ACTA

ALIMENTARIA

An International Journal of Food Science

Editor

J. HOLLÓ

Volume 17 **March 1988** **Number 1**

Akadémiai Kiadó
Budapest

Kluwer Academic Publishers
Dordrecht/Boston/London

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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Kluwer Academic Publishers
P.O. Box 17, 3300 AA Dordrecht, Holland
and 101 Philip Drive, Norwel, MA 02061 U. S. A

Publication programme, 1988: Volume 17 (4 issues)
Subscription prices per volume: Dfl. 276,—/$ 130.00 including postage.

Acta Alimentaria is published 4 times per annum: March, June, September and December

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.

ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

VOLUME 17

1988

AKADÉMIAI KIADÓ
BUDAPEST

KLUWER ACADEMIC PUBLISHERS
DORDRECHT /BOSTON/ LONDON

Acta Alimentaria, Vol. 17 (1), pp. 3—11 (1988)

DETERMINATION OF AMINO ACIDS IN LUPINE BY NEAR INFRARED REFLECTANCE SPECTROSCOPY

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(Received: 16 January 1986, revision received: 13 October 1986; accepted: 17 October 1986)

Preliminary studies were carried out to determine if NIR spectroscopy could be used to estimate the concentrations of amino acids in lupine.

Seventeen amino acids were analyzed in the lupine calibration samples. The chemical analyses were performed with an ion exchange amino acid analyzer type OE 914 Aminochrom II. The log (1/R) spectra of the ground lupine samples were obtained and recorded in the 1100–2500 nm wavelength range on the NEOTEC (Pacific Scientific) Research Composition Analyzer, Model 6450.

The relationship between concentration data and values of the second derivative spectra measured at different wavelengths was determined by multiple linear regression analysis using two different equation forms (two mathematical models).

Results as characteristic wavelengths, multiple correlation coefficients, standard errors of calibration and coefficients of variability for seventeen amino acids in lupine are summarized in Tables 2 and 3. For lysine, isoleucine and threonine (limiting acids) the correlation coefficients were around 0.85, the SEC values around 0.08 and the CV values around 5.5. The higher CV value for methionine determination may be related to the inherent higher error of methionine analysis by ion exchange chromatography due to hydrolysis losses.

These results prove that NIR technique can be used as a rapid, non-destructive method for determining the amino acid concentrations in lupine.

Keywords: amino acid analysis, near infrared spectroscopy, lupine. nutrition

The determination of amino acid concentrations of animal and plant materials is of major interest to those involved in the food and feed industry. From the point of view of nutritional physiology the most important amino acids are considered to be the ten essential ones. These include : lysine, methionine, threonine, tryptophan, isoleucine, arginine, leucine, phenylalanine, valine and histidine. The first five amino acids listed are limiting ones. In many plant materials one or more of them are present in a minor concentration limiting the nutritive value of the protein.

The standard chemical determination of amino acid concentration involves hydrolysis of the proteins followed by purification and ion exchange chromatography. This is a fairly complicated and time consuming process, hindering the efficiency of the research programs in food and feed industry.

The promising results of the NIR technique in the different fields of agriculture and food industry have encouraged us to extend our research work to determine the amino acid concentration by this technique. Provided

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this research activity is succesful, the introduction of this rapid and non-destructive analytical method might give great impetus to research programs mentioned above.

At the time of starting our research work RUBENTHALER and BRUINSMA (1978) already published successes in lysine estimation in cereals by near infrared reflectance. In 1979 GILL and co-workers gave account of lysine and nitrogen measurement by infrared reflectance analysis as an aid to barley breeding. In 1981 at an International Symposium (held in Sorrento, Italy, 19–22 October) BISTON and co-workers (1983) presented a paper on the determination of protein in lupine seeds and of amino acids and protein in *Vicia faba* seeds. Later WILLIAMS and co-workers (1984) published a paper describing the application of NIR for the rapid analysis of four limiting amino acids in wheat and up to fourteen amino acids in barley.

We carried out the research work for determining the amino acid concentrations by NIR technique in lupine samples from different parts of Hungary harvested in different years. So far, such measurements on up to seventeen amino acids in lupine have not been reported in the scientific literature.

The present study aims at finding the possibility for applying the NIR technique for the rapid determination of amino acid concentrations in lupine; and at the same time, to predict accuracy for the different amino acids, to choose the most suitable form of the regression equations, determine the parameters (characteristic wavelength, coefficients, constants, smoothing segments, gaps at producing derivatives).

The results of these studies will hopefully enable us to develop a single-purpose instrument or to extend the use of one already existing to be able to perform such measurements.

1. Materials and methods

Seventeen amino acids were analyzed and we received concentration data in mass %. All these analyses were performed with an ion exchange amino acid analyzer (type OE 914 Aminochrom II. made by Labor MIM, Hungary).

The concentration data of the $n = 30$ lupine calibration samples characterized by minimum–maximum values, means and standard deviations are summarized in Table 1.

Since the NIR technique is strictly correlative, the accuracy of the analytical data of the calibration samples are of critical importance. In order to determine the relationship between optical properties and concentrations we needed a set of calibration samples in which amino acid concentrations varied between minimum and maximum values of samples found in every day prac-

Table 1

The concentration data of the n = 30 lupine calibration samples characterized by minimum–maximum values, mean values and standard deviations

Amino acids	Min.	Max.	Mean	SD
Lysine	1.28	2.08	1.57	0.19
Methionine	0.17	0.44	0.24	0.06
Isoleucine	1.22	1.69	1.48	0.14
Threonine	0.97	1.42	1.16	0.12
Arginine	2.24	4.23	3.07	0.50
Histidine	0.62	1.68	0.95	0.26
Leucine	1.78	2.84	2.37	0.33
Phenylalanine	1.08	1.70	1.38	0.17
Valine	1.10	1.77	1.43	0.16
Cisteine	0.17	0.78	0.34	0.18
Alanine	1.00	1.53	1.27	0.15
Asparagine	2.77	3.83	3.32	0.30
Glutamine	5.21	8.68	6.52	0.96
Glycine	1.14	1.58	1.36	0.11
Proline	0.91	1.93	1.38	0.18
Serine	1.45	2.29	1.75	0.22
Tyrosine	0.76	1.23	1.02	0.18

tice. Consequently these lupine samples for calibrations were carefully selected, prepared and analyzed at Labor MIM, Department of Chemistry.

The log (1/R) spectra of the ground lupine samples were obtained and recorded in the 1100–2500 nm wavelength range on the NEOTEC (Pacific Scienfific) Research Composition Analyzer, Model 6450. The log (1/R) spectra were transposed to the second derivative for analysis. The relationship between concentration data and values of the second derivatives measured at different wavelengths was determined by multiple linear regression analysis first using a two term homogeneous linear equation form and thereafter a single term ratio equation form where the value of the second derivative at the first characteristic wavelength was divided by the value of the second derivative at the second characteristic wavelength. The log (1/R) spectra were smoothed by a running average of 16 nm segments using triangular weighting function. The gap used at forming the second derivatives was 12 nm.

2. Results

Preliminary studies were carried out to determine if NIR spectroscopy could be used to estimate the concentrations of amino acids in lupine.

First log (1/R) spectra of pure powdered amino acid samples were recorded in order to see the differences. We selected the log (1/R) spectra of pure methionine, cisteine and alanine samples which are shown in Fig. 1.

The figure illustrates that log (1/R) spectra of pure amino acids show great and characteristic differences.

We investigated the correlation between the amino acid concentrations and the values of the second derivative spectra at different wavelengths of lupine samples. The correlation spectra (correlation plot) for lysine and isoleucine are shown in Fig. 2. and Fig. 3. The figures illustrate that not only

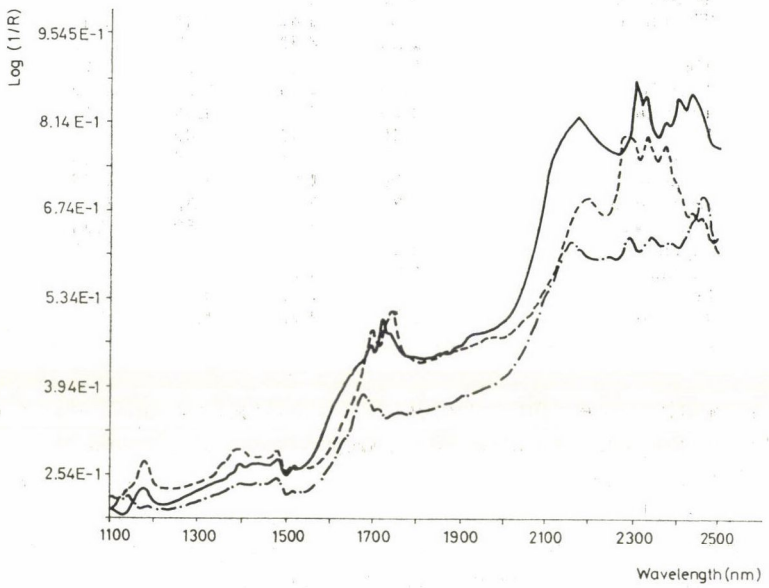

Fig. 1. The log (1/R) spectra of pure methionine, cisteine and alanine samples in the 1100–2500 nm wavelength range. Measuring geometry: 0°/45°. Diameter of the illumination beam: ∅ 25 mm. Spectral bandpass: 12 nm. Spectra were recorded in 2 nm steps. Triangular smoothing, segment 16 nm. Cisteine: ———, methionine: — — —, alanine: —·—·—

pure amino acids but also the amino acids built in protein molecules show differences in their spectra.

We investigated the effect of change in amino acid concentrations by comparing the second derivative spectra of samples with maximum and minimum concentrations for each amino acid. Figure 4. shows the second derivative spectrum of the lupine sample No. 3. with minimum lysine concentration and the second derivative spectrum of the lupine sample No. 23. with maximum lysine concentration.

The relationship between spectral values and amino acid concentrations of the 30 lupine samples was determined by use of an iterative procedure with the multiple regression analysis. The computer program determined the char-

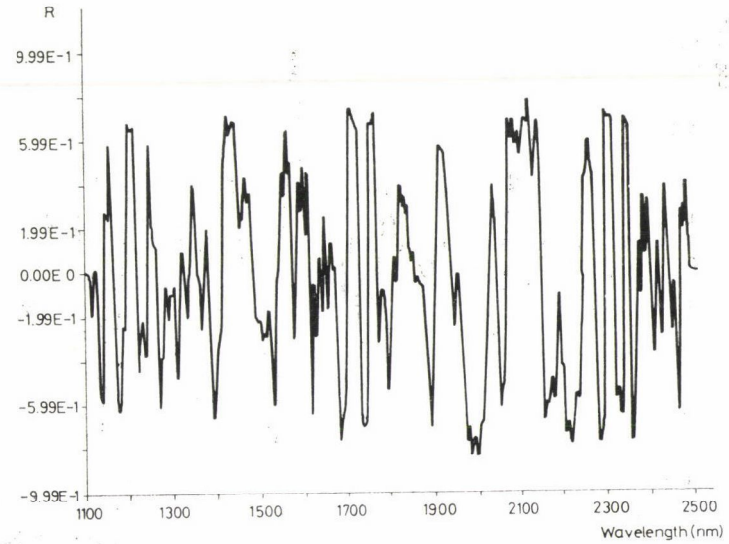

Fig. 2. Correlation spectra for lysine. The values of the correlation coefficients between lysine concentrations and values of the second derivatives at different wavelength as function of the wavelength

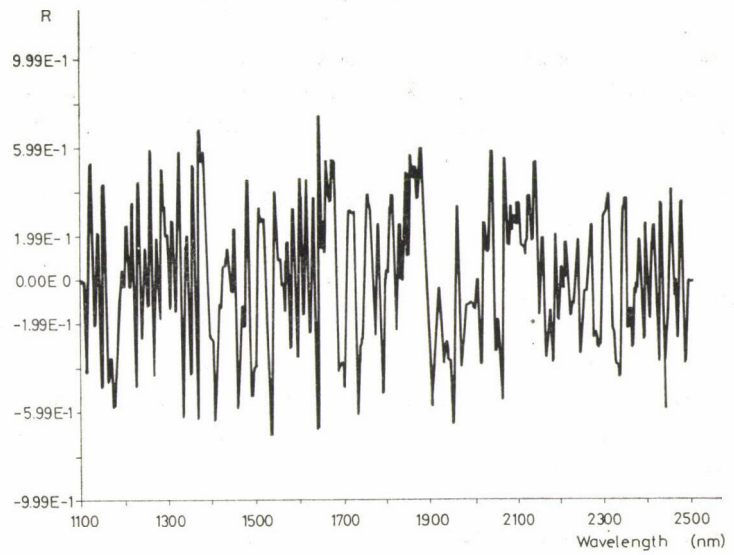

Fig. 3. Correlation spectra for isoleucine. The values of the correlation coefficients between isoleucine concentrations and values of the second derivatives at different wavelengths as function of the wavelength

