKARIA et al., 1979). Among the pigments of tomato fruit pheophytin, lutein, neoxanthin, violaxanthin, prolycopene, lycopene, neurosporene, β-carotene and ζ-carotene were separated and identified by using Liquochrom system with Chromsil C_{18} column. Using a mobile phase containing aceton–water 9 : 1 a good separation of both major and minor colour substances of tomato fruit was achieved without derivatization or saponification prior to separation. Using acetone with the Chromsil C_{18} column was found to be harmful and analysis of about 200 samples is enough to render the column unusable and repacking is required. Therefore the replacement of acetone with acetonitrile and isopropanol was important. This was successful. Moreover the new mobile phase showed no oxidation or isomerization products which occurred on the chromatograms with acetone elution in the form of small unidentified peaks (Fig. 5). The other

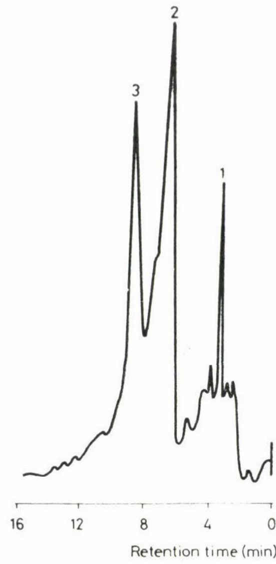

Fig. 2. HPLC separation of tomato fruit pigments dissolved in the eluent which consists of acetonitrile–isopropanol–water 200 : 288 : 13. 1: Lutein; 2: lycopene; 3: β-carotene

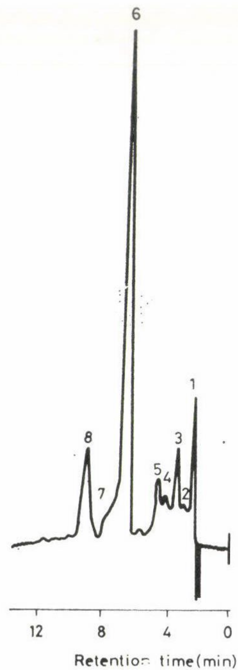

Fig. 3. HPLC separation of tomato fruit pigments dissolved in chloroform-eluent 1 : 1 and eluted with acetonitrile–isopropanol–water 200 : 288 : 13. 1: Solvent, 2: chlorophyll; 3: lutein; 4: neoxanthin; 5: violet xantophylls; 6: lycopene; 7: neurosporene; 8: β-carotene

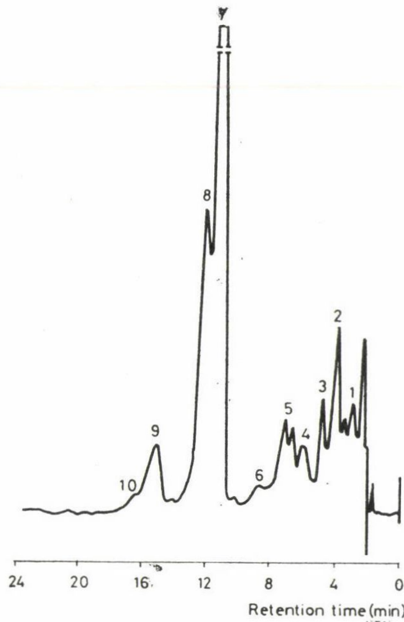

Fig. 4. HPLC chromatogram of tomato fruit pigments at high concentration and with acetone–water 9 : 1 as eluent. 1: Chlorophyll; 2: lutein; 3: neoxanthin; 4: unidentified; 5: violet xanthophylls; 6: prolycopene; 7: lycopene; 8: neurosporene; 9: β-carotene; 10: ζ-carotene

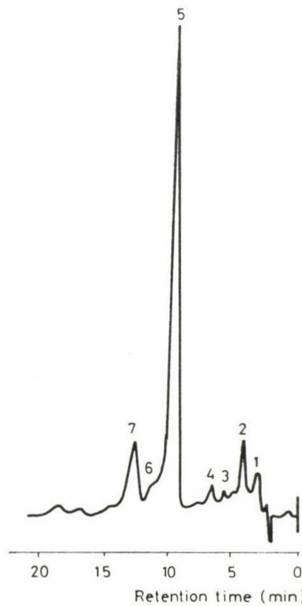

Fig. 5. HPLC chromatogram of tomato fruit pigments dissolved in chloroform–acetone 1 : 1 and eluted with acetone–water 9 : 1. 1: Chlorophyll; 2: lutein; 3: neoxanthin; 4: violet xanthophylls; 5: lycopene; 6: neurosporene; 7: β-carotene

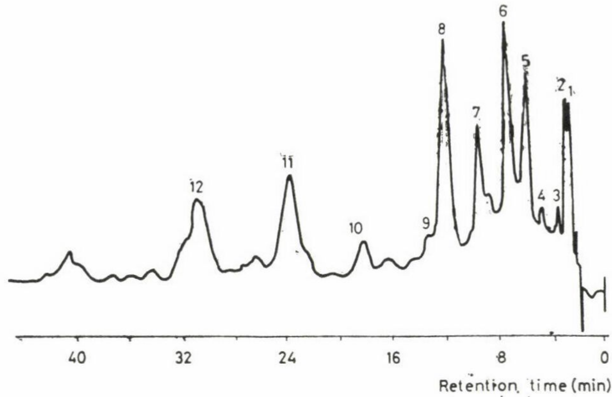

Fig. 6. Separation of red pepper powder colour substances by HPLC using acetonitrile--isopropanol-water 200 : 288 : 13 as eluent. 1: Capsorubin; 2: capsanthin; 3: zeaxanthin; 4: lutein; 5: capsanthin ester I; 6: capsanthin ester II; 7: zeaxanthin ester; 8: β-carotene: 9: capsorubin ester I; 10: capsorubin ester II; 11: capsanthin ester III; 12: capsanthin ester IV

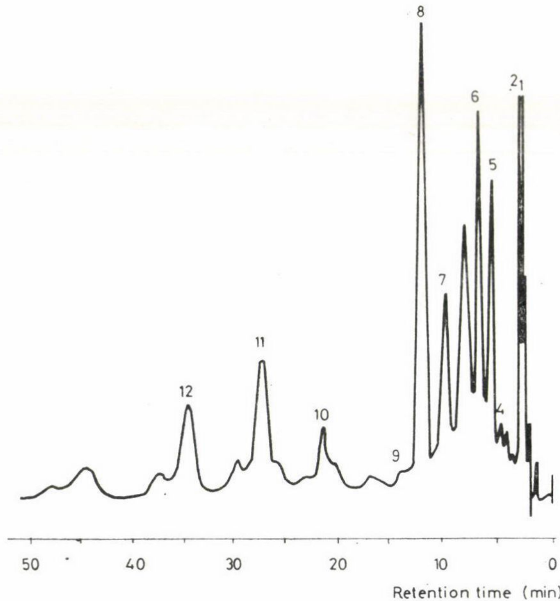

Fig. 7. Separation of red pepper powder colour substances by HPLC using acetone-water 9 : 1 as eluent. Identification of components is the same in Fig. 6

advantage of the new mobile phase is that complete separation can be achieved with shorter retention times.

Separation of red pepper pigments with both mobile phases (Figs. 6 and 7) supported our finding on tomato fruit. An acceptable separation was obtained with acetonitrile-isopropanol-water (200 : 288 : 13) eluent. The most

important pigments of red pepper and their esters were detected with modified separation characteristics.

Table 3 illustrates the proportion (%) of each component in the extracted pigments. Lycopene, β-carotene, lutein constituted about 76, 12.4 and 3.5%, respectively, in overripe tomato fruit, whereas, the minor components comprise about 3–5% of the total pigments. These proportions differ with the variety of tomato and the stage of ripening. The ripe red fruit, in addition to the above mentioned pigments, contains neurosporene and ζ-carotene as major pigments. The decrease of the neurosporene and ζ-carotene content of over ripe fruit is due to the continuous interconversion of these intermediates to lycopene and β-carotene.

Table 3

Pigments of tomato fruit at different stages of ripening

Pigment	Proportion of each pigment (%) ripe yellow		ripe red		over ripe red	
	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s
Chlorophyll	7.79	0.35	3.41	0.27	3.36	0.3
Lutein	7.23	0.28	1.9	0.16	4.02	0.148
Neoxanthin	0.07	0.001	0.66	0.005	nil	
Violet xanthophyll	nil		2.1	0.16	0.53	0.05
Prolycopene	0.33	0.05	1.04	0.065	1.43	0.053
Lycopene	24.9	0.84	50	1.52	75.8	1.83
Neurosporene	25.1	0.75	29	0.78	nil	
β-carotene	15.4	0.15	7.9	0.42	12.86	0.17
ζ-carotene	18.88	0.32	3.6	0.52	*a*	

The values represent the mean of three measurements ($\bar{x}$) and the standard deviations (± s)

a: The component was not detected at the concentration used

Neurosporene and carotenes are the predominant pigments in yellow fruit, whereas lutein was the major xanthophyll. These results are of nutritional importance, since β-carotene is vitamin A precursor, and its amounts have to be determined during the stages of ripening.

3. Conclusion

It could be concluded that a HPLC system with a suitable mobile phase for separation of tomato fruit pigments is a sensitive, reproducible, and rapid analytical method causing minimum changes in the composition of the pigment. The new mobile phase can be used for following the biochemical changes occurring in pigment composition during maturation, storage and technological processing of tomato.