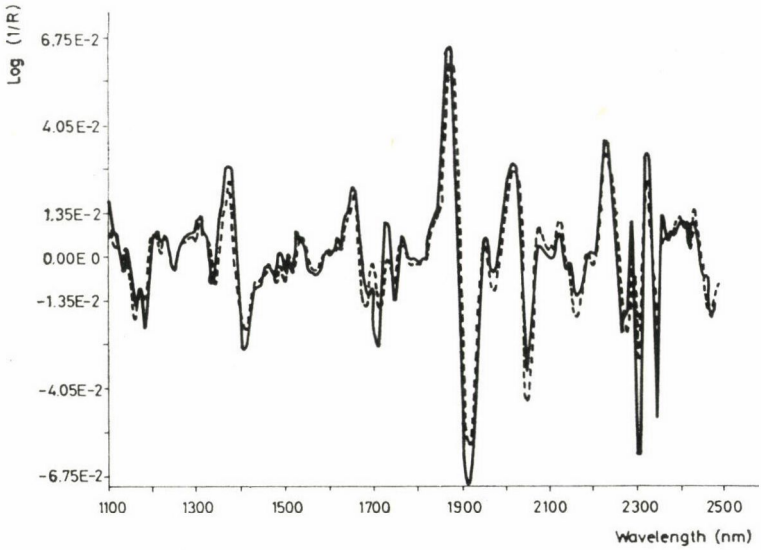

Fig. 4. Second derivative spectra of lupine samples No. 3 and No. 23 containing maximum and minimum lysine. Triangular smoothing, segment 16 nm, gap 12 nm. Second derivative spectrum of lupine sample No. 3: ———, second derivative spectrum of lupine sample No. 23: — — —

acteristic wavelength, coefficients and constants that gave the best fit to the following equation for each amino acid:

$$Q_i = K_{0i} + K_{1i} V''_{\lambda 1i} + K_{2i} V''_{\lambda 2i}$$

where Q_i stands for the amino acid concentrations (in mass %); K_{0i}, K_{1i}, K_{2i} constants and coefficients, resp.; λ_{1i} and λ_{2i} are characteristic wavelengths; $V''_{\lambda 1i}$ and $V''_{\lambda 2i}$ are the values of the second derivatives at these characteristic wavelengths.

We used only two term equations because of the limited quantity of calibration samples in order to avoid overfitting.

The results are summarized for seventeen amino acids in Table 2 containing the characteristic wavelengths, multiple correlation coefficients, standard errors of calibration and coefficients of variability (relative errors).

We tried to find the relationships between spectral values and amino acid concentrations at other absorption bands using the same equation form. The results in multiple correlation coefficients and standard errors of calibration were almost as good as those shown in Table 2.

The relationship between spectral values and amino acid concentrations were also studied in the following form of equation:

$$Q_i = K_{0i} + K_{1i} \frac{V''_{\lambda 1i}}{V''_{\lambda 2i}}$$

Table 2

Summary of linear regression analyses relating concentration data from chemical analyses and values of the second derivative of log (1/R) curves at two characteristic wavelengths for 30 lupine samples, searching 1000 to 2500 nm
Equation form: $Q = k_0 + k_1 V''_{\lambda 1} + k_2 V''_{\lambda 2}$

Amino acids	λ_1(nm)	λ_2(nm)	R	SEC (mass %)	CV (%)
Lysine	1312	1684	0.878	0.092	5.48
Methionine	1156	2000	0.778	0.042	14.00
Isoleucine	1926	2140	0.831	0.079	5.41
Threonine	2042	2140	0.816	0.071	5.92
Arginine	1690	1694	0.863	0.261	8.06
Histidine	1420	1592	0.856	0.139	12.09
Leucine	1210	1950	0.910	0.142	6.16
Phenylalanine	2140	2444	0.862	0.092	6.62
Valine	1220	2268	0.759	0.107	7.43
Cisteine	2072	2392	0.875	0.090	18.75
Alanine	1700	2452	0.787	0.098	7.72
Asparagine	1338	1592	0.804	0.185	5.60
Glutamine	1168	1416	0.813	0.583	8.39
Glycine	1636	2326	0.784	0.071	5.22
Proline	2312	2424	0.783	0.120	8.45
Serine	1352	2272	0.803	0.134	7.13
Tyrosine	1232	2024	0.730	0.128	12.80

The marks are the same as in the above equation.

Characteristic wavelengths, multiple correlation coefficients, standard errors of calibration and coefficients of variability for the seventeen amino acids are summarized in Table 3.

3. Conslusions

The pure amino acid spectra — from which some have been demonstrated in Fig. 1 as an example — show very different and characteristic shapes. Consequently it has been assumed on this basis that amino acids built in protein molecules also show different spectra.

Plotting the correlation between the amino acid concentrations and the values of the second derivative spectra at different wavelengths of lupine samples as a function of the wavelength, these correlation spectra were different and characteristic for each amino acid from which two can be seen in Fig. 2 and Fig. 3. These results proved our assumption that amino acids incorporated into protein molecules also have different spectra. The fact that peaks of the correlation spectra are at different wavelengths for each amino acid refer to the possibility of determining the amino acid concentrations from the spectral data measured at different wavelengths.

The correlation spectra for lysine in lupine samples show peaks in the 1650–1750 nm and the 1950–2020 nm wavelengths ranges. Here also the

Table 3

Summary of linear regression analyses relating concentration data from chemical analyses and values of the second derivative of log (1/R) curves at two characteristic wavelengths for 30 lupine samples, searching 1000 to 2500 nm
Equation form: $Q = k_0 + k_1(V_{\lambda 1}/V_{\lambda 2})$

Amino acids	λ_1(nm)	λ_2(nm)	R	SEC (mass %)	CV (%)
Lysine	1984	2424	0.871	0.093	5.54
Methionine	2120	2424	0.764	0.042	14.00
Isoleucine	2344	1640	0.826	0.079	5.41
Threonine	1644	1594	0.762	0.078	6.50
Arginine	2120	1624	0.858	0.260	8.02
Histidine	2154	1616	0.838	0.143	12.43
Leucine	1710	1372	0.898	0.148	6.40
Phenylalanine	1186	1686	0.864	0.089	6.40
Valine	1472	1156	0.714	0.113	7.85
Cisteine	2394	1444	0.882	0.086	17.83
Alanine	1640	1778	0.757	0.102	8.03
Asparagine	1592	1338	0.790	0.186	5.63
Glutamine	2186	1594	0.803	0.618	8.89
Glycine	2326	1454	0.784	0.070	5.14
Proline	2424	1684	0.762	0.122	8.59
Serine	1338	1592	0.781	0.137	7.40
Tyrosine	2408	1352	0.727	0.126	12.70

second derivative spectra of the lupine samples containing the lowest and highest lysine concentration — as seen in Fig. 4. — show great differences. Thus it is not by chance that in the two mathematical models one of the wavelengths from the pair found most suitable for lysine by regression analysis was chosen from these wavelength ranges.

The results published in Table 2 and Table 3 — provided we observe the SEC and CV values — show that they are similar to the results received for the amino acid concentration data of wheat and barley referred to in the quoted paper. Here also higher value was obtained for methionine determination. This higher value may be related to the inherent higher error of methionine analysis by ion-exchange chromatography due to hydrolysis losses. The relatively smaller R values received for lupine can be attributed to the amino acid concentration data for lupine being of a narrower range than in the case of wheat and barley samples as cited in the literature.

In our studies, none of the samples were omitted for the calculations, although the results could have been improved by omitting samples with a large deviation.

If we compare the two mathematical models and the respective data contained in Table 2 and Table 3 we can conclude that the results are practically of the same value.

These results prove that NIR technique can be used as a rapid, non-destructive method for determining the amino acid concentrations in lupine.

These results might be improved by using mathematical models with more terms requiring, however, a greater number of calibration samples and higher computational capacity, further improvements can be achieved by optimizing for segments and gaps, and using replicates.

*

Thanks are due to Ms Magda NÁDASI for her conscientious technical assistance in performing measurements and data processing. I am most grateful to Labor MIM, Budapest for providing me with specially prepared and analysed lupine samples.

Literature

BISTON, R., DARDENNE, G., SINDIC, M., DARDENNE, P., WATHELET, B. & DE SOUSA, B. (1983): Near infrared spectroscopy as a rapid and accurate technique for estimating Lupinus seed protein and *Vicia faba* seed amino acids and protein. — in: THOMPSON, R. & CASEY, R. (Eds.) *Perspectives for peas and lupins as protein crops. Proceedings of an International Symposium on Protein Production from Legumes in Europe.* Martinus Nijhoff Publ., The Hague, pp. 247–255.

GILL, A. A., STARR, C., SMITH, D. B. (1979): Lysine and nitrogen measurement by infrared reflectance analysis as an aid to barley breeding. *J. agric. Sci., 93,* 727.

RUBENTHALER, G. L., BRUINSMA, B. L. (1978): Lysine estimation in cereals by near infrared reflectance. *Crop Sci., 18,* 1039.

WILLIAMS, P. C., PRESTON, K. R., NORRIS, K. H. & STARKEY, P. M. (1984): Determination of amino acids in wheat and barley by near infrared reflectance spectroscopy. *J. Fd Sci., 18,* 17.

Acta Alimentaria, Vol. 17 (1), pp. 13—31 (1988)

THE EFFECT OF PACKAGING AND STORAGE CONDITIONS ON THE KEEPING QUALITY OF WALNUTS TREATED WITH DISINFESTATION DOSES OF GAMMA RAYS

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(Received: 17 April 1986; revision received: 29 September 1986; accepted: 9 October 1986)

Shelled walnuts were packed in polyethylene and laminated foil (PVDC–polyethylene) pouches as well as cans, under nitrogen or air atmospheres. The packaged samples were irradiated with a dose of 0, 0.5 and 1.0 kGy, resp., and stored up to 4 months at 5 °C, 20 °C or 40 °C. Peroxide and *p*-anisidine values and free fatty acid content were followed during storage to record changes in the lipids of the nuts. Retention of tocopherols was estimated directly after irradiation and after 8 weeks of post-irradiation storage. Also the nuts were scored by a panel for colour, odour, taste and texture. Radiation treatment did not affect the sensory qualities or the indices of lipid oxidation. No significant differences were found in the content of tocopherols between untreated and irradiated samples. At 40 °C, the sensory quality decreased fast and the nuts became inedible within 2 months. At 5 °C, the nuts could be stored for more than four months. Nuts packed under nitrogen kept their organoleptic quality better than nuts packed under normal air, particularly at 5 °C. Polyethylene was not a satisfactory packaging material for shelled walnuts. Packaging in cans gives a much better result, laminated foil can also be used. The peroxide and *p*-anisidine values as well as the free fatty acid content increased faster for nuts stored at 40 °C, compared to nuts stored at 5 °C and 20 °C. Also nuts stored with nitrogen had lower peroxide and *p*-anisidine values and free fatty acid content.

Keywords: keeping quality, walnut, irradiation disinfestation, packaging

Insect infestation and oxidative deterioration of stored nuts including walnuts is a serious problem in several countries (TALHOUK, 1966). The magnitude of storage losses varies with the postharvest storage practice. It has been estimated that these losses in walnuts may vary between 15 and 20% in Pakistan.

Chemical disinfestation methods such as fumigation require repeated application because they may not eliminate the eggs of insects. Furthermore,

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there is a growing concern about occupational health hazard and residue problems associated with the use of chemical pesticides. These are the reasons for a widespread interest in disinfestation by ionizing radiation.

Numerous studies on the sensitivity of stored product insects to the effects of ionizing radiation summarized by TILTON and BURDITT (1983) show that 0.5 kGy could control even the most resistant beetle species. The reproduction of moths and development of mite populations could be controlled by 1 kGy although the mites present would not be killed (BROWER & TILTON, 1970). Radiation disinfestation studies of shelled pecans, almonds and walnuts by BROWER and TILTON (1972) have shown that about 0.4 kGy dose would be sufficient to prevent insect development in consumer packages and to eliminate feeding damage to the product. However, proper packaging is essential to prevent re-infestation.

Neither chemical pesticides nor irradiation can prevent oxidative deterioration. Because of a high content of unsaturated fatty acids, walnuts are particularly susceptible to oxidative rancidity (LABUZA, 1971). The rancid walnuts may be considered as inedible. Oxidative deterioration is facilitated at high storage temperature and by exposure to light (WAHID et al., 1985).

To minimize storage losses, the present studies were undertaken to investigate the effect of packaging and storage conditions on the degree of lipid oxidation and sensory quality of shelled walnuts untreated or irradiated with disinfestation doses of gamma rays.

1. Materials and methods

1.1. Packaging and irradiation treatment

The shelled walnuts, in cartons imported from India, were obtained from a local company in Amsterdam, the Netherlands. For the experiments the unsorted walnuts were packed in bags of polyethylene, low density, 0.022 mm thickness, ICI product and laminated foil Combithen-XXB/13 k 1530 (components: polyvinylalcohol (PVOH) 15 μm + polyethylene (PE) 30 μm a product of Wolffs, Walsorde, Germany, and cans. Each package, containing 150–160 g nuts, was filled with normal air or evacuated and filled with nitrogen gas. For each treatment and packaging condition three replicates were used. The batches were gamma irradiated at the ^{60}Co source of the Pilot Plant for Food Irradiation in Wageningen, The Netherlands, using doses of 0, 0.5 and 1.0 kGy, respectively, with a dose rate of 1.0 kGy h^{-1}. The samples were stored at 5 °C, 85% R. H.; 20 °C, 50% R. H. or 40 °C, 30% R. H. for a period of 4 months. (Relative humidities refer to the R. H. of the storage space outside the packages.)

1.2. Sample preparation

The following sample preparation was carried out in 3 replicates. Approximately 30 g nuts were randomly taken from the bags or cans. These nuts were ground in a pestle and mortar in order to get a well-mixed and pulverized sample. The ground sample was extracted in a Soxhlet extraction apparatus for 4 hours with petrolether (boiling point 40–60 °C) as solvent. The extracted oilwas recovered in a rotating vacuum evaporator after evaporating the solvent. The oil was kept under nitrogen at 4 °C until the analyses could be done.

1.3. Analytical methods

The samples were periodically tested for chemical and sensory properties. Also the moisture content and water activity were tested in the beginning and at the end of the storage to check the possibility of microbial growth. In order to follow the rate of oxidation the following analyses were carried out:

— Peroxide value, which gives an indication about the first step of the oxidation, peroxide formation.

— *p*-Anisidine value which gives an indication about the amount of oxidation products, namely carbonyl compounds.

— Free fatty acid content which gives information about lipolysis by micro-organisms and enzymes or further oxydation of secondary products.

Irradiation can have some effect on the vitamin content of some products therefore the tocopherol composition and content were studied.

1.3.1. Peroxide value. The peroxide value was estimated according to a method described by LEA (1952). Approximately 5 g of oil to the nearest 0.01 g, were weighed into stoppered Erlenmeyer flask and dissolved in 10 cm^3 chloroform. Fifteen cm^3 glacial acetic acid were added and the solution was deaerated by flushing with nitrogen gas for 5 min. Two cm^3 of saturated potassium iodide solution were added and after mixing the sample was kept in dark for 1 h. Then the sample was diluted with 30 cm^3 water and the liberated iodine was titrated by 0.01 N sodium thiosulphate solution using 1% starch solution as indicator. The peroxide value was calculated according to the following equation:

$$\text{PO-value [meq kg per oil]} = \frac{[0.01\,N\ \mathrm{Na_2S_2O_3}\ [\mathrm{cm_3}[\times 2]}{\text{mass of sample}\frac{1}{4}[\mathrm{g}]}$$

1.3.2. p-Anisidine value. The *p*-anisidine value was estimated according to the method described in the A. O. A. C. (1975).

Approximately 1 g oil, to the nearest 0.01 g, was weighed into a 25 cm^3 volumetric flask, dissolved and diluted to volume with iso-octane. The optical density of this solution (OD1) was estimated at 350 nm using iso-octane as blank. Ten cm^3 of the oil solution were pipetted into stoppered test tubes and

2 cm^3 of *p*-anisidine reagent (0.25 g *p*-anisidine in 100 cm^3 glacial acetic acid) were added to each tube. After shaking and keeping at room temperature for exactly 10 min, the optical density of the reaction mixture (OD2) was estimated at 350 nm using iso-octane *p*-anisidine mixture as blank. The *p*-anisidine value was calculated according to the following equation:

$$\text{PA-value} = \frac{25 \times (1.2 \times (\text{OD2}) - (\text{OID}))}{\text{mass of sample [g]}}$$

1.3.3. Free fatty acid content. The free fatty acid content was determined according to the method described in the Nederlandse Norm 6332 (ANON., 1980). Approximately 5 g oil, to the nearest 0.01 g, were weighed into an Erlenmeyer flask and dissolved with 30 cm^3 neutralized ethanol–ether mixture (1 : 1) and a few drops of phenolphtalein indicator (1%). The solution was titrated with potassium hydroxide solution (0.01 *N*) until the pink colour was stable for at least 20 sec. The free fatty acid content (as % oleic acid) was calculated according to the following equation:

$$\text{FFA-value [\% oil]} = \frac{0.01\,N\ \text{KOH [cm}^3\text{]} \times 0.282}{\text{mass of sample [g]}}$$

1.3.4. Tocopherol determination. The tocopherols were determined according to the following modified IUPAC method (ANON., 1982).

To a 100 cm^3 ground-necked round bottom flask 1 cm^3 of a squalane solution (50 mg per 100 cm^3 hexane) was added. The solvent was evaporated using vacuum. Approximately 1 g of the oil, to the nearest 0.01 g, was weighed into the flask and 4 cm^3 of ethanolic pyrogallol (5 g per 100 cm^3) solution were added. An air condenser was attached and the mixture was brought to boil. When the boiling started, 1 cm^3 of potassium hydroxide solution (1.6 g per cm^3) was added. After boiling for 3 min, the heating was stopped, the flask was cooled by running water and 25 cm^3 of distilled water were added. The content of the flask was transferred quantitatively to a separating funnel. The flask was rinsed with 40 cm^3 diethyl ether, transferring the rinsings to the separating funnel. Two further extractions were made of the aqueous layer with 25 cm^3 of diethyl ether each time, combining the three extracts in a second separating funnel. During each extraction, the separating funnel was shaken and the emulsion was broken with a few drops of ethanol.

The layers were allowed to separate, then the water layer was taken off. The combined extracts were washed with 20 cm^3 portions of water with vigorous shaking until the washing liquids did not turn pink after addition of a few drops phenolphtalein solution (1 g per 100 cm^3 ethanol). The extracts were dried by filtering over sodium sulphate. The ethereal layer was transferred into a 100 cm^3 flask and the diethyl ether was evaporated by using a rotating vacuum evaporator.

The residue was dissolved in 5 cm^3 of diethyl ether and transferred quantitatively to a 15 cm^3 test tube using a minimum of diethyl ether rinsing. The diethyl ether was evaporated completely using vacuum. To the test tube 0.2 cm^3 N-methyl-N-trimethylsilyltrifluoroacetamid was added, the tube was closed and allowed to stand for 15 min at 70 °C. One μl of the silylethers was injected to a gas chromatograph equipped with a flame ionization detector and a 25 m $\times$ 0.22 mm i.d. fused silica CP Sil 5 CB (similar to SE 30) capillary column. Oven temperature was 280 °C.

The content of tocopherol (i) was calculated according to the following equation:

$$\text{Tocopherol (i) content [mg per kg oil]} = A(\text{i}) \times m_2 \times 1000 / A(\text{s}) \times m_1$$

where:

m_1 = the mass [g] of the test portion,
m_2 = the mass [mg] of the squalane added to the round bottom flask,
A(i) = the area under the peak corresponding to tocopherol (i),
A(s) = the area under the peak corresponding to squalane.

1.3.5. Head space oxygen content. The oxygen content in the packaging was estimated using a Teledyne model 320B oxygen analyser.

For the bags, 2 injection needles were used to circulate the gas through the analyser. For the cans a self made adapter was used.

1.3.6. Moisture content. The moisture content was estimated according to the method described in an EC-publication (ANON., 1968).

Approximately 5 g nuts, broken in pieces, were weighed into an aluminium dish and placed into an oven at 102 $\pm$ 2 °C. The samples were kept at that temperature for 4 h. After this period the dish was cooled to room temperature using a desiccator and again weighed. The moisture content was calculated from the weight loss of the sample under described conditions.

1.3.7. Water activity. The water activity was estimated according the method of NORTHOLT and HEUVELMAN (1982).

A sample flask of 100 cm^3 was half filled with nuts. The flask was left closed for stabilizing for 3 h at room temperature. A microscopic slide, divided into 4 areas with a marker, was coated with a layer of vaseline. On the vaseline layer were placed, within the areas, crystals of known water activities: 15, 30, 45 and 60% A_w, respectively. The slide was placed into the flask and left for stabilizing for 20 h. Afterwards the crystal-liquifaction was estimated.

1.4. Sensory testing

For the sensory testing the quick rank test for multiple comparison of KRAMER (1956) was used. The samples of different treatments were tested monthly for the properties colour, odour, taste and texture using 9 to 11 non-trained panel members. The possible scores were: 1 = poor; 2 = unacceptable; 3 = acceptable; 4 = good; 5 = excellent.

The results were calculated using an error level of 5% single tailed. Because of the impossibility of comparing all treatments at the same time the following effects were studied:

— Effect of irradiation and gas condition in cans with the 5 °C storage temperature : 0 kGy, 0.5 kGy, 1.0 kGy, normal air and nitrogen.

— Effect of packaging and gas condition on unirradiated samples (0 kGy) with the 5 °C storage temperature: can, laminated foil, polyethylene, normal air and nitrogen.

— Effect of storage temperature and gas condition on unirradiated samples (0 kGy) in cans: 5 °C, 85% R. H.; 20 °C, 50% R. H.; 40 °C, 30% R. H., normal air and nitrogen.

2. Results and discussion

2.1. Moisture and water activity

The average moisture content and approximate water activity of three samples of walnuts irradiated and non-irradiated, packed in different packages and stored at different temperatures are shown in Table 1.

Initial moisture contents were similar to those reported by SEDLAČEK (1985) and SOUCI and FACHMANN (1981/82). As shown in Table 1, the walnut samples packed in polyethylene and laminated foil and stored at higher temperature and low relative humidity, were clearly dried out within 8 weeks. Samples packed in polyethylene and laminated foil and stored at high outer relative humidity, had slightly increased moisture content after 8 weeks of storage, however still sufficiently low to prevent mould growth (BEUCHAT, 1978).

2.2. Head space oxygen content of samples packed under nitrogen

The average oxygen contents in head spaces of 9 samples of walnuts irradiated and non-irradiated, packed with nitrogen gas, are given in Table 2.

The data of the table revealed clearly that the oxygen penetrated very easily into the polyethylene bags. Under this circumstance the nitrogen filling of the polyethylene bags had only an initial protection against oxidation and not during storage. Also the oxygen contents were higher in the laminated foil bags than in the tins.

2.3. Lack of radiation effects on chemical properties of lipids in walnuts

To test first the effect of irradiation on the chemical properties, an analysis of variance carried out for all treatments showed no significant difference, with a probability of error of 5% single tailed; neither directly after irradiation, nor during storage.

Table 1

Moisture content and approximate water activity of shelled walnut, packed in different packages, stored under different conditions at different temperature

Storage				Polyethylene			Laminated foil			Can		
Temperature (°C)	Relative humidity (%)	Gas	Time (week)	Moisture content (%) $\bar{x}$	± s	A_w	Moisture content (%) $\bar{x}$	± s	A_w	Moisture content (%) $\bar{x}$	± s	A_w
5	85	Air	0	4.4	0.1	0.45	4.4	0.1	0.45	4.4	0.1	0.45
			15	4.7	0.1	0.45	4.9	0.1	0.45	4.8	0.1	0.45
		Nitrogen	0	4.4	0.1	0.45	4.4	0.1	0.45	4.4	0.1	0.45
			15	4.9	0.1	0.45	5.3	0.1	0.45	5.1	0.2	0.45
20	50	Air	0	4.4	0.1	0.45	4.4	0.1	0.45	4.4	0.1	0.45
			13	4.3	0.3	0.45	3.7	0.4	0.45	4.6	0.2	0.45
		Nitrogen	0	4.4	0.1	0.45	4.4	0.1	0.45	4.4	0.1	0.45
			13	3.7	0.2	0.45	4.3	0.3	0.45	4.4	0.3	0.45
40	30	Air	0	4.4	0.1	0.45	4.4	0.1	0.45	4.4	0.1	0.45
			8	2.0	0.1	0.3	2.6	0.2	0.3	4.0	0.4	0.45
		Nitrogen	0	4.4	0.1	0.45	4.4	0.1	0.45	4.4	0.1	0.45
			8	1.7	0.1	0.3	1.7	0.1	0.3	3.7	0.8	0.45

$\bar{x}$ = average of 3 measurements
± = standard error

Table 2

Oxygen content walnut irradiated and non-irradiated, packed with nitrogen gas

Storage		Oxygen content of walnut packed in					
Temperature (°C)	Time (week)	Polyethylene		Laminated foil		Can	
		$\bar{x}$	$\pm$ s	$\bar{x}$	$\pm$ s	$\bar{x}$	$\pm$ s
5	0	—	—	—	—	4.1	0.4
	3	20.0	0.1	8.0	1.6	3.4	0.2
	7	20.8	0.0	6.9	1.7	3.7	0.2
	11	20.7	0.1	6.0	1.8	3.9	0.7
	15	20.7	0.0	5.3	1.6	4.4	0.7
20	0	—	—	—	—	4.1	0.4
	2	20.6	0.0	9.2	2.6	3.0	0.1
	5	20.8	0.0	5.4	1.3	1.9	0.2
	9	20.3	0.0	4.4	1.7	0.6	0.2
	13	19.9	0.3	5.0	2.1	0.3	0.1
40	0	—	—	—	—	4.1	0.4
	1	20.3	0.1	4.2	0.6	0.9	0.2
	4	20.6	0.0	2.1	0.8	0.4	0.3
	6	20.5	0.1	2.9	1.7	0.2	0.1
	8	20.5	0.1	2.7	1.9	0.2	0.1

— = not measured
$\bar{x}$ = average value of 9 measurements
$\pm$ s = standard error

This is in agreement with the data published so far in the literature on the effects of gamma irradiation on lipids of nutmeats. Very slight, insignificant changes in the lipid composition of walnuts were observed by BANCHER and co-workers (1972a,b,c) at 5 kGy, i.e. at a dose 5- to 10-times higher than the disinfesting dose. KASHANI and VALADON (1984) found that 0.5 kGy did not affect the lipids of pistachio kernels, and even 10 kGy had very little effect, only a slight increase of the peroxide value, on the lipids of pistachio kernels.