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Acta Alimentaria, Vol. 16 (4), pp. 351—384 (1987)

DECONTAMINATION, INCLUDING PARASITE CONTROL, OF DRIED, CHILLED AND FROZEN FOODS BY IRRADIATION[a]

J. Farkas

Institute of Food Technology, University of Horticulture and Food Industry, Budapest, 1118 Villányi u. 35–43. Hungary

(Received: 19 November 1986; accepted: 12 December 1986)

Extensive literature supports the conclusion that radiation treatment at doses that do not cause unacceptable changes in organoleptic qualities can effectively eliminate potentially pathogenic non-sporing bacteria from red meat, poultry and fishery products under normal commercial conditions for products which are marketed in both fresh and frozen stage. Irradiation of frozen products appears to be more feasible commercially than processing chilled products. A dose of 3 to 5 kGy is sufficient to control potentially-pathogenic, non-sporing bacteria without affecting technical, sensory and nutritional qualities.

Radiation processing at reasonably low doses, less than 1 kGy under specific conditions, appears feasible for control of food-borne parasites. Existing data promise significant reduction of risk of human infections by *Trichinella spiralis*, *Taenia solium (Cysticercus cellulosae* in pork), *Taenia saginata (Cyticercus bovis* in beef), *Toxoplasma gondii* and *Opisthorchis viverrini.* Published data indicate these parasites, are controlled by a dose of 0.15–0.6 kGy making infected foods safe for human consumption.

Radiation decontamination of dry ingredients, herbs and enzyme preparations is a feasible alternative to fumigation with microbicidal gases. Radiation doses of 3 to 10 kGy proved to be sufficient to reduce the viable cell counts to a satisfactory level. The flavour, texture and other important technological and sensory properties of most ingredients are not influenced at radiation doses necessary for satisfactory decontamination.

The microflora surviving irradiation are more sensitive to subsequent food processing treatments than the microflora of unirradiated products. Radiation decontamination can be applied best as a terminal treatment of packaged products, and as such, it results in considerable saving of energy and labour as compared to alternative decontamination techniques. Radiation decontamination is an emerging technology in several countries and more-and-more clearances on radiation decontaminated foods are issued or expected to be granted in the near future.

1. Introduction

1.1. Problems related to food contaminated by biological agents

The report of the Joint FAO/WHO Expert Committee on Food Safety (WHO, 1984) stated "illness due to contaminated food is perhaps the most widespread health problem in the contemporary world and an important cause of reduced economic productivity". The U. S. Public Health Service

[a] Background paper for the Task Force Meeting on the Use of Irradiation to Ensure Hygienic Quality of Food, International Consultative Group on Food Irradiation, Vienna, 14–18 July 1986.

Akadémiai Kiadó, Budapest
D. Reidel, Dordrecht

estimated that as many as 81 million cases of diarrhoea of food-borne origin may occur in the United States each year (ARCHER & KVENBERG, 1985). Numerous chronic sequellae to enteric infections are documented. "Morbidity associated with food hygienic problems affects large portions of populations in industrialized nations and its magnitude and gravity in developing countries seems overwhelming" (YOUNG, 1985).

The Joint FAO/WHO Expert Committee on Food Safety meeting in June 1983 (WHO, 1984) emphasized preventive measures to provide adequate supplies of safe and nutritious food supplies. The committee noted that in 1980 the total impact of food-borne disease related to microbiological and parasitic infectious agents enter the body through the ingestion of food cause a subtantial number (more than 1000 million in children alone) of diarrhoea cases which can also cause malabsorption and thus have a serious affect on nutritive values of foods. Some of these important agents in food-borne diseases are listed in Tables 1/a and 1/b. Microbial and parasitic infections of foods are tremendous problems hampering food industry and trade (FAO, 1983). Food must satisfy health and sanitary regulations, meet established quality standards and otherwise satisfy requirements of buyers. There is a need to adopt and apply measures/methods that will assure maintenance of quality at all stages of production, post-harvest handling, processing, packaging, storage, transportation and distribution of food. The safety of food should be guaranteed at the retail and possibly at the consumer level.

In spite of all past efforts, relatively high percentages of foods of animal origin are contaminated with pathogenic bacteria such as Salmonella, *Campylobacter*, staphylococci, *Clostridium perfringens*, etc., resulting in food infections and food-borne disease/illness in all countries for which there are good statistics available (KAMPELMACHER, 1985).

This is due to the explosive growth in the mass rearing of animals, pollution of the environment, mass production of foods, increasing international trade of food and feeds, mass migration of people both as tourists, guest workers or emigrants, and a build-up of microbiological contamination in the environment. Infected animals which are clinically healthy, but are carriers of pathogens such as Salmonella, are a significant reason that poultry and red meats play a major causative role in human salmonellosis. Especially high contamination rate by *Campylobacter fetus* subsp. *jejuni* observed in poultry and poultry products has recently been recognised as frequent sources of infection by this pathogen (DOYLE, 1981).

Hazards associated with human activities require greater stress on food hygiene. Traveller's diarrhoea is a widespread phenomenon, affecting about 20–25% of nearly 500 million persons per year. Environmental factors and certain ethnic food consumption patterns can also significantly affect the risk of food-borne infections. For instance, halophilic *Vibrio parahaemolyticus* in

Table 1/a

Some agents of important food-borne diseases of animal origin

Bacteria	Reservoir/Carrier	Multiplies in food	Examples of some incriminated foods
Campylobacter jejuni	Poultry, cattle, pigs	sometimes	Raw milk, poultry
Escherichia coli	Man to food	Yes	Raw vegetables, prepared foods
Salmonella typhi	Man to food	Yes	Meat products, shell fish
Salmonella (non-typhi)	Man and animals	Yes	Meat, poultry, eggs, etc.
Shigella	Man to food	Yes	Prepared products
Staphylococcus aureus	Man to food	Yes	Ham, poultry, prepared products
Vibrio sp	Man, animals, marine life	Yes	Shellfish, raw fish
Yersinia enterocolitica	Pigs, poultry	Yer	Milk, pork, poultry

Table 1/b

Some agents of important food-borne diseases of animal origin

Parasites	Resevoir/Carrier	Multiplies in food	Examples of some incriminated foods
Protozoa			
Toxoplazma gondii	Pig	No	Raw and partially processed products
Sarcocystes carnii	Pig	No	Raw and partially processed products
Roundworms			
Trichinella spiralis	Pig	No	Raw and partially processed products
Gnathostoma spinigerum	Fish (pig, poultry)	No	Raw and partially processed products
Anisakis sp.	Marine fish	No	Raw and partially processed products
Flukes			
Opisthorchis sp.	Fish	No	Raw and partially processed products
Fasciopsis buski	Aquatic plants	No	Raw and partially processed products
Tapeworms			
Cysticercosis cellulosae (Taenia solium)	Pig (In man)	No	Raw and partially processed products
Cysticercus bovis (Taenia saginata)	Beef (In man)	No	Raw and partially processed products
Diphyllobothrium latum	Fish	No	Raw and partially processed products

marine foods consumed raw is a significant cause of foodborne diseases in Japan and other countries. Maintaining a healthy nutritional status while minimizing the adverse effects of intercurrent diseases of microbial origin

should be given a high priority in preventive programs. Food processing technologies must continue to be developed to combat these diseases. Of particular interest is low-dose food irradiation for insect disinfestation, destruction of microorganisms and parasites to prevent quantity and quality losses (WHO, 1984).

Results of a recent survey of retail meats sold in the United States revealed that *C. jejuni* or *Campylobacter coli* or both were present in 3.6% of ground beef, 4.7% of beef flank, 8.1% of lamb stew, 4.2% of pork sausages, 5.0% of pork chop, and 29.7% of chicken samples (STERN et al., 1985). In the Netherlands in an earlier comprehensive survey, 13% to 74% of samples of frozen poultry (VAN SCHOTHORST et al., 1976) and 9% to 37% of unpasteurized egg products were positive for Salmonella (VAN SCHOTHORST and VAN LEUSDEN, 1978) These raw foods are the indirect sources of food-borne infections occuring during culinary preparation of foods, causing cross-contamination of surfaces, kitchen utensils and human hands. Other foods consumed raw or insufficiently heated, such as minced meat and raw sausages, etc. are direct sources of infection.

Parasitism of humans and livestock persists in some degree in all parts of the world, despite the measures already taken to control it. Foods of animal origin play a major role in infections caused by helmiths and parasitic protozoa, for example, trichinellosis and toxoplasmosis transmitted by meat and meat products. The major species of parasites in human food of animal origin are shown in Table 1/b.

Food-borne illnesses are usually under-reported and underrated as a human health problem because the effects of enteritis, though sometimes acute, are usually self-limiting and rarely fatal. However, food-borne infections can cause severe illness in the very young, the elderly and the debilitated (ARCHER & KVENBERG, 1985). Consequent economic and social effects related to increased medical costs, loss of productivity, loss of business and possible legal action can be extremely high. According to ROBERTS (1985), it is conservatively estimated that medical costs and productivity losses attributed to trichinellosis, toxoplasmosis, salmonellosis, campylobacteriosis and *T. saginata* taeniasis total roughly 10^9 dollars annually in the USA. This estimation excludes the impact of outbreaks on the food processors and food service establishments which may be equal or exceed in economic terms, the direct human illness costs (LINTON, 1983; TODD, 1985a, b, c).