For this reason, our analytical data for both untreated and irradiated samples were pooled as replicates at each particular packaging and storage condition for evaluating effects of packaging and storage on chemical parameters of lipids in walnuts.

2.4. Effects of packaging and storage conditions on chemical properties of lipids in walnuts

2.4.1. Peroxide value. The average peroxide values of 9 samples of walnuts irradiated and non-irradiated are shown in Fig. la,b,c.

Results indicated that the peroxide value passed through a maximum and was very much influenced by the temperature of storage. A ten- to fifteen-

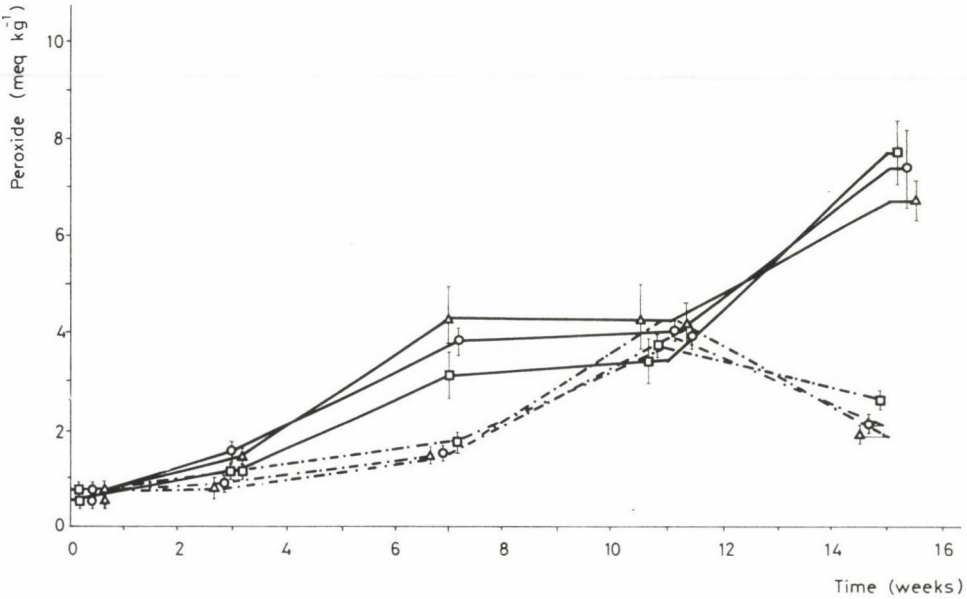

Fig. 1a. Peroxide value of oil from shelled walnuts, irradiated and non-irradiated, packed in different packages and stored at 5 °C, 85% R. H.
□——□: Polyethylene, air; ○——○: laminate, air; △——△: can, air; □— — —□: polyethylene, nitrogen; ○—·—·—○: laminate, nitrogen △—·—·—△: can, nitrogen

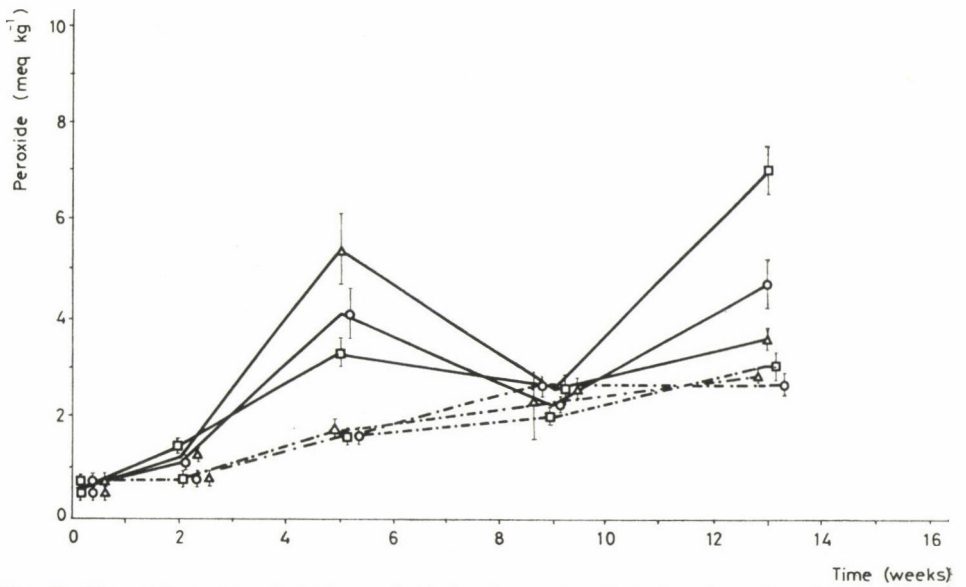

Fig. 1b. Peroxide value of oil from shelled walnuts, irradiated and non-irradiated, packed in different packages and stored at 20 °C, 50% R. H. For symbols see Fig. 1a

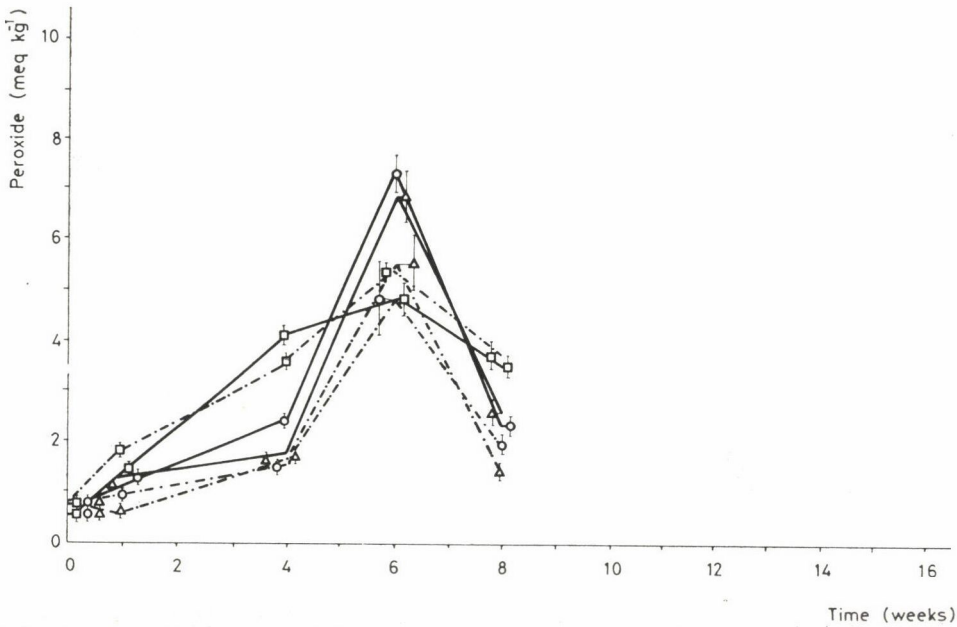

Fig. 1c. Peroxide value of oil from shelled walnuts, irradiated and non-irradiated packed in different packades and stored at 40 °C, 30% R. H. For symbols see Fig. 1a

fold increase in peroxide value was observed in all packages and both gas conditions after 6 weeks of storage at 40 °C. Peroxide value increased as the storage time advanced also in the samples kept at 5 °C and 20 °C in all the packages under both gas conditions. Measurements revealed, however, that the residual oxygen level (Table 2) in the nitrogen-gas-filled bags was sufficient to allow development of rancidity.

The results indicate that peroxidation was lowest in laminated foil and cans under nitrogen gas during storage at 5 °C and 20 °C.

2.4.2. p-*Anisidine value.* The average *p*-anisidine values of 9 samples of walnuts irradiated and non-irradiated are shown in Fig. 2a, b, c.

The highest value was found in polyethylene at 40 °C as compared to laminated foil and cans. The effect of cans and laminated foil under nitrogen gas on the formation of carbonyl compounds was not significantly different at 5 °C and 20 °C.

2.4.3. Free fatty acid content. The average free fatty acid contents of 9 samples of walnuts irradiated and non-irradiated are shown in Fig. 3a, b, c.

There was some increase in the free fatty acid content in all the walnut samples during the storage period.

HARDRON and co-workers (1981) reported that the free fatty acids in walnuts were initially low and increased to 2.6% at 12–14 °C, 70–80% R. H. during a storage period of 12 months. In our experiments, samples stored at

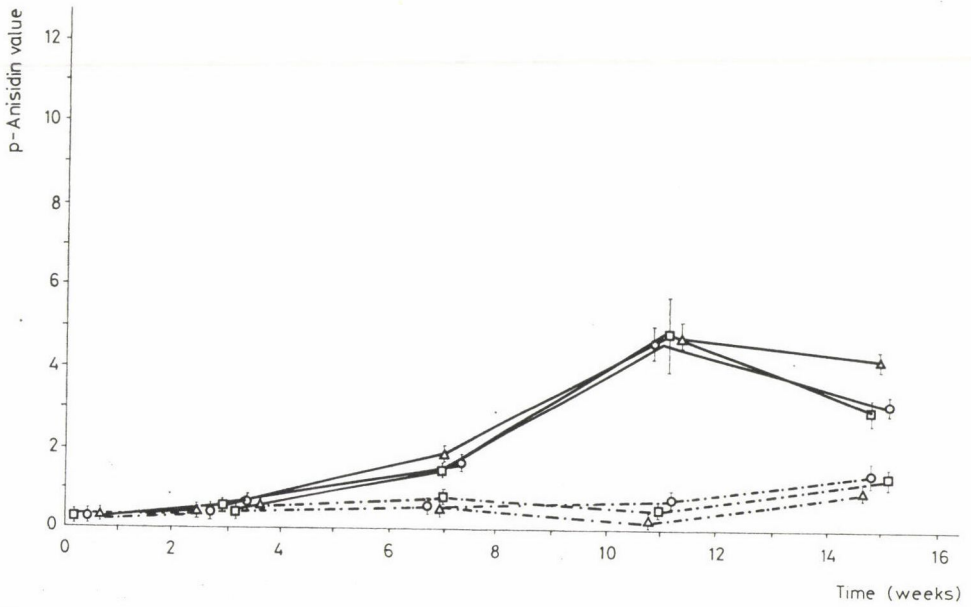

Fig. 2a. Anisidine value of oil from shelled walnuts, irradiated and non-irradiated, packed in different packages and stored at 5 °C, 85% R. H. For symbols see Fig. 1a

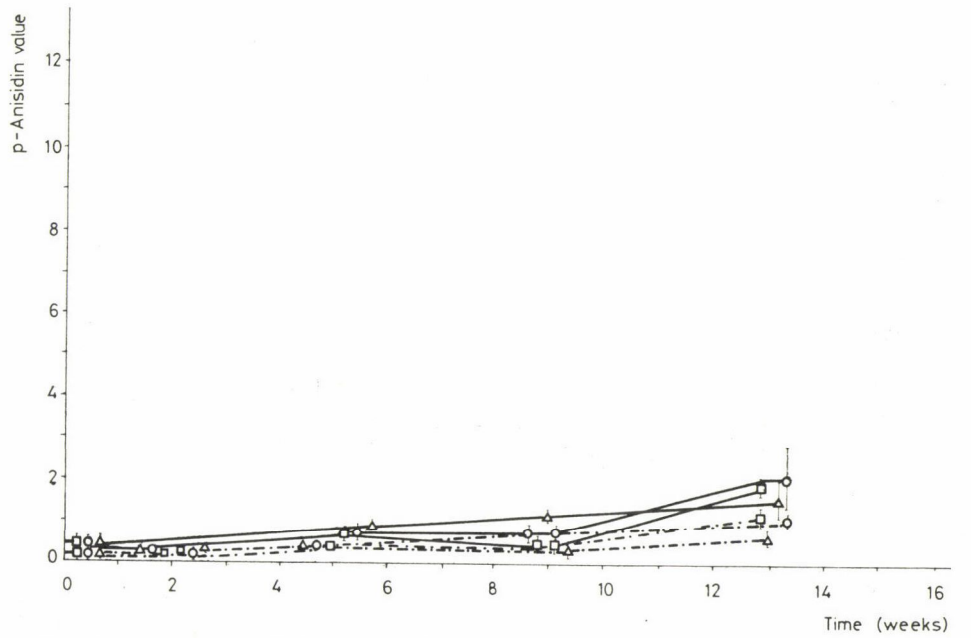

Fig. 2b. Anisidine value of oil from shelled walnuts, irradiated and non-irradiated, packed in different packages and stored at 20 °C, 50% R. H. For symbols see Fig. 1a

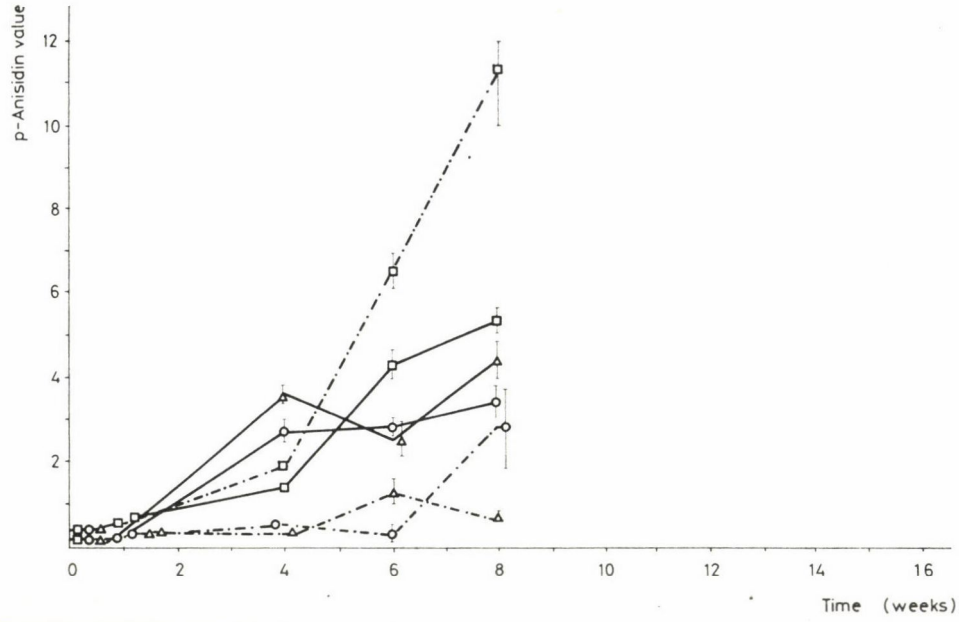

Fig. 2c. Anisidine value of oil from shelled walnuts, irradiated and non-irradiated, packed in different packages and stored at 40° C, 30% R. H. For symbols see Fig. 1a

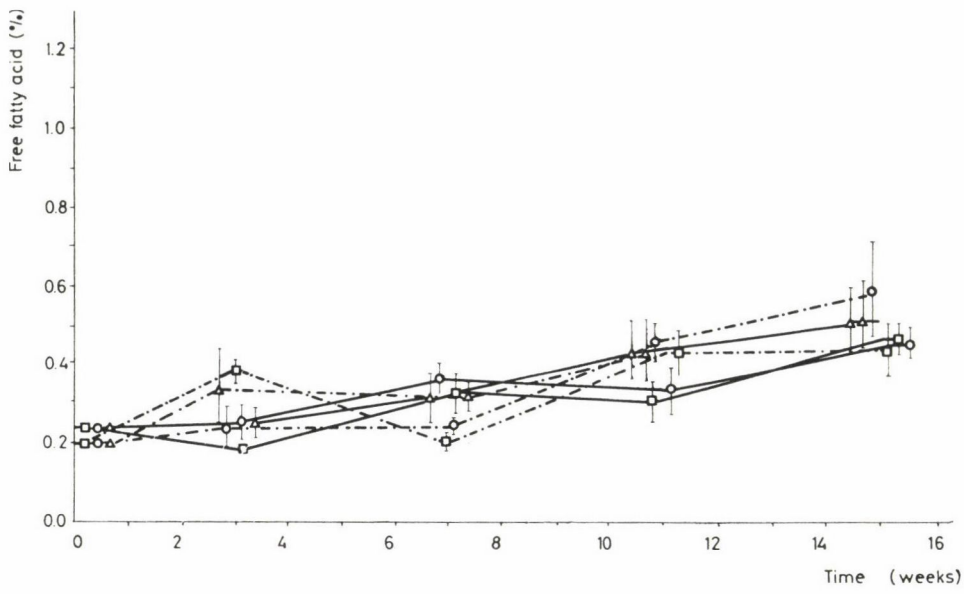

Fig. 3a. Free fatty acid content of oil from shelled walnuts, irradiated and non-irradiated, packed in different packages, stored at 5 °C, 85% R. H. For symbols see Fig. 1a

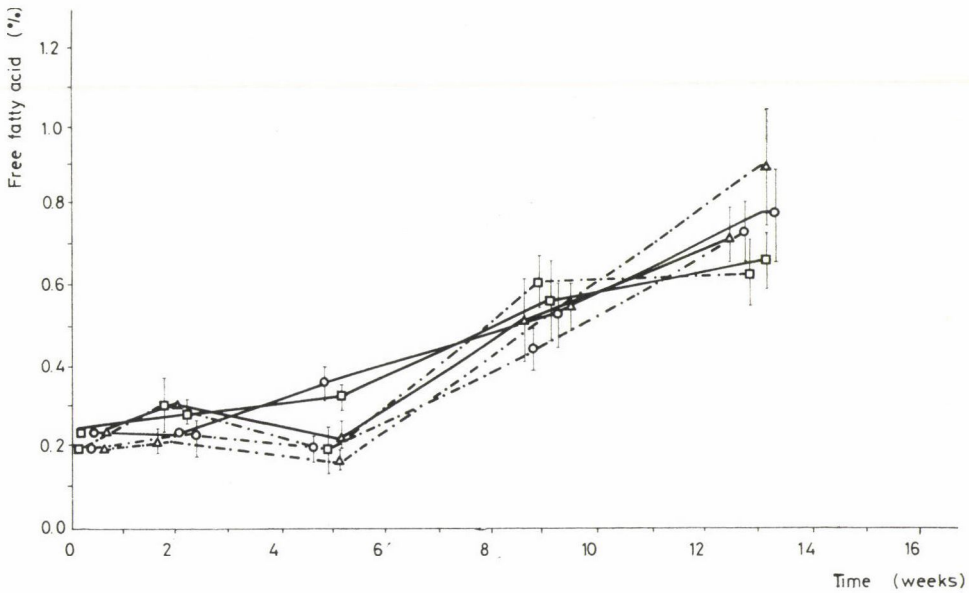

Fig. 3b. Free fatty acid content of oil from shelled walnuts, irradiated and non-irradiated, packed in different packages, stored at 20 °C, 50% R. H. For symbols see Fig. 1a

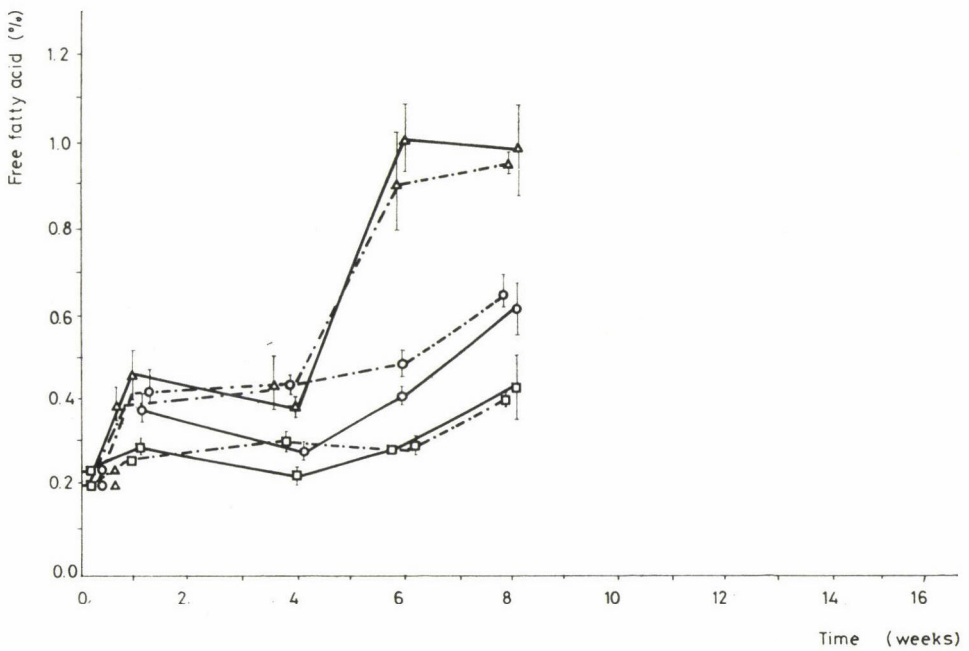

Fig. 3c. Free fatty acid content of oil from shelled walnuts, irradiated and non-irradiated, packed in different packages, stored at 40 °C, 30% R. H. For symbols see Fig. 1a

5 °C showed much less increase than samples stored at 20 °C or 40 °C. The composition of gas atmosphere had no significant effect on these lipolytic changes. At 40 °C, the free fatty acid content was lower in the polyethylene-packaged than in the laminated pouch-packed samples, and even much less than in the canned samples. This seems to indicate that desiccation during storage (see Table 1) inhibits lipolysis.

2.4.4. Tocopherol content. The results of tocopherol determinations are given in Tables 3 and 4.

There seemed to be no significant difference between the different treatments and storage conditions.

Table 3

The effect of packaging conditions on the retention of tocopherols (mg per kg oil) in untreated walnuts stored at 5 °C

Tocopherol	Storage (week)	Polyethylene		Laminate		Can	
		air	N_2	air	N_2	air	N_2
Alfa	0	9	—	—	—	12	11
	8	10	10	11	12	9	14
Beta + gamma	0	307	—	—	—	342	384
	8	337	351	328	370	286	374
Delta	0	34	—	—	—	36	42
	8	40	42	42	42	46	51

— = not investigated

Table 4

The effect of irradiation and storage temperature on retention of tocopherols (mg per kg oil) in walnuts packed in cans under air or nitrogen

Storage temperature °C	Storage time (week)	Radiation dose (kGy)	Alfa		Beta + Gamma		Delta	
			air	N_2	air	N_2	air	N_2
5	0	0	12	11	342	384	36	42
	8		9	14	286	374	46	51
	0	0.5	8	12	281	353	46	46
	8		9	9	295	242	47	44
	0	1.0	10	8	305	288	43	48
	8		10	11	248	296	36	43
20	0	0	11	12	369	368	51	46
	8		11	12	361	354	42	40
40	0	0	14	11	375	346	46	40
	8		11	12	342	336	45	48

Table 5

Results of sensory test of irradiated and non-irradiated walnuts, stored at 5 °C and packed in cans under nitrogen or air

Storage time (week)	Sensoric aspect	Sensory test	0 kGy				0.5 kGy				1.0 kGy			
			Air		Nitrogen		Air		Nitrogen		Air		Nitrogen	
			AS	RS	AS	RS	AS	RS	AS	RS	AS	RS	AS	RS
0		c	3.6	39	3.7	36	3.6	34.5	3.7	38.5	3.8	36	3.2	47
3		b	3.4	36	3.8	25.5	3.2	42.5	3.5	33.5	3.3	39	3.5	33.5
7	Colour	b	3.4	36	3.4	34	3.4	36	3.4	35.5	3.4	36.5	3.6	32
11		c	3.3	44.5	3.7	32.5	3.5	39	3.3	43	3.6	34	3.6	38
15		a	3.6	29.5	3.4	32.5	3.4	30.5	3.6	27.5	3.1	40.5	3.6	28.5
0		c	3.6	39	3.6	39	3.6	37.5	3.8	32.5	3.5	42.5	3.4	40.5
3		b	3.5	36.5	3.3	32.5	3.4	39	3.7	31.5	3.4	38.5	3.7	32
7	Odour	b	3.6	39	3.5	33	3.9	30	3.8	33	3.6	39	3.7	36
11		c	3.2	44.5	3.4	27	3.4	40.5	3.3	12.5	3.5	37	3.4	39.5
15		a	3.6	29.5	3.4	35.5	3.4	32.5	3.4	32.5	3.3	35.5	3.8	23.5
0		c	3.8	31.5	3.7	35	3.5	43	3.9	32.5	3.1	51.5	3.6	37.5
3		b	3.1	43	4.3	18.5	3.1	45	3.5	34.5	3.4	38	3.7	31
7	Taste	b	3.3	41.5	3.4	38.5	3.8	27	3.6	32.5	3.2	41.5	3.7	29
11		c	3.0	44	3.6	32.5	3.7	31.5	3.1	42.5	3.6	36	3.2	44.5
15		a	3.6	28.5	3.2	38.5	3.0	41	3.9	21.5	2.9	40.5	4.0	19
0		c	3.7	40.5	3.9	37	3.9	36.5	3.9	38	3.9	36	3.6	43
3		b	3.5	39.5	4.3	21.5	3.3	44	3.7	33.5	3.9	29	3.4	42.5
7	Texture	b	3.7	35	3.7	35	3.9	30.5	3.8	32.5	3.4	41.5	3.7	35.5
11		c	3.2	47	3.9	28	3.8	31	3.4	43.5	3.6	38.5	3.4	43
15		a	3.7	26.5	3.2	36.5	3.6	29.5	3.7	26	3.3	35.5	3.3	35

AS: average score
RS: rank sum
a: 9 panel members, rank sum borders 19–44 at P=0.05 probability level
b: 10 panel members, rank sum borders 22–48 at P=0.05 probability level
c: 11 panel members, rank sum borders 25–52 at P=0.05 probability level
—: significantly differ (better)

Table 6

Results of sensory test of non-irradiated walnuts packed in different packages and conditions, stored at 5 °C and 90% R. H.