Many dry food ingredients, particularly spices and herbs, the major portion of which are produced in developing countries and are an important source of their foreign exchange earnings, are frequently highly contaminated thus causing difficulties during further processing and incorporation into intermediate and high-moisture products, e.g. meat products. Contamination of dried spices and other vegetable products are usually spoilage organisms,

but frequently pathogenic microorganisms are also found and such contamination has led to infection in man.

Improved control of microbiological and parasitic contamination of food can help reduce diarrhoea and other mainly acute and chronic food-borne diseases. The recognized safety and freedom from additives creates an opportunity for implementation of radiation processing and good manufacturing practices can lead to improved public health.

1.2. General considerations on radiation processing of critical food products to improve hygiene

While the primary responsibility for food safety lies with those who handle and prepare food for consumption, and public education is probably the single most important measure to prevent food-borne diseases and unnecessary food losses (LINTON, 1983), the importance of processing critical food products for safety cannot be overemphasized (MOSSEL, 1984a; MAXCY, 1982). Of course, control of potential contamination of foods must not create hazards for public health, nor alter the acceptability of the food for the consumer.

After decades of research efforts, irradiation of food is attracting attention as an effective mode of improving food safety (MOSSEL & STEGEMAN, 1985). The appropriate use of ionizing radiation may safely minimize the load of contamination without causing undesirable alteration in composition or acceptability of products.

This paper deals with food items which rate high on the list of commodities where irradiation can be used in order to reduce microbial load, minimize the presence of pathogenic microorganisms, and to control parasite infestation. Radiation can inactivate organisms in foods that are in hermetically sealed packages or in the frozen state without thawing. Freezing reduces the available water and tends to increase the radiation dose required to reduce the number of microorganisms. However, organoleptic quality of high moisture foods can be protected by irradiation in the frozen state, e.g. shelled pooled eggs.

Increasing awareness of the safety and acceptability of food irradiation as a tool for alleviating food supply problems, by controlling food contamination and post-harvest losses, is mainly due to research results compiled and evaluated by national and international scientific bodies.

The Joint FAO/IAEA/WHO expert Committee on Wholesomeness of Irradiated Food (JECFI, 1981) concluded that irradiation of any food commodity up to an overall average dose of 10 kGy dose presents no toxicological hazard nor introduces special nutritional or microbiological problems. (Note: Concerning microbiological and nutritional acceptability of irradiated food, the Committee emphasized that attention should

be given to the significance of any changes to each particular food and its role in the diet.)

The Codex General Standard for Irradiated Foods and the *Recommended International Code of Practice for the Operation of Irradiation Facilities used for the Treatment of Foods* were adopted by the Codex Alimentarius Commission in July 1983, and were subsequently circulated to all member countries of the Codex Alimentarius Commission for acceptance.

This international recognition of safety and benefit should facilitate implementation of radiation decontamination of dried, chilled and frozen foods. The processing method should have immediate potential for application in many countries.

The number of clearances at national levels, except on spices, is still very limited, partly because public health authorities are concerned that allowing radiation processing treatment may lead to the use of such processing to mask hygienic failures and might lead to relaxation of hygienic measures. However, as it was pointed out by MOSSEL (1984b), this fear is not at all substantiated by almost a century of experience in the dairy industry using a terminal microbiological control treatment by heat pasteurization of milk. Radiation would be an adjunct to and not a substitute for good hygienic, sanitary and manufacturing practices.

1.3. Dose limitations related to quality changes and costs

Since maintaining organoleptic and nutritional quality and keeping costs down are important factors, it is desirable to use the lowest possible doses necessary to achieve desired levels of microbiological or parasite control on a commercial scale. It is important, therefore, to establish the efficacy of the radiation treatment and threshold doses for quality changes. Actually, the exact dose required for each individual application should be established by risk analysis, taking into consideration the contamination level, the hazard involved, the efficacy of the radiation treatment and the fate of critical organisms during manufacturing, storage, distribution and culinary preparations of foods (MOSSEL & DRION, 1979).

Threshold doses at which detectable irradiated flavours occur in red meats and chicken are shown in Table 2 (SUDARMADJI & URBAIN, 1972). The raw meats were irradiated at 5 to 10 °C and the flavour was determined after cooking the meats. Too high a dose of irradiation will also discolour meat. Beef is most sensitive in this regards. Discolouration of phosphate-treated, vacuum-packaged, refrigeration stored beef occurs at about 4 kGy (URBAIN, 1973).

A reduction of salmonellae numbers by a factor of 10^7 attempted in the early stage of food irradiation research usually requires doses which exceed

Table 2

Threshold doses for some foods of animal origin for an organoleptically detectable "off-flavour"
(SUDARMADJI & URBAIN, 1972)

Food[a]	Threshold dose (kGy)
Turkey	1.50
Pork	1.75
Beef	2.5
Chicken	2.5
Shrimp	2.5
Frog	4.0
Lamb	6.25
Horse	6.50

[a] Irradiated at 5° to 10 °C

the threshold levels for development of irradiated flavours in high moisture fresh foods when the irradiation is carried out above 0 °C. Irradation in frozen state increases the threshold doses before off-flavours develop. Therefore irradiation in the frozen state allows use of radiation dose levels in excess of those indicated in Table 2. For example, LICCIARDELLO and co-workers (1968) suggested a dose of 4.75 kGy for treatment of poultry meat to obtain a 7-log cycle reduction. This level of security may be excessive, when it is noted that chemical or physical treatments used as alternatives to ionizing radiation are usually considered successful when they achieve 2–3 log cycles reduction of Salmonella (MORRISON & FLEET, 1985).

2. Radiation decontamination of chilled and frozen food of animal origin

In case of food of animal origin, the pathogens of greatest concern, and the ones which have been given the greatest attention for control by irradiation, are the salmonellae which are present in many foods.

2.1. Reduction of pathogenic microorganisms in poultry and meat

Poultry. Salmonellosis is frequently associated with consumption of poultry products (BRYAN, 1981). Poultry are mass-produced in many countries and high infection rates with pathogens are reported nearly all over the world. Many surveys in different countries have shown that 30 to 50% of frozen and refrigerated chicken carcasses are contaminated with Salmonella (SILLIKER, 1982). However the actual number of Salmonella colony forming units (CFU) per carcass are quite low and generally range between 5 and 1000 CFU. In Dutch studies CFU-s of Salmonella expressed per 100 g of skin of slaughtered

Table 3

Radiation resistance of some pathogenic non-sporing bacteria in meat and poultry

Pathogens/Serotypes	Product	D_{10} values (kGy)	Reference
Campylobacter fetus subsp. jejuni	"filet americain"	0.08—0.16	KAMPELMACHER, 1983
Escherichia coli	poultry, chilled	0.30—0.37	KRAMOMTONG & EL FOULY, 1980; EL FOULY, 1983
Escherichia coli	poultry, frozen	0.55	KRAMOMTONG & EL FOULY, 1980
Salmonella anatum	minced beef	0.35	TANASUGARN, 1968
Salmonella anatum	"filet americain" with mayonnaise sauce	0.50	KAMPELMACHER, 1983
Salmonella anatum	horse meat, frozen	1.0	LEY, 1968
Salmonella choleraesuis	ground pork	0.68	SZCZAWINSKA, 1983
Salmonella derby	minced beef	0.31	TANASUGARN, 1968
Salmonella dublin	ground pork	0.77	SZCZAWINSKA, 1973
Salmonella gallinarum	ground pork	0.42	SZCZAWINSKA, 1983
Salmonella good	horse meat, frozen	0.7	LEY, 1968
Salmonella manchester	minced beef	0.35	TANASUGARN, 1968
Salmonella meleagridis	minced beef	0.38	TANASUGARN, 1968
Salmonella meleagridis	horse meat, frozen	0.9	LEY et al., 1963
Salmonella minnesota	horse meat, frozen	0.6	LEY 1968
Salmonella newport	minced beef	0.50	TANASUGARN, 1968
Salmonella niloese	poultry, chilled	0.55—0.68	KRAMOMTONG & EL FOULY, 1980
Salmonella niloese	poultry, frozen	1.25	KRAMONTONG & EL FOULY, 1980
Salmonella oranienburg	horse meat, frozen	0.9	LEY, 1968
Salmonella paratyphi B	minced beef	0.27	TANASUGARN, 1968
	horse meat, frozen	1.1	LEY et al., 1963
Salmonella saintpaul	minced beef	0.50	TANASUGARN, 1968
Salmonella senftenberg	horse meat, frozen	0.9	LEY, 1968
Salmonella stanley	minced beef	0.35	TANASUGARN, 1968
Salmonella typhimurium 266/78	ground pork	0.57	SZCZAWINSKA, 1983
Salmonella typhimurium 77	ground pork	0.78	SZCZAWINSKA, 1983
Salmonella typhymurium	"filet americain" with mayonnaise sauce	0.71	KAMPELMACHER, 1983
Salmonella typhymurium	horse meat, frozen	1.0—1.3	LEY et al., 1963 LEY, 1968
Salmonella virchow	minced beef	0.40	TANASUGARN, 1968
Salmonella weltevreden	minced beef	0.45	TANASUGARN, 1968
Staphylococcus aureus	poultry, chilled	0.34	EL FOULY, 1983
Streptococcus faecalis	poultry, chilled	0.95	EL FOULY, 1983
Streptococcus faecalis	poultry, chilled	0.69	KRAMOMTONG & EL FOULY, 1980
Streptococcus faecalis	poultry frozen	1.22	KRAMOMTONG & EL FOULY, 1980
Yersinia enterocolitica 0.3	"filet americain" with mayonnaise sauce	0.043	KAMPELMACHER, 1983
Yersinia enterocolitica 0.3	minced meat	0.10	KAMPELMACHER, 1983
Yerisinia enterocolitica 0.5,27	"filet americain" with mayonnaise sauce	0.065	KAMPELMACHER, 1983
Yersinia enterocolitica 0.5,27	minced meat	0.16	KAMPELMACHER, 1983
Yersinia enterocolitica 0.9	minced meat	0.21	KAMPELMACHER, 1983