Storage time (week)	Sensoric aspect	Sensory test	Polyethylene				Laminated foil				Can			
			air		nitrogen		air		nitrogen		air		nitrogen	
			AS	RS	AS	RS	AS	RS	AS	RS	AS	RS	AS	RS
0		c	3.6	39	3.7	36	3.6	39	3.7	36	3.6	39	3.7	36
3		b	3.7	35	3.5	41	3.6	38	3.8	32	3.4	35	3.8	29
7	Colour	b	3.7	37	3.7	37	3.7	37	4.0	31	3.4	34	3.4	34
11		c	3.6	45	3.7	39	3.8	36	3.7	39	3.3	30.5	3.7	41.5
15		a	3.6	36.5	3.6	36	3.7	33.5	3.9	28.5	3.6	26	3.4	28.5
0		c	3.6	39	3.6	39	3.6	39	3.6	39	3.6	39	3.6	39
3		b	3.1	43.5	3.7	31	3.4	39	3.3	39	3.5	33.5	3.3	24
7	Odour	b	3.5	36	3.6	35	3.7	32	3.5	37	3.6	36	3.5	34
11		c	3.6	37	3.6	39	3.4	44.5	3.2	50	3.2	22	3.4	38.5
15		a	3.9	27	3.9	27	3.6	33.5	3.3	36	3.6	36.5	3.4	29
0		c	3.8	31.5	3.7	35	3.8	31.5	3.7	35	3.8	31.5	3.7	35
3		b	3.7	33	3.4	39.5	3.4	42.5	3.7	33	3.1	32.5	4.3	29.5
7	Taste	b	3.2	42.5	3.8	29.5	3.7	33	3.6	33	3.3	35	3.4	37
11		c	2.8	56	3.6	36	3.7	33	3.6	37.5	3.0	26	3.6	42.5
15		a	3.0	39.5	3.7	26	3.6	29	3.3	30.5	3.6	31.5	3.2	32.5
0		c	3.7	40.5	3.9	37	3.7	40.5	3.9	37	3.7	40.5	3.9	37
3		b	3.8	34	3.3	43.5	3.6	39.5	3.9	31	3.5	34	4.3	28
7	Texture	b	3.6	34.5	3.6	36.5	3.6	35.5	3.8	29.5	3.7	37.5	3.7	36.5
11		c	3.7	40	3.8	31	3.6	46	3.8	37	3.2	37	3.9	40
15		a	3.8	28	3.7	30	3.8	27.5	3.6	32.5	3.7	29.5	3.2	41.5

AS: average score
RS: rank sum
a: 9 panel members, rank sum borders 19–44 at P = 0.05 probability level
b: 10 panel members, rank sum borders 22–48 at P = 0.05 probability level
c: 11 panel members, rank sum borders 25–52 at P = 0.05 probability level
—: significantly differ (better)
- - - : significantly differ (worse)

Table 7

Results of sensory test of non-irradiated walnuts packed in cans under nitrogen or air, stored at different temperatures (°C) and relative humidities (% R. H.)

Storage time (week)	Sensoric aspect	Sensory test	5 °C – 90% R. H.				20 °C – 50% R. H.				40 °C – 30% R. H.			
			air		nitrogen		air		nitrogen		air		nitrogen	
			AS	RS	AS	RS	AS	RS	AS	RS	AS	RS	AS	RS
0		c	3.6	39	3.7	36	3.6	39	3.7	36	3.6	39	3.7	36
3		b	3.4	33.5	3.8	36.5	3.3	36.5	3.6	28.5	3.0	38.5	3.3	36.5
7	Colour	b	3.4	27.5	3.4	29.5	3.5	29.5	3.8	22	1.6	56.5	2.5	45
11		c	3.3	24.5	3.7	32.5	3.5	29.5	3.8	23.5	1.1	62.5	1.5	58.5
15		a	3.6	21	3.4	25	3.7	23	3.6	21	1.1	51	1.4	48
0		c	3.6	39	3.6	39	3.6	39	3.7	39	3.6	39	3.6	39
3		b	3.5	38	3.3	31.5	3.2	34	3.3	31.5	2.9	40.5	3.2	34.5
7	Odour	b	3.6	21.5	3.5	27.5	3.6	26.5	3.6	26	1.6	53.5	1.6	55
11		c	3.2	17	3.4	30	3.3	31.5	3.3	31.5	1.1	61	1.2	60
15		a	3.6	20	3.4	25.	3.8	20	3.2	25	1.2	50	1.3	49
0		c	3.8	31.5	3.7	35	3.8	31.5	3.7	35	3.8	31.5	3.7	35
3		b	3.1	42.5	4.3	25	3.0	34.5	3.5	26	2.9	26.5	2.4	45.5
7	Taste	b	3.3	23.5	3.4	29.5	3.4	28.5	3.6	25	1.4	55.5	1.5	54
11		c	3.0	17	3.6	32	2.6	37	3.4	25	1.0	60	1.0	60
15		a	3.6	21	3.2	20	3.1	25	3.2	24	1.0	49.5	1.0	49.5
0		c	3.7	40.5	3.9	37	3.7	40.5	3.9	37	3.7	40.5	3.9	37
3		b	3.5	31.5	4.3	29.5	3.7	29.5	3.8	26.5	2.8	49	3.1	44
7	Texture	b	3.7	21.5	3.7	30.5	3.4	32.5	3.7	25	2.2	51.5	2.6	49
11		c	3.2	25.5	3.9	34	3.3	28.5	3.6	23.5	1.4	60.5	1.5	59
15		a	3.7	23.5	3.2	20	3.3	23.5	3.4	23	1.1	49.5	1.1	49.5

AS: average score
RS: rank sum
a: 9 panel members, rank sum borders 19–44 at P = 0.05 probability level
b: 10 panel members, rank sum borders 22–48 at P = 0.05 probability level
c: 11 panel members, rank sum borders 25–52 at P = 0.05 probability level
- - - : significantly differ (worse)
—: significantly differ (better)

2.5. Sensory evaluation

The results of sensory evaluation of walnuts, irradiated and non-irradiated, packed in different packages and stored under different conditions are given in Tables 5, 6 and 7.

All walnut samples stored at 5 °C and 20 °C were within the range of acceptability scores at all aspects up to 15 weeks storage, investigated. No significant differences could be observed between irradiated and non-irradiated samples. The packaging also did not effect the sensory quality of the walnuts. However, high temperature storage (40 °C) drastically reduced the acceptability period.

The colour of the walnuts, which was quite attractive at the beginning of the experiment, changed from light to dark shades during storage at 40 °C. This dark colour increased considerably with the storage period. The texture also changed to become less brittle and softer and some objectionable odour and taste was observed in walnuts stored at 40 °C for 7 weeks or longer. These results can be explained by the fact that the high temperature storage facilitated lipid decomposition in nuts, causing objectionable changes in all sensory parameters.

In conclusion then, our results — in agreement with data reported on similar products — showed that radiation disinfestation doses do not cause off-flavour or rancidity of walnuts.

*

Special recognition and thanks are due to Mr. J. LABRIJN and Mrs. D. van MAZIJK and all the members of the Central Laboratory and the Department of Sensory Evaluations of the RIKILT for providing assistance and some of the necessary research facilities and equipments during completion of this study.

Literature

ANON. (1968): Publikatieblad van de Europese Gemeenschappen L 239/12, Bijlage III Staatsdrukkerij en Uitgeversbedrijf, The Hague, The Netherlands.

ANON. (1980): Nederlandse norm NEN 6332. Nederlands Normalisatie-instituut, Rijswijk (ZH), The Netherlands.

ANON. (1982): Tocopherol determination, Method 2404, International Union of Pure and Applied Chemistry. *J. appl. Chem., 54*, 233–245.

A. O. A. C. (1975): Methods of analysis. Association of Official Analytical Chemists, Washington, D. C., USA, No. 2.504.

BANCHER, E., WASHÜTTL, J. & GOLLER, H. J. (1972a): Untersuchungen der Lipide in den Sphaerosomen- und Mitochondrienfraktionen γ-bestrahlter Samen von Erdnuss *(Arachis hypogaea)* und Walnuss *(Juglans regia)*. *Z. Pflanzenphysiol., 67*, 399–403.

BANCHER, E., WASHÜTTL, J. & GOLLER, H. J. (1972b): Einfluss der Gamma-Bestrahlung auf die Lipide von Erd- und Walnüssen *(Arachis hypogaea* L. und *Juglans regia* L.). — Part I.: Qualitative und quantitative Untersuchungen der Neutrallipide und freien Fettsäuren. *Seifen-Öle-Fette-Wachse, 98*, 370–372.

BANCHER, E., WASHÜTTL, J. & GOLLER, H. J. (1972c): Einfluss der Gamma-Bestrahlung auf die Lipide von Erd- und Walnüssen *(Arachis hypogaea* L. und *Juglans regia* L.). — Part II. Qualitative und quantitative Untersuchungen der polaren Lipide. *Seifen-Öle-Fette-Wachse, 98*, 505–507.

BEUCHAT, L. R. (1978): Water activity as affected by moisture content of tree nuts. *J. Fd Sci.*, 43, 754.

BROWER, J. H. & TILTON, E. W. (1970): Insect disinfestation of dried fruit by using gamma radiation. *Fd Irradiat., 11*, (1–2), 10.

BROWER, J. H. & TILTON, E. W. (1972): Insect disinfestation of shelled pecans, almonds and walnuts by gamma radiation. *J. econ. Ent., 65*, 222–224.

HARDRON, H., KEME, I., KLEINER, J., MESSERLI, M. & ZURCHER, K. (1981): The behaviour of some nuts under different storage conditions. *CCB Review for Chocolate, Confectionery and Bakery, 6*, (3), 27–31.

KASHANI, G. G. & VALADON, L. R. G. (1984): Effects of gamma irradiation on the lipids, carbohydrates and proteins of Iranian pistachio kernels. *J. Fd Technol., 19*, 631–638.

KRAMER, A. (1956): A quick rank test for significance of differences in multiple comparisons. *J. Fd Technol., 10*, 391–392.

LABUZA, T. (1971): Auto-oxidation of nuts. *C. R. C. Crit. Rev. Fd Sci. Technol., 2*, 355.

LEA, C. H. (1952): Methods for determining peroxide in lipids. *J. Sci. Fd Agric., 3*, 386.

NORTHOLT, M. D. & HEUVELMAN, C. J. (1984): The salt crystal liquefaction test. A simple method for testing water activity of foods. *J. Fd Protection, 48*, 537.

SEDLAČEK, B. A. J. (1958): Beurteilung der Walnusskerne durch objektive Ranzigkeit-Methoden. *Z. Lebensm. Unters. u. -Forsch., 107*, 28–32.

SOUCI, S. W. & FACHMANN, W. (1981/82): *Food composition and nutrition tables.* Wissenschaftliche Verlagsgesellschaft GmbH, Stuttgart, Germany, pp. 1124–1125.

TALHOUK, A. S. (1966): Prospects and needs for radiation disinfestation of packed fruits and vegetables. — in: *Application of food irradiation in developing countries.* Tech. Rep. Ser. no. 54. IAEA, Vienna, pp. 105–113.

TILTON, E. W. & BURDITT, A. K. (1983): Insect disinfestation of grain and fruit. — in: JOSEPHSON, E. S. & PETERSON, M. S. (Eds.) *Preservation of food by ionizing radiation.* CRC Press, Inc., Boca Raton, Florida, USA. Vol. 2. pp. 216–229.

WAHID, M., SATTAR, A., NEELOFAR, ATTA, S., AHMAD, A., JAN, M. & KHAN, I. (1985): Radiation disinfestation and chemistry of dried fruits and nuts. — in: *NIFA, Annual Report 1984–85*, Nuclear Institute for Food and Agriculture, Tarnab, Peshawar, Pakistan, pp. 25–40.

Acta Alimentaria, Vol. 17 (1), pp. 33—35 (1988)

CAROTENOID COMPOSITION AND VITAMIN A VALUE OF RAMI *(BOHEMERIA NIVEA)* LEAVES

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(Received: 20 December 1985; revision received: 3 December 1986; accepted: 12 December 1986)

The carotenoids of the fresh and dried leaves of *Bohemeria nivea* were extracted and identified. Lutein as well as α- and β-carotene were found in fresh leaves but only β-carotene and lutein were present in the dried leaves. The high vitamin A value, 10 666 and 16 933 IU per 100 g for the fresh and dried leaves, respectively, is due to the β-carotene content.

Keywords: rami, carotenoids, vitamin A, chromatography

Rami, the source of an excellent textile fiber, is an important crop in Brasil and several other tropical countries. A considerable amount of unused leaves is a by-product of the fiber production (from the stem) and has not yet found any practical use.

The dried and milled leaves, due to their high content of protein and calcium, might become a valuable feed for cattle and preliminary experiments indicated the presence of considerable amounts of carotenoids, the extraction, separation and quantification of which we are reporting in the present paper.

1. Material and methods

Three samples each of the fresh and dried leaves were analyzed separately. For the fresh leaves, each sample was prepared from about 30 leaves. The dried sample was prepared by air drying about 8 kg of leaves. Twenty-two gram samples were taken for analysis. The dried sample was stored at −20 °C in tightly sealed plastic bags, until the analysis could be initiated.

The water content of sample was determined by drying in a vacuum oven at 65–70 °C for 24 h.

Carotenoids were extracted with cold acetone (Rodriguez et al., 1976), transferred to hexane, saponified with 10% KOH–MeOH, washed, concentrated and separated on a MgO : Hyflo-Supercel (1 : 2)column, using as developer, 2% Et_2O/hexane, 5% Et_2O/hexane followed by pure acetone. Two zones

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were separated from the extract of dried leaves and three, from the fresh leaves.

The material eluted with acetone, suspected to be a hydroxyl-carotenoid, was methylated with acidified MeOH (DAVIES, 1976).

Purity of each fraction was checked by rechromatography on silica gel plates and Al_2O_3 column.

Spectra of each purified fraction were taken with a Pye Unicam 8000 recording spectrophotometer (England) using hexane as solvent.

The vitamin A values were calculated assuming that 0.6 g of β-carotene is equivalent to 1 IU per vitamin A (NAS-NRC, 1980).

2. Results

Table 1 summarized the results of the quantitation and spectra characteristic of each carotenoid isolated from rami leaves.

Table 1

Carotenoid composition of fresh and dried rami leaves

Carotenoid	Concentration (μg per g)			
	Dried leaves (9% H_2O)		Fresh leaves (73% H_2O)	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
α-Carotene	—		2.30	0.01
β-Carotene	101.53	0.06	64.03	0.06
Lutein	34.67	0.46	14.22	0.10

$\bar{x}$: mean value of 3 separate analysis
$\pm s$: standard deviation

Chromatographic behaviour (zone sequence and elution characteristics) were indicative of α- and β-carotene and lutein like structure.

The visible spectra in petroleum ether were consistent with α-carotene for fraction 1 (λ_{max} at 420, 442, 471 nm), β-carotene for fraction 2 (λ_{max} at 424, 447, 474 nm) and lutein for fraction 3 (λ_{max} at 422, 444, 471 nm) (DAVIES, 1976). The latter pigment was also characterized by its positive response to methylation producing a compound with an R_f equivalent to a monohydroxy-carotenoid. This is consistent with the presence of 2 hydroxyl groups, one of which is in an allylic position. The absence of substituents in the first two pigments, already indicated by the facile elution from the column, was confirmed on a silica gel plates developed with 3% methanol in benzene, where they ran with the solvent front.

The vitamin A values, from the β-carotene content only, is 16 933 IU per 100 g dried leaves or 10 666 IU per 100 g of fresh leaves. These values are higher than those obtained in eleven common Brasilian leafy vegetables, the highest value being that of parsley (9 800 $\pm$ 4 027).

The results of this study provide another justification for the utilization of rami leaves as animal feed. Aside from its high protein and calcium contents, it is also very rich in provitamin A.

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We gratefully acknowledge the kindness of Mr. R. BENATTI JR., (Instituto Agronomico, Campinas) of putting at our disposal the fresh and dried leaves.

Literature

DAVIES, B. H. (1976): Carotenoids. — in: GOODWIN, T. W. (Ed.) *Chemistry and biochemistry of plant pigments*. Academic Press, London, Vol. 2. pp. 108–111, 144, 150.

NAS-NRC (1980): *Recommended dietary allowances* (9th ed.). National Academy of Science, Washington, D. C., pp. 56–57.

RODRIGUEZ, D. B., RAYMUNDO, L. C., LEE, T. C., SIMPSON, K. L. & CHICHESTER, C. O. (1976): Carotenoid pigment changes in ripening *Momordica charantia* fruits. *Ann. Bot.*, *40*, 615–624.

Acta Alimentaria, Vol. 17 (1), pp. 37—41 (1988)

APPLICATION OF OVERPRESSURE LAYER CHROMATOGRAPHY IN RED PEPPER ANALYSIS

STUDY OF THE CAROTENOIDS GIVING THE RED COLOUR TO GROUND RED PEPPER

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(Received: 21 April 1986; revision received: 5 November 1986; accepted: 31 January 1987)

An overpressure layer chromatography apparatus made on the basis of a Hungarian patent was used to examine the carotenoid composition of red pepper and of the millings prepared from it. Overpressure layer chromatography was found to ensure a more selective separation of the carotenoids and a possibility of their more exact evaluation than traditional layer chromatography. Quantitative determination was carried out photometrically after scraping off and extracting the spots. About 92 to 98% of the carotenoids examined was recovered by the method used. In the case of the paprika variety cultivated on the largest scale it was possible to follow up the change of chloroplast pigments into chromoplast pigments during ripening. It was established that during ripening the amount of capsanthin and capsorubin decisively affecting the colour of red pepper continuously increased. In the ripe stage the red pigments form about 60% of the total colouring substance content and of these about 54% is provided by capsanthin.

Keywords: ground red pepper, overpressure layer chromatography (OPLC), carotenoids

Ground red pepper from Hungary, and especially from Szeged, is a popular and generally used seasoning in many countries of the world. Apart from the features relating to the soil, the climate and the cultivation technology, the fame of this ground pepper is based to a considerable extent on the individual preparation and milling technology, and on the extremely strict, wide-ranging quality control. Up-to-date quality control is performed by means of organoleptic, physical, chemical and microbiological examinations. As regards chemical control, any type of chromatography can be used in testing of the colouring material, the capsaicin content, pesticide residues and the browning number. The results of our studies connected with the capsaicin content were earlier reported at a conference (Aczél, 1984). In the present paper we deal with overpressure layer chromatographic investigations of the carotenoids in red pepper. We had two main aims in this: to determine how exactly the characteristic carotenoids of paprika can be measured photometrically after chromatography and elution, and to establish the change in the amounts of these carotenoids during the ripening process.

Overpressure layer chromatography was developed on the basis of a Hungarian patent (Tyihák et al., 1981a). It is a closed-system, planar liquid chromatographic technique where the sorbent layer is completely sealed off

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by the cushioning system under external pressure and the solvent is forced at overpressure onto the layer through the cushioning system. Development can be performed in one direction, in two directions, concentrically, by overrunning or in two dimensions.

The method was introduced in the laboratory practice by the inventors (TYIHÁK et al., 1979, 1981b) and was first for analysis of foodstuffs by SELMECI and co-workers (1983) and of ground pepper ACZÉL and co-workers (1983) and ACZÉL (1985, 1986), respectively.

For the separation of the carotenoids from ground pepper the method introduced by VINKLER and KISZEL-RICHTER (1972) was adapted. The results were compared to those obtained by the reduction method of BARANYAI and SZABOLCS (1976). Total colouring matter was determined as specified in HUNGARIAN STANDARD (1976).

1. Materials and methods

1.1. Preparation of the sample

Red paprika variety Sz-20 was cut up evenly, dried carefully at 65 °C, ground and the ground product passed through a mesh 0.50 sieve. Analyses were carried out in the part falling through the sieve.

1.2. Extraction

One g of ground red pepper was shaken for 1 h in 100 cm^3 benzene. The extract was filtered and the solvent was evaporated at 50 °C in an atmosphere of N_2 in a rotary evaporator. The residue was dissolved in 1 cm^3 benzene and the solution was stored in ampoulles at −10 °C for about 2 weeks.

1.3. Chromatography

Chromatography was carried out on a Chrompres (Labor MIM, Hungary) apparatus using silicagel 60 F_{254} (Merck) layer and petroleum ether–benzene–acetone–acetic acid (40 : 10 : 2 : 2.5) running reagent. Parameters of the apparatus: 1.2 MPa cushion pressure; 0.3 cm^3 per min running rate; 25 min running period.

The 100 μl extract was transferred to the plate in stripes in diffuse light with a Hamilton syringe.

1.4. Quantitative determination

Subsequent to running the plates were dried in diffuse light in nitrogen atmosphere. The spots were sucked into a microcolumn and carotenoids were eluted from the absorbent in benzene. Uniformly 5 cm^3 of the solution was used to determine the extinction on the Unicam SP-6550 type photometer.

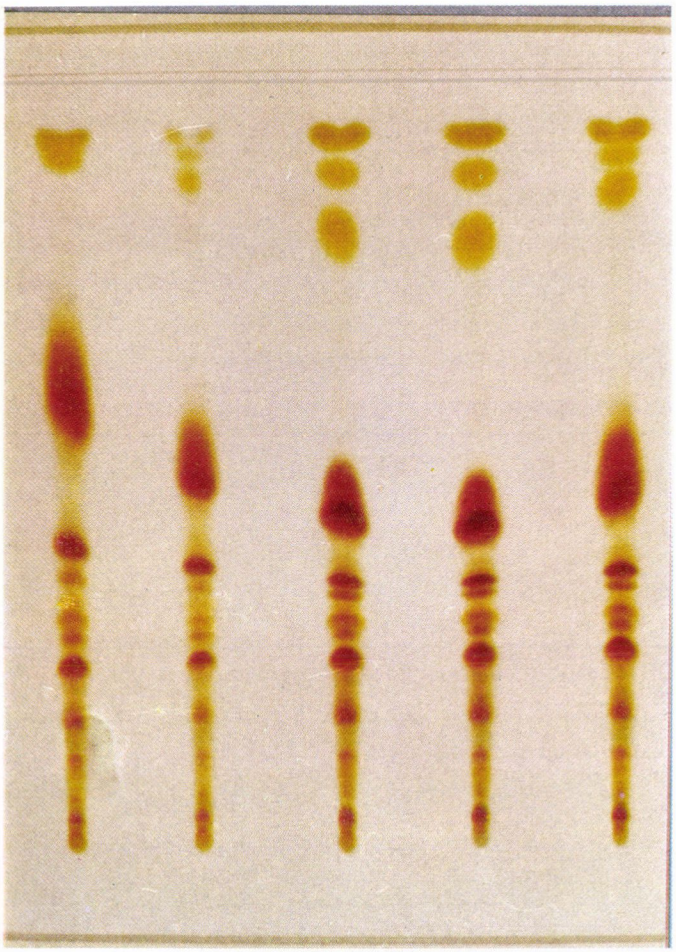

Fig. 1. Separation by overpressure layer chromatography of the red pigments of ground red pepper. 1: capsanthin; 2: capsorubin

2. Results and discussion

Recovery values of the red carotenoid components of ground paprika are summed up in Table 1.

Capsanthin was found to be recovered at 95.2% (± 2.99%) and capsorubin at 91.8% (± 3.88%) level on the average.

The carotenoid content of Sz-20 paprika variety during ripening is shown in Table 2.

In the unripe fruit carotenoid content could not be detected. In the half-ripe fruit both capsanthin and capsorubin are present. Of the total pigment

Table 1

Quantitative determination of the recovered red carotenoid pigments by photometry

Pigment	Solvent (benzene per cm^3)	Recovery (%)		n
		$\bar{x}$	$\pm s$	
Capsanthin	5	95.2	3.02	13
Capsorubin	5	91.8	3.90	9

$\bar{x}$: mean value
$\pm s$: standard deviation
n: number of measurements

Table 2

Carotenoid content[a] of paprika variety Sz-20 in different phases of ripening

Colour	Pigment	Carotenoid content (g per kg)					
		unripe		half-ripe		mature	
Red	Capsanthin	—	—	0.82 $\pm$2.124	0.96 $\pm$1.617	3.32 $\pm$0.980	3.40 $\pm$1.724
Red	Capsorubin	—	—	0.17 $\pm$0.991	0.28 $\pm$1.223	0.57 $\pm$2.190	0.62 $\pm$3.216
Total colouring matter (MSz 9681/5—76)		0.48 $\pm$0.961	0.61 $\pm$0.842	2.14 $\pm$1.090	2.35 $\pm$1.118	6.22 $\pm$0.097	6.24 $\pm$1.240

[a] number of measurements (n) = 4

Table 3

Red pigment content in the total colouring matter of ground red pepper of different quality (variety: Sz-20) in g per kg unit

Quality		Hungarian standard method Total colouring matter	OPLC method			Reduction method	
			Capsanthin	Capsorubin	Total red pigment	Total colouring matter	Total red pigment
Special	$\bar{x}$	5.19	2.80	0.48	3.28	5.27	3.46
	$\pm s$	0.112	0.062	0.018	0.252	0.096	0.128
	v	2.72	3.17	4.21	2.68	2.11	1.89
Dessert	$\bar{x}$	3.82	2.14	0.16	2.30	4.06	2.57
	$\pm s$	0.171	0.121	0.219	0.399	0.861	0.412
	v	3.00	2.19	3.11	3.31	1.67	2.01
Sweet-noble	$\bar{x}$	3.29	1.71	0.10	1.81	3.51	2.02
	$\pm s$	0.285	0.098	0.062	0.179	0.043	0.122
	v	3.79	3.29	3.99	2.17	3.43	2.94
Gulyás	$\bar{x}$	2.67	1.31	0.31	1.62	2.69	1.70
	$\pm s$	0.098	0.281	0.107	0.217	0.101	0.098
	v	3.22	4.02	3.17	3.11	2.19	3.27
Rose	$\bar{x}$	2.16	1.14	0.13	1.27	2.32	1.36
	s	0.198	0.081	0.044	0.321	0.376	0.299
	v	2.97	2.71	1.98	1.97	2.86	2.12

$\bar{x}$: mean value of 5 measurements; $\pm s$: standard deviation; v: variation coefficient (%)

content of the ripe fruit capsanthin forms on the average 54% while capsorubin 9.5%.