Table 4

Effect of irradiation on the microbial quality of frozen chicken
(PRACHASITTHISAKDI et al., 1984)

Organism	$\log_{10}$ CFU g^{-1}				
	0 kGy	1 kGy	2 kGy	3 kGy	4 kGy
Mesophilic colony count	6.8	5.8	4.6	4.1	3.6
Physychrotophic colony count	5.8	5.7	4.0	<2.8	<1.8
Enterobacteriaceae	5.5	<2.8	1.0	0.4	−0.4
Lactobacillus	6.0	4.1	4.2	3.1	<2.8
Lancefield D streptococci	5.1	3.7	3.9	3.2	>2.0
Staph. aureus	4.6	2.2	<−0.5	<−0.5	<−0.5

chicken or 1500 cm^3 of drip water were found to vary between 2 and 1400 CFU with 90% of the counts being between 5 and 60 CFU (KAMPELMACHER, 1985). Most commercially raised poultry have *Campylobacter* in their inestinal flora. In the USA, poultry is estimated to be the cause of half of the campylobacteriosis cases and the direct cause of 9.5 per cent of the salmonellosis cases (ROBERTS, 1985).

Many approaches have been adopted to reduce this incidence of contamination. These include measures aimed at controlling dissemination of pathogenic bacteria by applying microbiological controls at the stages poultry breeding, farming, processing and post-processing (TODD, 1980). Control of potential microbiological pathogens in the end-product is most attractive since it is applied just before retail distribution of the carcasses and the numbers of other potentially pathogenic and spoilage bacteria are also reduced.

Alternative sanitizing procedures in poultry processing operations such as in-plant chlorination, surface heating or spray application of lactic acid on carcasses may be helpful in reducing the contamination pressure, but are limited by either regulatory limitations or by consumer acceptability. Thus irradiation of poultry has a high priority among candidates for radiation disinfection (KAMPELMACHER, 1981). Typical published data on radiation resistance of Salmonella and other pathogens in meat and poultry are given in Table 3.

Campylobacter and *Yersinia* have low resistance to irradiation, *Escherichia coli* also seems to be quite susceptible, while Salmonella serotypes vary in their radiation sensitivity. Hence *E. coli* is unlikely to be useful as an "indicator" organism for Salmanella in irradiated foods. It can be noted from Table 4 that irradiation in the frozen state increases the radiation dose required for control of vegetative bacteria by about two-fold.

Pre-packaging of poultry after slaughter and subsequent irradiation with a dose of 2–5 kGy have been effective in reduction of the most resistant serotype of Salmonella by about 3-log cycles and *Campylobacter* by a still

greater rate whether the birds had been chilled or deep-frozen. MULDER (1982) reported 93 per cent reduction in Salmonella contamination of chicken carcasses as the result of irradiation at 2.5 kGy level. Other organisms, e.g. staphylococci and the vegetative form of *Clostridium perfringens* are also effectively eliminated at this dose level. The effect of irradiation on the microbial quality of frozen chicken is shown in Table 4 (PRACHASITTHISAKDI et al., 1984).

Deep freezing has the additional advantage that it not only reduces to some degree the viable counts of Salmonella, but also increases the threshold dose level for sensory changes. Sensory evaluations in Hungary showed that irradiation of frozen chicken with 3 to 5 kGy had no effect on the culinary properties of various dishes prepared from chicken (KISS, 1984). A process under implementation in France is the electron beam processing of frozen blocks of mechanically separated poultry meat to reduce the risk of salmonellosis (GALLIEN et al., 1985). A treatment with 4–5 kGy average absorbed dose appears to be adequate for this purpose.

Frozen meat. This important commodity in international and domestic trade is frequently found to be contaminated with salmonellae and similar organisms. Drip water released upon thawing of frozen meats is a frequent source of cross-contamination in food preparation. In the USA, 31 per cent of salmonellosis outbreaks reported to the Centers for Disease Control in 1981 were caused by beef (CDC, 1983). Favourable results with the radiation decontamination of frozen meats have been reported and the process was first considered for products to be used as pet food (LEY et al., 1963; NEAL, 1965). A 10^5-fold inactivation of *S. typhimurium* in frozen horse meat could be achieved with 6.5 kGy. Radiation decontamination of frozen meats and game may be of particular interest to countries producing and exporting large amount of such products (MOSSEL & DE GROOT, 1965).

Low-dose irradiation of certain pre-packaged meat products such as ground beef, minced meat, hamburgers may help in controlling meat-borne pathogens and parasites. Pathogenic microorganisms and parasites in meat products which are commonly consumed raw, e.g. "filet Americaine", or semi-cooked are of particular importance. Experiments in the Netherlands showed that doses as low as 1 kGy were effective in reducing Salmonella up to approx. 2-log cycles in such products (KAMPELMACHER, 1984). *C. jejuni* and *Y. enterocolitica* are even reduced by more than 4-log cycles with this dose.

2.2. Reduction of potentially pathogenic microorganisms in fishery products

Fin fish and shell fish can be contaminated with certain nonsporing pathogenic bacteria and virus. This can occur when fishery products are taken from polluted waters or as a result of post-harvest contamination.

Crustaceans and moluscs as well as frog-legs, originating from polluted aquatic environments, frequently harbour pathogenic microorganisms and pose publich health hazard as shown by a number of disease outbreaks in recent years with high number of persons sickened and with relative high number of death cases (KAMPELMACHER, 1985). Irradiation to control pathogens has been found very useful with frozen fishery products and considerable amounts of frozen shrimps, prawns and frog-legs are irradiated commercially in the Netherlands and Belgium with doses up to 4 kGy.

It was found in studies at International Facility for Food Irradiation Technology (IFFIT) that a radiation dose of 4 kGy resulted in 3-log cycles reduction of the aerobic psychrotrophic and mesophilic colony counts of Malaysian shrimps (PRACHASITTHISAKDI et al., 1984). In addition, *Enterobacteriaceae,* Lancefield D *streptococci* and *Staph. aureus* could not be detected in 1 g samples, after application of doses between 2 and 4 kGy.

Regarding frog-legs, Indonesian studies (TAMBUNAN, 1985) demonstrated that a combination of washing in chlorinated water, freezing and irradiation at a dose between 3 and 6 kGy eliminated Salmonella from the product. D_{10} values of Salmonella serotypes in artificially contaminated shrimp were found to range from 0.3 to 0.5 kGy for refrigerated samples and from 0.4 to 0.6 kGy for frozen samples (NOUCHPRAMOUL, 1985).

Vibrio parahaemolyticus has been considered the leading causative agent of bacterial gastroenteritis in Japan from eating fish. Sixty per cent of fresh shrimps obtained from local markets in Bombay, India, was contaminated with this organism (LEWIS, 1983). NOUCHPRAMOUL (1985) detected *V. parahaemolyticus* in 85% of fresh or frozen shrimp in Thailand. This organism is quite radiosensitive. Various strains inoculated into crabmeat and irradiated at a dose of 0.25 kGy caused a 2 to 5 log cycle reduction in numbers (MATCHES & LISTON, 1971). Lewis, 1985 reported D_{10} values of 0.04–0.05 kGy in shrimp homogenates. Therefore, a dose as low as 1 kGy is sufficient to eliminate *V. parahaemolyticus* in frozen seafoods.

Similarly, Shigella, identified as a causative agent isolated from a shrimp cocktail in a December, 1983, epidemic which resulted in 14 death in the Netherlands was found to be more radiation sensitive in frozen precooked peeled shrimp than Salmonella (KAYSER & MOSSEL, 1984). WONGCHINDA and co-workers (1985b) found that a dose of 2.5 kGy reduced the number of survivers of four Shigella serotypes by more than 6-log cycles in inoculated pack studies.

Acceptability. Variable results are found in the literature on the effect of irradiation on crustaceans, probably because of variable experimental conditions and because sensory quality as compared to that of untreated control may depend very much on the time elapsed between irradiation and sensory testing. For example, RHODES (1964) reported that raw prawn,

treated with 3 kGy of ionizing radiation, had a slight "irradiation" odour but was normal in appearance while COLEBY & SHEWAN (1965) stated that the maximum permissible dose of ionizing radiation for raw shrimps is approx. 9 kGy. After a shipping test, fresh unshelled shrimps treated with 2 kGy of ionizing radiation were judged to be slightly better in consumer-type testing than unirradiated samples (NOVAK et al., 1968). In experiments in Thailand, gamma radiation with 2.2 kGy did not significantly ($P \leq 0.05\%$) affect the colour, flavour and texture, but had an effect ($P \leq 0.05\%$) on odour. A difference in acceptance of irradiated shrimp was noted among consumers at various localities (NOUCHPRAMOUL, 1985).

2.3. Elimination of enteropathogenic bacteria from egg products

Table 5 lists the doses for a 7-log reduction of salmonellae in various egg products (THORNLEY, 1963). In the determination of the listed doses in most cases the resistant serotypes *S. typhimurium* and *S. senftenberg* were employed.

Here again changes of sensory and functional properties limit the applicable doses. One direction of research to overcome this problem was the reduction of dose requirements for disinfection by means of combination treatments. LICCIARDELLO (1964) has found a synergistic effect of simultaneous application of thermal and ionizing energy on destruction of salmonellae in egg magma, i.e. reduced D_{10} values of *S. typhimurium* to 0.25–0.35 kGy at elevated (40–50°) temperatures, still below the thermal denaturation level.

The other direction in minimizing the undesirable effects of radiation is radiation treatment of frozen egg products. Irradiation of 4–5 kGy does not impair the quality of frozen whole egg or of foods prepared with the irradiated egg product (LEY et al., 1962; KISS, 1985). The same dose resulted in 6-log

Table 5

Irradiation doses for the inactivation of salmonellae in various egg products to obtain a 10^7 reduction of count
(THORNLEY, 1963)

Product		Dose (kGy)
Whole egg	(liquid)	2.0—4.42
	(fozen)	4.76—5.40
	(dried)	3.70
Egg yolk	(frozen)	3.20
	(dried)	5.70
Egg white	(liquid)	2.60
	(fozen)	2.12
	(dried)	5.85
Sugared egg white (dried)		8.40

cycles reduction of the mesophilic aerobic counts and reduced coliforms and *S. aureus* below the detectable level (KISS, 1985). A pasteurizing dose of 5 kGy gives a 7 to 8-log reduction in the most radiation resistant Salmonella tested in frozen whole eggs (see Table 5).

Although heat pasteurizing process of whole eggs (liquid) and egg yolk was established well in the last decades, for bulk frozen eggs and particularly for pasteurizing egg albumen, radiation disinfection seems to be well suited (NEAL, 1965).

2.4. Effect of radicidation on the microflora in foods of animal origin

Investigations with frozen chicken and frozen shrimps have shown that the percentage of Gram-positive cocci increased whereas Gram-negative rods decreased in the microflora surviving irradiation of 2 and 4 kGy (PRACHASITTHISAKDI et al., 1984). Storage studies with untreated or irradiated samples of frozen precooked shrimps (WONGCHINDA et al., 1985a) have shown that at 12 °C the spoilage flora developed in control shrimps consisted mainly of psychrotrophic and mesophilic *Moraxella* spp. and the coryneform group. (Note: Shrimp will be spoiled and inedible within 24 hours to 36 hours if held at 12 °C or higher). At 21 °C, they were followed by *Acinetobacter* as the most prevalent organisms. After irradiation at 4 kGy, *Moraxella* spp. at 12 °C storage irradiation at 4 kGy, *Moraxella* spp. at 12 °C storage, and *Moraxella* plus *Acinetobacter* spp. at 21° storage were predominant in the spoilage flora.

These observations together with earlier findings (WELCH & MAXCY, 1975, 1979) corroborate that *Moraxella-Acinetobacter* spp. and catalase positive cocci are the predominant organisms in food of animal origin irradiated at radicidation doses, and support the view that irradiation does not present a hazard resulting from immediate flora changes or shifts in the microbial community structure secondary to temperature abuse (MOSSEL & STEGEMAN, 1985).

3. Parasite disinfection in foods of animal origin

Although insufficient research work has been done on this significant public health problem (ANON., 1985), it appears that irradiation of carcasses can be used under specific conditions as an effective alternative for preventing diseases caused by some meat-borne parasites such as cysticerci, trichinella larvae and toxoplasma cysts. As for trichinellosis, an average of 152 human cases were reported annually from 1975 to 1981 in the USA, and pork and "ground beef" contaminated with pork were responsible for roughly 86 per cent of the reported cases (SCHANTZ, 1983). In addition to other types of toxoplasmosis syndromes observed in adults, an estimated 3300 babies born every

year in the USA with congenital toxoplasmosis arising from pregnant women consuming undercooked infected pork (ROBERTS, 1985).

Radiation effects on parasitic protozoa and helminths are associated with loss of infectivity, loss of pathogenicity, interruption or prevention of completion of life cycle, and death of the parasite. Relatively high doses (4–6 kGy) are required to kill outright food-borne parasites. Objectionable sensory changes would be induced at these dose levels in raw foods which carry the parasites (URBAIN, 1978). However, much lower doses are adequate to prevent reproduction and maturation resulting in loss of infectivity. Therefore, the majority of authors consider that doses below 1 kGy are sufficient to prevent infections from food-borne parasites. Such low doses do not alter the flavour or texture of the meat (GIBBS et al., 1964). (See also Table 2.)

Gamma irradiation of *Trichinella spiralis*-infected pork with a dose of 0.15 to 0.30 kGy made the parasite sexually sterile and blocked the maturation of ingested larvae in the host gut (GIBBS et al., 1961; 1964; BRAKE et al., 1985; SIVINSKI, 1985a, b). Neither the age of the encysted muscle, nor the oxygen tension in the meat, significantly affected the radiosensitivity. The data indicate that a radiation dose of 0.3 kGy can provide a substantial margin of safety for human consumption of infested meat. The US FDA consequently approved the use of irradiation to control *Trichinella spiralis* in pork at a minimum absorbed dose of 0.3 kGy and not to exceed 1.0 kGy on 16 July 1985 (FDA, 1985).

Feasibility studies of pork irradiation in commercial operations have shown the process to be technically, and economically feasible in the USA (SIVINSKI, 1985b). Similarly, irradiation at 0.3–0.5 kGy kills *Toxoplasm gondii* (KING & JOSEPHSON, 1983; DUBEY et al., 1985) and 0.4 kGy irradiation of beef contaminated with *Cysticercus bovis (Taenia saginata* in man) would prevent development of the parasite in a human host (KING & JOSEPHSON, 1983). A combined treatment of 3.0 kGy with a minimum storage of 7 days at 2° post-irradiation is fatal to cysts in beef (VAN KOOIJ & ROBIJN, 1968). VERSTER and co-workers (1977) suggest that pork carcasses infested with *Cysticercus cellulosae (Taenia solium* in man) might be fit for human consumption after irradiation with doses of 0.2–0.6 kGy.

Although doses that would kill the larvae of the parasitic nematode *Anisakis* in salted herring were reported to be high (higher than 6–10 kGy, VAN MAMEREN & HOUWING, 1968), further experiments should be undertaken to determine what sublethal dose might render the larvae noninfectious or nonpathogenic. As far as organoleptic changes are concerned, inconsistent results are published in the literature on herring. RHODES (1964) reported that irradiation treatment up to 10 kGy level was found to have no effect on the appearance or odour of herring vacuum packed in oxygen-impermeable wraps. COLEBY and SHEWAN (1965) state that the maximum permissible dose

of ionizing radiation for kippered herring (lightly salted and lightly smoked) is approx. 9 kGy. Bismarck herring treated with ionizing radiation at levels above 0.75 kGy was found by WITTFAGEL (1965) to have an off-flavour.

Investigations in Thailand demonstrated that low dose irradiation of freshwater fish can prevent infectivity of metacercariae of liver fluke *(Opisthorchis viverrini)* when such fish are prepared into popular local dishes made from raw or semi-processed fish (BHAIBULAYA, 1985). At 0.5 kGy, the metacercariae could not develop in hamsters and caused no infection in their livers.

4. Radiation decontamination of dry ingredients

Under the prevailing production and handling practices many dry commodities of agriculture and food industry such as spices, dried vegetable seasonings, herbs, dried vegetables, texturizing agents, enzyme preparations, etc. may contain large number of micro-organisms which may cause spoilage, defectiveness in foods, or, more rarely disease. For example, examination of 357 specimens of 47 different dried food products in Germany in the period 1980–1983 revealed a Salmonella contamination in 29 samples (8.1%) (BOCKEMÜHL & WOHLERS, 1984). The destruction of thermoduric bacterial spores introduced to the food by spice or flavour ingredients often requires a severe heat treatment which ensure the microbiological stability only at the cost of substantial reduction of nutritional and sensory quality of the manufactured product. Thus, a major concern of food processors is to assure themselves that the microbial load of ingredients and processing aids does not contribute to spoilage of food and does not diminish its microbial safety.

Practical control of microbial contamination of food products by organisms from ingredients would lie logically in the selection of materials relatively free from significant microorganisms. The work involved in carrying out a routine microbiological examination of all ingredients makes this selection, particularly, because of the heterogeneous distribution of microbiological contamination, an unrealistic requirement. Some ingredients may inherently carry troublesome microorganisms, in spite of all efforts to improve their production conditions. All these problems account for attempts to reduce the viable cell counts of these ingredients by some sterilization treatment methods. In most of the cases, it is unnecessary to achieve full sterility, only a proper reduction of the viable cell count is needed.

Fumigation. Until recently, fumigation with volatile microbicide compounds, particularly with ethylene oxide (EtO) has been widely used for disinfection and disinfestation of ingredients and various dried foods. However, there is a growing opposition to this fumigation, due to the mounting evidence gathered on health-related problems involved in its use.

Table 6

Major dry commodities for which radiation decontamination is a technically feasible alternative to fumigation

Cereal products (flours, malt, muesli like products)
Dehydrated vegetables
Dehydrated vegetable seasonings
Dried egg products
Dried fruits
Gelatin
Gum arabic, tragacanth and agar-agar
Herbal teas and dried medicinal plants
Industrial enzyme preparations
Pectin
Protein preparations
Spices and herbs
Starches
Sugar
Talc

Ethylene oxide and some of its more persistent interaction products with food, particuarly ethylene-halohydrins proved to be mutagens, and they are suspected causing other chronic and delayed toxic effects. Due to this, fumigation represents a significant occupational health hazard for workers in fumigation plants while the presence of residues and reaction products in fumigated commodities gives rise to more-and-more toxicological misgivings (HOGSTEDT et al., 1979; EHRENBERG & HUSSAIN, 1981).

The mounting concern in recent years has restricted the permitted uses of fumigants, and more restrictive regulatory controls are coming into power (NEUMAYR et al., 1983, OSHA, 1984). Therefore, it seems highly desirable and timely to introduce an appropriate physical method for disinfection.

Irradiation is a challenging alternative to fumigation. Table 6 lists groups of dry commodities for which radiation disinfection has been studied and found technically feasible. The largest amount of research work has been devoted so far to irradiation of spices, herbs and dry vegetable seasonings.

4.1. *Spices, herbs and dehydrated vegetable seasonings*

These commodities contain microorganisms which are indigeneous to the soil and plants in which they are grown, and that survive the drying process. The source of contamination may be dust, insects, faecal material from birds and rodents, and possibly the water used in some processes. The viable cell counts of spice samples of various origin might show deviations of several log cycles.

Black pepper, turmeric, paprika, allspice and marjoram are the spices most highly contaminated with bacteria (ICMSF, 1980). Aerobic bacterial spores may frequently form more than 50% of the mesophilic total viable cell count.

POWERS and co-workers (1976) found *Bacillus cereus* in 53% of the spices. Although its viable count is usually under 10^4 per gram in extreme cases *B. cecereus* up to 10^5 per gram were also found (BAXTER & HOLZAPFEL, 1982). DE BOER and BOOT (1983) found recently *Clostridium perfrigens* in 80% of 54 different kind of spices. Since spores of these organisms may survive cooking temperature and will grow in foods at temperatures between 20 to 50 °C, spices harbouring these spores must be considered as a potential health hazard.

Spices may not be suitable substrates for the growth or long survival of salmonellae, nevertheless, occasional Salmonella contamination is a reality (BOCKEMÜHL & WOHLERS, 1984). Indeed, black pepper and white pepper have both been implicated as sources of *Salmonella* epidemics in Canada and Norway (DAIDLEY et al., 1974; WHO, 1982).

White pepper, black pepper, chilli and coriander seems to be most heavily contaminated with molds (ICMSF, 1980). Relatively high incidence of toxigenic molds has been detected and aflatoxins have been found in a range of spices although the levels of aflatoxin recorded were generally low (SHANK et al., 1972; FLANNIGAN & HUI, 1976).

Depending on the number and types of microorganisms present, and the chemical composition of the spice, radiation doses of 3 to 10 kGy can reduce the total aerobic viable cell counts below a level of 10^3 to 10^4 g^{-1}, generally required and accepted as a maximal count of decontaminated spices demanded in the spice trade (FARKAS, 1986). This dose range is approximately equal in microbicidal effect to the commercially established fumigation process. A dose of 4–5 kGy can eliminate molds at least as efficiently as ethylene oxide treatme nt.

Thermophilic bacteria of great importance to the canning industry, can be practically eliminated with the same radiation dose as those necessary to a sufficient reduction of the total aerobic viable counts. Bacteria of the Enterobacteriaceae family are relatively radiation sensitive even in dry ingredients, and in most cases a dose of approx. 5 kGy seems to be sufficient for their elimination (ZEHNDER & ETTEL, 1982).

Flavour change in spices, herbs, etc. No substantial changes were found in the volatile oil content in most spices when they were treated with doses up to 10–15 kGy. Canadian and Hungarian authors found less damage to the volatile oil content of allspice and black pepper disinfested by gamma irradiation than in the same spices treated with EtO (VAJDI & PEREIRA, 1973; FARKAS & ANDRÁSSY, 1985). Similarly, non-volatile oil content of these spices plus celery seeds, oregano, garlic and colour of paprika were more affected by ethylene oxide than by gamma irradiation.

Several authors have carried out detailed gas-chromatographic studies on the quantitative and qualitative composition of volatile oils from numerous

Table 7

Dose requirements for radiation decontamination of spices, herbs and dry vegetable seasonings as compared to the retention of their volatile oil content, and threshold doses of organoleptic changes (FARKAS, 1986)

Product	Dose requirement (kGy)	Relative yield of volatile oils[a] at 8–10 kGy	Threshold dose of organoleptic changes (kGy)
Allspice	≤ 6	97–100	~ 15
Anise	≤ 5	100	
Basil	4–10	99	~ 12.5
Bay	< 5		
Caraway	≤ 7.5	88–111	
Cardamom	< 5	81	~ 7.5
Cayenne	5–6		~ 10
Celery leaves	4	97–100	
Celery seeds	6		> 10
Charlock	5		~ 10
Chive	4–8		4–8
Cinnamon	4–5	97	> 10
Clove	< 5	98	< 20
Coriander	≤ 5	98–120	< 5 but also < 16
Cumin	≤ 4	100–105	6–10
Curry	6–8		> 10
Dilltips	10		> 10
Fenuel	8	98–123	> 15
Fenugreek	4–10		< 5 but also > 10
Garlic powder	5–7.5		3–4.5 but also > 16
Ginger	5–6	66–88	> 10
Juniper	7.5	71–82	> 15
Mace	< 5	95	
Marjoram	7.5–10	100–103	5–10 but also > 16
Mustard seed	< 5	100–103	> 10
Nutmeg	4–5	87–100	> 10
Onion powder	4–10		< 10 (optical index) 8–16 (flavour)
Oregano	≤ 4	99–100	> 10
Paprika	4–8		8–10 but also > 16
Parsley	5–8		
Pepper, black	5–10	67–112	≤ 10 but also > 16
white	5–8	70–102	> 10
Red pepper	≤ 5		≥ 10
Sage	4		10
Savory	< 5		
Thyme	5–7.5	101	≥ 10
Turmeric	5–8		5–10 but also ≥ 10

[a]As percentage of the yield of untreated sample

irradiated spices, which show that the percentage distribution of components of volatile oils remained almost unchanged, nevertheless, slight quantitative changes may occur in some cases (BACHMAN & GIESZCYNSKA, 1973; BACHMAN et al., 1978). Also from many studies on other chemical constituents of spices it can be concluded that dry products such as spices are less affected chemically by irradiation than items of high moisture content. The small changes

occurring in some cases in the chemical composition of spices at dose levels sufficient for radiation disinfection seem to have no practical significance or speak in favour of the radiation treatment. Antioxidant properties of spices remained unaltered by the radiation disinfection treatment (KURUPPU et al., 1985).

Although "sterilizing" doses of 15–20 kGy may slightly or noticeably change the flavour characteristics of some spices, the 3–10 kGy doses sufficient to decontaminate do not influence the sensory properties of an overwhelming majority of spices and herbs. Recent comparative flavour-profile studies with highly diluted samples of selected spices and dry vegetable seasonings (black pepper, paprika, onion powder and garlic powder) showed less change in the flavour profile of radiation decontaminated than in fumigated samples (FARKAS & ANDRÁSSY, 1985). It should be noted that even in cases when the sensitive methods of sensory panels may detect statistically validated difference between untreated and irradiated spices, the spicing power is usually either than influenced or not changed to an extent which will influence the applicability of irradiated spices in the food industry (HANSEN, 1966; EISS, 1984; FARKAS, 1986).

On the basis of a large number of references, Table 7 summarizes the dose requirements for radiation disinfection of various spices, herbs and dry vegetable seasoning, as compared to the retention of volatile oils and threshold doses of organoleptic changes.

Herbal teas and dry medicinal plants have similar feasibility for radiation disinfection as spices and condiments (FARKAS, 1986; GRÜNEWALD, 1984; KATUSIN-RAZEM et al., 1985; SAINT-LEBE et al., 1985).

4.2. Dehydrated vegetables, dry mixes and cereal products

Dried vegetables are rarely involved in food-borne illnesses. However, spores of *B. cereus*, *C. botulinum* and *C. perfringens*, if present in the soil, are likely to carry through into the final dried product and may become harmful if permitted to grow on reconstitution of such vegetables. *B. cereus* is a frequent contaminant in dried vegetables and its occurrence in dry potatoes or onion products needs particular attention (MICHELS, 1978). Notably high incidences of Salmonella contamination have been found recently among samples of various dried vegetables (BOCKEMÜHL & WOHLERS, 1984).

A dose range of 4 to 10 kGy seems to be sufficient to reduce the aerobic plate count to the 10^3–10^4 per gram level, usually without changing the flavour qualities (FARKAS, 1986). In asparagus powder, some darkening and "burned" taste and flavour were noted at doses of 8 and 10 kGy, irradiation of mushroom powder and dry mushroom slices with 10 kGy did not influence odour or taste, but resulted in some darkening (GRÜNEWALD, 1983; 1984).

In addition to reduction of microbial populations, radiation treatment of specific dehydrated items at doses at 5 kGy or higher may have a tendering action, resulting in a decreased cooking time requirement (VAN KOOIJ, 1967; FARKAS et al., 1970).

Clostridium perfringens or *Bacillus cereus* are often present in dry soup and gravy mixes (KIM & GOEPFERT, 1971; KEOSEYAN, 1971). Although rarely, Salmonella contamination may also occur (ANON., 1979). The microbiological quality of the so-called instant soups which need not be cooked before consumption, is of particular importance. If the reconstituted product is held warm, particularly between 30 °C and 50 °C, eventual pathogens may grow to levels that will cause illness. In addition to strict hygiene in preparation, radiation disinfection of critical ingredients or the dry mixes in the final package can be reliable method for improving microbiological safety of such products.

Salmonellae have been detected often in soy meal and sometimes in other oil seed meals (ICMSF, 1980b). ZEHNDER & ETTEL (1981) reported that soy meal used as ingredient of nut pastes could be effectively decontaminated with a radiation dose of 5 kGy. Although the sensory panel found statistically significant difference between the flavour of untreated and that of irradiated soy meal samples, the flavour change tended to be favoured by the panelists.

Irradiation can be employed to control thermophilic bacteria in cereal ingredients of canned foods, which should meet the requirement of very low levels of thermophilic spore-formers. Similarly, those muesli-like cereal products which are used as ingredients in some dairy products and can not be thermally processed because it would be detrimental to their sensory quality, are also considered suitable for radiation decontamination (HENON, 1984).

4.3. Dried egg and protein preparations

Sensitive foods. Salmonella are the principal microbial problem with dried egg products (ICMFS, 1980c). Doses about 6 kGy seem to be adequate for radicidation of dried egg albumen (THORNLEY, 1963; KAHAN, 1969; INGHLESI et al., 1975) without impairing its functional and organoleptic properties, while irradiation under aerobic conditions causes off-flavour and radiation-induced undesirable oxidative changes in whole egg powder or egg yolk solids at 3 kGy or higher (KATUSIN-RAZEM, 1984). A sensory acceptable dose of 2 kGy would result in 2–3 log cycle reduction of Salmonella contamination (BOMAR, 1970; KATUSIN-RAZEM, 1984; FARKAS, 1986). Irradiation of sensitive products in oxygen-free packaging could minimize oxidation, improve flavour retention, and thus improve the feasibility of the radiation treatment.

Protein preparations. Powdered whey, sodium caseinate, dehydrated blood and plasma, defatted fish protein concentrate, etc. may be treated with

5–10 kGy dose which effectively decontaminate them without appreciably altering physico-chemical and organoleptic characteristics (Brankova & Dimitrova, 1975; Tsuji, 1983; Anon., 1984).

4.4. *Thickening and gelling agents*

Plant hydrocolloids (vegetable gums) seem to be more sensitive to radiation treatment than spices or dry vegetables and some of them show impairment of rheological properties even at dose as low as 0.2–0.5 kGy (Zehnder & Ettel, 1981; Grünewald, 1984).

Agar-agar is less sensitive to irradiation than other hydro-colloids (Rogachov et al., 1972) and a considerable reduction of microbial contamination can be achieved in gum arabic and tragacanth with relatively low doses which do not harm seriously the functional properties (Henon, 1984; Farkas, 1986).

Viable cell counts of commercial starches could be satisfactorily reduced by doses around 3–4 kGy (Saint-Lebe & Berger, 1972; Delattre et al., 1975). Chemical changes and alternation of functional properties occur when irradiated at greater than 4 kGy (Farkas, 1986).

In the dry state, the microbiological quality of pectin and gelatin can also be effectively improved with doses up to 5–10 kGy without seriously affecting their technological or oganoleptical properties (Bachman et al., 1974; Skinner & Kertész, 1960).

4.5. *Other ingredients and processing aids*

Commercial granulated sugar utilized by the beverage, confectionary and canning industries can be sterilized by radiation doses of 10–20 kGy (Kiss et al., 1968) and probably considerably lower doses could sufficiently eliminate thermoresistant spore-contamination. However, at doses higher than 5 kGy, various degradation products can be detected and a discolouration of the solid sugar can be observed.

The reduction of microbial count in industrial enzyme preparations by ionizing radiation is another viable application (Diehl, 1975; Farkas, 1986). It has been shown by several laboratories that radiation doses of around 10 kGy or less are sufficient to reduce microbial load of many dry enzyme preparations without appreciably affecting enzyme activity. However, relatively large differences in the various enzyme preparations have been noted which may be due to the grade of their purity, the presence or absence of some protecting substances, etc. (Delincee et al., 1975; Vas & Proszt, 1966).

Talc is used both in pharmaceutical and food industries and its radiation sterilization is commercially established in several countries for the pharmaceutical industry.

4.6. Irradiation of dry ingredients as an emerging commercial technology

Increasing amounts of dry food ingredients are radiation treated commercially in the recent years, first of all in Holland (Gammaster b.v., Ede and Pilot Plant of Food Irradiation, Wageningen), in Belgium (I. R. E.—Mediris, Fleurus) and the South African Republic (Iso-Ster/Pty/Ltd, Johannesburg) (FARKAS, 1984; 1985; DU PLESSIS & STEVENS, 1985).

Significant quantities of spices are being irradiated in France by the "Conservatome", in the USA (Radiation Technology, Inc., Neutron Products, Inc., and several others), in Hungary (AGROSTER, Budapest), in Norway and Brazil. The French Atomic Energy Commission is developing a project which aims at the construction of a large commercial food irradiation plant in the South-east of France. Commercial activity of the plant will soon start with dry and dehydrated products (HENON, 1985).

5. Increased sensitivity of the residual microflora surviving radiation treatment and microbiological safety of decontaminated food

Sublethal damage to microorganisms taking place during irradiation can increase their sensitivity to other injurious agents and synergistic effects of irradiation and certain processes applied in food technology can be encountered (SZCZAWINSKA et al., 1983). For example, salmonellae, which survived irradiation of artificially inoculated meat (with a dose of 1–3 kGy) died slightly faster during storage of meat at 0–2 °C and showed retarding growth during storage at 8–10 °C compared to unirradiated control. Similarly, salmonellae irradiated with a dose of 1 kGy were sensitized against curing salts ($NaNO_2$ and NaCl) in meat (SZCZAWINSKA et al., 1984; SZCZAWINSKI et al., 1985).

The surviving microbial flora of ingredients treated with a "pasteurizing" dose of radiation is proved to be sensitized to further antimicrobial actions and certain environmental effects. The survivors have lower heat resistance and salt-tolerance, and they are more demanding as regards their pH-, moisture- and growth-temperature requirements than the microorganisms of untreated ingredients (FARKAS et al., 1973; FARKAS & ANDRÁSSY, 1985). The heat sensitizing effect of irradiation increases with increasing radiation doses (KISS & FARKAS, 1981). The sensitization of the surviving microflora of irradiated dry ingredients is a permanent feature and dose not diminish upon a regular storage of the products (FARKAS & ANDRÁSSY, 1984).

Microbiological concerns. Questions regarding concerns on microbiological implications of food irradiation such a flora shifts and eventual induction of increased radiation resistance and other genetic changes of surviving micro-

organisms have been reviewed elsewhere (INGRAM & FARKAS, 1977; MOSSEL, 1977; ICMSF, 1980d; TEUFEL, 1981; FARKAS, 1981; MAXCY, 1982). These questions have been carefully considered by expert bodies including the Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food (JECFI, 1981) and the Board of the International Committee on Food Microbiology and Hygiene of the International Union of Microbiological Societies (ICMFH, 1982). Analysing the scientific knowledge available, they conluded that food irradiation is an important addition to the methods of control of food-borne pathogens and would not present any additional hazards from shifts in the microflora or changes in the attributes of microorganisms.

Viral concerns. Due to the high radiation resistance of viruses, food irradiation has never been intended seriously to be used for the eradication of viruses. It is clear, however, that irradiation offers certain benefits toward reducing any virus load at doses intended to eliminate non-sporing pathogenic bacteria. Nevertheless, in radiation disinfection of food, as in most food preservation technologies without sufficient heating to remove all danger of live viruses (e.g. dehydration, freezing, salting) control of virus transmission must be based on good hygienic and manufacturing practices (GRECZ et al., 1983).

6. Packaging of radiation disinfected food

For disinfected foods, packaging suitable to protect the food from microbial recontamination is essential. One of the main advantages of irradiation as a treatment for reduction of microbiological risks is the fact that radiation can be applied as terminal treatment, i.e. after packaging. Since radiation treatment causes practically no temperature rise in the product, packaging materials including those, which can not withstand heat, can be used. Actual radiation processing practices show that most standard packaging materials used for sealed packaging of products concerned are satisfactory for use with irradiated foods, too.

7. Legislative status of radiation decontaminated food

National legislative recognition of safety of irradiated foods is still lagging behind the achievements at the U. N. level. Nevertheless, the JECFI recommendations and Codex Alimentarius documents greatly facilitated a positive attitude of many national authorities. As regards irradiated foods, a number of clearances have been issued, most of them subsequent to the 1980-JECFI Report. At present, 24 countries have granted specific clearances for one or more radiation decontaminated food products or product-groups

PRODUCT \ COUNTRY	ARGENTINA	AUSTRALIA	BANGLADESH	BELGIUM	BRASIL	CANADA	CHILE	CHINA	DENMARK	FINLAND	FRANCE	FRG	GDR	HUNGARY	INDIA ˣ	ISRAEL	NETHERLANDS	NEW ZEALAND	NORWAY	SOUTH AFRICA	UNITED KINGDOM	USA	USSR	YUGOSLAVIA
BATTERMIX POWDER																	△							
CEREAL FLAKES											▦●													
CHEESE POWDER																				▦●				
CHICKEN, CHILLED/FROZEN			▦●		▦●	△	▦●							▦△		▦●	●			●			△	▦●
COCOA POWDER																				▦●				
DEHYDRATED POTATOES																				▦●				
DEHYDRATED VEGETABLES				▦○							▦●						▦●			▦●				▦●
DRIED COCONUT																				▦●				
DRIED YEASTS																				▦●				
DRY BLOOD PROTEIN				▦○													▦○							
DRY VEGET. SEASONINGS			▦●	▦○		▦●	▦●				▦●			▦○		▦●	○			▦●		▦●		▦●
EGG POWDER																	▦○							▦●
ENZYME PREPARATIONS									▦●								●					▦●		
ENZYME SOLUTIONS													▦●											
FISH, FROZEN & FISH PROD.			▦●		▦●		▦●										▦○							
FROGLEGS, FROZEN			▦○												▦●		○							
FRUITJUICES, FROZEN																				▦●				
GUM ARABIC				▦○							▦●													
HERBAL TEAS																	▦○			▦●				▦●
MALT																	▦○							
MEALS FOR IMMUN-REPR. PATIENTS												+					+				+			
MECH. DEBONED POULTRY M.											▦●													
PORK (TRICHINELLA DESINF.)																						▦●		
SAUSAGE								▦●																
SHRIMPS, FROZEN / BOIL.		○	▦○												▦●		○							
SPICES AND HERBS	▦●		▦●	▦○	▦●	▦●	▦●		▦●	▦●	▦●		▦○	▦○	▦●	▦●	▦○	▦○	▦●	▦●		▦●		▦●
TEA EXTRACTS																								▦●
WHEY POWDER																				▦●				

Fig. 1. Survey of radiation decontaminated food products cleared for human consumption in different countries. ●: Unlimited; ○ provisional; +: for hospital use only; △: test marketing; ×: for export only; ▦: issued since 1981

(Fig. 1), although a total of 32 countries have given clearance to at least one or more irradiated food items in general.

Comprehensive lists of the status of clearances for food irradiation on a world-wide basis are published from time to time by the Food Preservation Section of the Joint FAO/IAEA Division, Vienna, in the Food Irradiation Newsletter.

The growing interest toward radiation processing of foods is shown by the fact that further petitions for radiation decontaminated products are pending, at least in Australia, Belgium, Federal Republic of Germany, France, Hungary, the Korean Republic, Spain, Sweden, the United Kingdom and the USA.

8. Conclusions

Contamination of foods, especially of those of animal origin, with microorganisms, particularly pathogenic non-sporing bacteria, and with parasitic helminths and protozoa is an enormous public health problem and important cause of human suffering all over the world. Also many dry ingredients, particularly spices and herbs, whose major portion is produced in developing countries, giving an important source of their foreign exchange earnings, may be highly contaminated with spoilage organisms, causing difficulties during further processing into composite food products.

Pathogenic microorganisms are frequently found in dry and perishable food ingredients. Besides being a quite serious obstacle to the well-being of populations, contaminated food is the source of tremendous economic losses in relation to medical costs, loss of productivity, loss of business and possible legal action. It hampers producers of food as well as processors and traders.

The primary responsibility for food safety should rest with those who handle and prepare food for consumption, therefore, education continues as the most important measure to prevent food-borne diseases and food losses. However, the importance of processing critical food products for safety can not be overemphasized. Its benefit is demonstrated by the history of safety of heat-pasteurizated milk products.

While thermal pasteurization of liquid foods is a well established and satisfactory means of terminal disinfection treatment of such commodities, it does not suit solid foods and dry ingredients. Alternative chemical sanitizing procedures have regulatory limitations and/or inherent public health problems concerning occupational safety, residues, and environmental pollution.

Decades of research and development efforts have demonstrated that radiation disinfection of dried, chilled and frozen food by ionizing radiation is a safe, efficient, environmentally clean and energy efficient process. Irradiation of foods is particularly valuable as a disinfection procedure of end-products after processing and packaging.

Extensive literature reveals that radiation treatment with doses not causing unacceptable changes in organoleptic qualities can sufficiently eliminate pathogenic non-sporing bacteria such as salmonellae, Campylobacter, staphylococci, Vibrio, Yersinia, Shigella, etc. from red meat, poultry and fishery products under normal commercial conditions. With those products which are marketed in both fresh and frozen stage, irradiation after freezing appears to be more feasible commercially than the former one. A dose of 3 to 5 kGy is sufficient to control pathogenic bacteria, generally without affecting technical, sensory and nutritional qualities.

The exact irradiation dose to be used for decontamination of a certain food product must be determined by trial runs which provide the necessary

information on efficacy of the irradiation procedure and on its innocuity to the quality parameters of the product. A potential for use of the irradition technology exists under specific conditions for control of food-borne parasites by reasonably low doses. The existing data are promising for prevention of human infections of *Trichinella spiralis, Taenia solium (Cysticercus cellulosae* in pork), *Taenia saginata (Cysticercus bovis* in beef), *Toxoplasma gondii* and *Opisthorchis viverrini,* respectively. Regarding these parasites, a dose requirement of 0.15–0.6 kGy can be summarized from the published literature for making carrier foods safe for human consumption.

Radiation decontamination of dry ingredients, herbs and enzyme preparations is a feasible alternative to fumigation with microbiocide gases. Radiation doses of 3 to 10 kGy proved to be sufficient to reduce the viable cell counts to a satisfactory level. The flavour, texture or other important technological and sensory properties of most ingredients are not influenced at required doses of radiation.

The microflora surviving the cell count reduction by irradiation is more sensitive to subsequent food processing treatments than the microflora of unirradiated products. Radiation decontamination can be applied best as a terminal treatment of packaged products, and as such, it results in considerable savings of energy and labour as compared to alternative decontamination techniques.

Radiation decontamination is an emerging technology in several industrial countries. Proper regulatory action and socio-economic feasibility can produce in this direction a major radiation application area, following the well established record of the application of radiation treatment to sterilize disposable medical products for hospital and home health care.

However, public health authorities, medical groups, food handlers and consumers are not sufficiently aware of the contribution that food irradiation would make towards improved food safety. The number of clearances for this purpose is still limited and international trade of radiation-disinfected food is almost non-existent. Food exporting countries find it difficult to permit the radiation decontamination of foods as long as their main trading partners do not accept such commodities.

9. Recommendations

In order to facilitate technology transfer and a successful introduction of the radiation decontamination of foods, the following recommendations are suggested:

— Governments should be encouraged to accept the Codex General Standard for Irradiated Foods and to implement it towards a broad clearance

on radiation decontamination of food, included the permission of importation of commodities decontaminated abroad in accordance with the Codex General Standard and the Codex Recommended International Code of Practice for the Operation of Radiation Facilities for the Treatment of Food and all other Codex of Practice applicable to specific commodities or commodity groups.

— In harmony with Codex Codes of Hygienic Practice and Codex Commodity Standards technological guidelines for radiation decontamination of typical groups of commodities should be prepared, preferentially under the aegis of the interested UN-Agencies, and in a multi-disciplinary co-operation of experts of food technology, hygiene and radiation processing. These guidelines should define the critical requirements on raw materials and added substances, packaging, as well as product holding and handling before, during and after irradiation. They should include provisions for process control, record keeping, post-irradiation inspection and microbiological safety tests.

— The interested UN-Agencies are encouraged to establish regional programmes in cooperation with national agencies on the adaptation of the process to local conditions. WHO should take an active role informing national authorities and their official contact points of the safety and effectiveness of irradiation as a method for decontaminating food, with a view to request them to apply the process in practice wherever applicable.

— Sufficient data have demonstrated the effectiveness and efficacy of irradiation as the method for disinfecting fresh, chilled, dry and frozen food. National authorities are urged to adopt the use of this technique to reduce the incidences of food-borne diseases, thus contributing to better primary health care and increase the availability of a safe and wholesome food supply.

— It is recommended to perform detailed studies, in comparison with alternative treatments, on benefits of specific applications of radiation decontamination of selected commodities in radiation plants to be situated at the points of exports or imports of such products, and for other strategic localities or specific risk groups where possible public health and economical effects can be quantified.

— Case studies describing successful past implementation of the radiation disinfection process should be published.

— Special training courses/workshops on radiation disinfection should be organized under the aegis of the International Consultative Group on Food Irradiation (ICGFI) utilising the capabilities of the International Facility for Food Irradiation Technology (IFFIT) and other appropriate institutions elsewhere.

— Trials in which large quantitites of critical food products are disinfected by radiation should be performed to establish actual dose requirements, quality implications and process control criteria under conditions

which closely approximate actual industrial situations. Governments and international agencies are encouraged to support such industry initiated projects.

— Setting up pilot-scale facilities or utilizing existing radiation plants is a prerequisite for such efficacy tests. Pilot plants could serve both as demonstration units and service facilities. Market testing and consumer acceptance studies of treated products should be carried out. In order to facilitate progress by a concerned effort, cooperation with the Joint FAO/IAEA Division and the International Facility for Food Irradiation Technology is recommended in such activities.

— Further studies are required to establish efficacy and suitability of radiation control of those food-borne parasites for which either no or not enough information are available in this regard, using radiation alone and in combination with other measures. Particular attention should be given to the effect of irradiation on parasites in products which are usually consumed semi-cooked or without cooking.

— The benefits of irradiation should never be considered as replacement for poor product quality nor for poor handling and storage conditions, i.e. as a substitute for good manufacturing practice.

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Thanks are due to Professor A. M. DOLLAR and Mr. P. LOARANU (IAEA, Vienna) for their critical review of the manuscipt and their helpful suggestions.

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