The total and red pigment content of ground red pepper gained from the same paprika variety of different quality is shown in Table 3.

In comparing the results of determination by overpressure layer chromatography and the reduction method it can be seen that the results obtained by the reduction method show a 5–11% higher total red pigment content then those obtained by chromatography. On comparing the total pigment content as determined by the standard method and the reduction method again the results of the reduction method are higher. The proportion of capsanthin in the total colouring matter of different quality samples amounts from 49% to 56%

Literature

ACZÉL, A., SELMECI GY. & CSEH F. (1983): Overpressure thin layer chromatography in the examination of foodstuffs. *Proceedings of the 6th World Congress of Food Science and Technology*, Dublin, pp. 34–35.

ACZÉL A. (1984): Application of overpressure layer chromatography in foodstuff examinations. Variation in capsaicin content of red pepper millings as a function of storage. *Proceedings of the Euro Food Pack*, Vienna, pp. 325–330.

ACZÉL, A. (1985): Application of overpressure layer chromatography in red pepper analysis. *Proceedings of the Euro Food Chem.*, Antwerpen, Vol. 3. pp. 1–4.

ACZÉL, A. (1986): Application of overpressure layer chromatography in red pepper analysis. Study of the carotenoids responsible for the red color in ground red pepper. *J. High Resolution Chromat.*, *9*, 407–408.

BARANYAI, M. & SZABOLCS, J. (1976): Determination by reduction of the red and total pigment content in paprika products. *Acta Alimentaria*, *5*, 87–105.

HUNGARIAN STANDARD (1976): *Fűszerpaprika őrlemény vizsgálata. Összes színezéktartalom meghatározása.* (Test methods for ground paprika. Determination of total colouring matter content. MSz 9681) 5—76.

SELMECI, GY., ACZÉL, A. & CSEH, F. (1983): Overpressure thin-layer chromatography in the examination of foodstuffs. *Budapest Chromatography Conference*, pp. 18–19.

TYIHÁK, E., MINCSOVICS, E. & KALÁSZ, H. (1979): New planar chromatographic technique: overpressure thin-layer chromatography. *J. Chromat.*, *174*, 75–81.

TYIHÁK, E., KALÁSZ, M., KULACSY, L., NAGY, L., KÖRMENDI, F., KNOLL, J., MINCSOVICS, E. & GULYÁS, A. (1981a): *Túlnyomásos vékonyréteg kromatográfia* (Overpressure layer chromatography. (Hungarian Patent, No. 173 749.)

TYIHÁK, E., MINCSOVICS, E., KALÁSZ, H. & NAGY, J. (1981b): Optimization of operating parameters in overpressured thin-layer chromatography. *J. Chromat.*, *211*, 45–51.

VINKLER, M. & KISZEL-RICHTER, M. (1972): A thin-layer chromatographic method to determine the pigment content (components) in the pericarp of paprika. *Acta Alimentaria*, *1*, 41–58.

Acta Alimentaria, Vol. 17 (1), pp. 43—51 (1988)

INDICES OF LIPID METABOLISM DISTURBANCE INDUCED BY DIET

(RAT EXPERIMENTS)

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(Received: 15 May 1986; revision received: 6 February 1987; accepted: 6 February 1987)

A synthetic diet inducing derangement of fat metabolism was formulated of an adequate amount of protein, fibre and vitamins and in addition 20% fat, 1% cholesterol and 0.5% sodium cholate. The ratio of mineral salts was also changed toward a pathologic direction.

After feeding Wistar (OÉTI — National Institute of Food Hygiene and Nutrition, Budapest) sexually mature male rats for 6 weeks on this lipogenic diet the serum and liver lipid indices increased significantly in comparison with samples of control animals fed on normal diet (5% fat) or a synthetic normal diet of 20% sunflower seed oil content.

The effects of the following fats were investigated: sunflower seed oil (P/S = 5.2), lard (P/S = 0.24), coconut oil (used for dietetic purposes) (P/S = 0.03) and a mixture of fats characteristic of Hungarian dietary habits (P/S = 0.6). Sunflower seed oil applied in the lipogenic diet caused reduction of total serum cholesterol, while the hepatic total lipid and total cholesterol content was significantly higher than in rats fed on other fats.

Upon consumption of coconut oil the increase in hepatic lipids was less high than with the other fats.

The model experiments causing derangement of fat metabolism proved to be suitable to detect the fat metabolism influencing effect of different changes in the diet.

Keywords: lipogenic diet, fats of different P/S, serum lipids, liver lipids

Diseases related to derangement of fat metabolism (obesity, diabetes, blood vessel diseases) show in several countries, among them in Hungary, a trend to increase. In this phenomenon a number of factors of inadequate nutrition play a role. Many experiments and statistical data prove the effect of excessive energy intake, of the one-sided consumption of nutrients, vitamins, macro- and micro elements. These findings gave an impetus to research into the quantity and quality of edible fats, into the ratio of various fatty acids and their relation to other components.

In earlier rat experiments the effects of various kinds of fat most generally used in Hungary were studied. It was found that the one-sided and exces-

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sive consumption of sunflower seed oil, rich in unsaturated fatty acids, in a diet otherwise of satisfactory composition, had an advantageous effect on the serum lipid indices of rats. Simultaneously, however, it increased substantially certain lipids of the liver in comparison with lard, rich in saturated fatty acids, and with a diet containing sunflower seed oil of the same quality but forming only 5% of the diet (J. N. ZSINKA et al., 1987a, b).

The present study was aimed at clarifying the effect of an inadequate change in the proportions of the other dietary components with the summation of their deletarious effects on the lipid indices and to see whether in consequence fat metabolism is deranged.

1. Materials and methods

In the course of the experiments groups of 20–20 Wistar (OÉTI) (228 ± 9 g) sexually mature male rats were fed near isoenergetically for 6 weeks on lipogenic diet, developed by the authors. (Groups were fed according to the group with the lowest energy intake, pair fed.) The composition of the diet is shown in Table 1.

Table 1

Composition of the diets

Components	Diet		
	Synthetic normal (%)	Synthetic lipogenic (%)	
Casein	20	20	
Starch	40	23	
Sugar	—	15.5	
Cholesterol	—	1.0	
Na-cholate	—	0.5	
Salt mixture (normal)[a] + (normal vitamins)	5	5	(lipogenic) + (vitamins)[b]
Sawdust	15	15	
Fat	20	20	
Total	100	100	
Energy content:			
Joule per 100 g	1775	1737	
Cal per 100 g	424	415	

Lipogenic salt mixture (*g*)

KCl	81.0	Fe-citrate	5.0
NaCl	166.0	$MnCl_2$	2.0
$MgSO_4$	52.0	$ZnCl_2$	1.5
KH_2PO_4	261.0	$CuSO_4$	0.3
$CaCO_3$	431.0	KJ	0.2

[a] SÓS and SZELÉNYI (1974)
[b] Vitamins A and E cut by half

The diet contained the highest level an adequate amount of protein (but it is casein only), fibre and a moderate amount of vitamins. The amount of the other components was changed. A part of the starch content was replaced by beet sugar. Taking into consideration the so-called atherogenic diets (where 2% or more cholesterol is added) a diet was composed to achieve a moderate effect without destruction of the animals and therefore 1% cholesterol and 0.5% Na cholate were added. The proportions of the mineral salt mixture were changed also towards a pathogenic composition.

In this lipogenic diet 20% of different kinds of fat were added. These diets are shown in Table 2.

The composition of fat in group VI was calculated on the basis of "Hungarian Household Statistics, 1983", reflecting the proportions of fats generally consumed in Hungary. Two control groups were used, one of them was fed on normal rat chow (LATI — Laboratory Animals Institute, Gödöllő), containing about 5% fat. The second group was given synthetic normal diet containing 20% sunflower seed oil. (Earlier experiments have shown this latter, otherwise normal diet to increase the lipid content of the liver.) The other

Table 2

Type of diet and the fat composition

	Experimental groups					
	I	II	III	IV	V	VI
Diet	LATI normal (powdered)	synthetic normal	synthetic lipogenic diet			
Fat content (%)	5	20	20	20	20	20
Type of fat	—	sunflower oil	sunflower oil	lard	coconut oil	mixed fats 69% lard 22% sunflower oil 9% butter

Fatty acid composition of fats

	P/S	Medium-chain fatty acids ($C_8C_{10}C_{12}$) (%)
Sunflower seed oil	4.6	—
Lard	0.24	trace
Coconut-oil	0.03	57.8
Mixed fats	0.6	—

P/S = poly-unsaturated/saturated fatty acids

groups were fed on synthetic lipogenic diet, containing 20% fat of different kind. The fatty acid composition of the fats was determined by gas chromatography.

Body mass and diet consumption of the rats were measured and the average diet and the energy consumptions were calculated. The 6 weeks feeding was followed by 18 hours starving and after this, under ether narcosis, blood was taken from the abdominal aorta. In samples of the serum and from the liver extracted according to FOLCH and co-workers (1957), the total lipid content was determined by the method of ZÖLLNER and KRISCH (1962). The triglyceride content of the serum was determined by Boehringer's enzymatic test method, that of the liver by the REANAL- (extraction) test. The enzymatic test of Gödecke was used to determine the cholesterol content and DOLE and MEINERTZ's method (1960) to determine the free fatty acid content. The weight of the organs (liver, heart, kidneys, spleen) was measured and their relative weights were calculated. Group averages and standard deviations were calculated and the significance of differences was established by analysis of variance (SACHS, 1984). Histological examinations were made of the organs.

2. Results

The diet and energy consumption of the rats is shown in Table 3.

The control groups fed normal diets (I, II) consumed slightly more than the other groups. The energy consumption of the other groups did not differ substantially.

Data of body mass are given in Table 4.

As a result of consuming lipogenic diet the body mass lagged behind the normal growth (I, II) by about 10–20%. In six weeks (except of group V^x) there were no significant differences in the feed efficiency (cal per 1 g b. m.: 22.1, 22.2, 21.8, 20.1, 24.1^x, 22.0). Differences were only in the relative liver mass, these are shown in Table 5.

Table 3

Average diet and energy consumption

	Groups					
	I	II	III	IV	V	VI
Diet (g/day/rat)	15.2	13.5	12.5	12	13	13.1
Energy (J/day/rat)	263	240	217	215	223	225
(Cal/day/rat)	62.7	57.2	51.8	50	53.2	53.4

Table 4

Change in body mass
(after 6 weeks per rat)

		Groups					
		I	II	III	IV	V	VI
Change	$\bar{x}$	119	108	100	105	92	102
	$\pm s$	17	16	14	17	17	13

$\bar{x}$ = mean value; $\pm s$ = standard deviation

Table 5

Change in relative liver mass
(g per 100 g body mass)

		Groups					
		I	II	III	IV	V	VI
Change	$\bar{x}$	3.0[a]	3.1[a]	5.93[b]	6.1[b]	5.4	4.6
	$\pm s$	0.26	0.21	0.42	0.4	0.48	0.61

[b] I, II—III, IV, V, VI $P < 0.05$
[a] III, IV—V, VI $P < 0.05$

Table 6

Lipids in the serum

		Groups					
		I	II	III	IV	V	VI
Total lipids (g per dm^3)	$\bar{x}$	3.2	2.31[a]	3.47[d]	3.9	3.54	4.4
	$\pm s$	0.5	0.93	0.4	0.3	0.75	0.4
Triglyceride (mmol per dm^3)	$\bar{x}$	0.59	0.49[a]	0.7	0.65	0.76	0.55[e]
	$\pm s$	0.04	0.11	0.1	0.1	0.2	0.032
Total cholesterol (mmol per dm^3)	$\bar{x}$	1.7	1.92[a]	3.4[bd]	4.15	3.17	5.44[c]
	$\pm s$	0.4	0.18	0.9	0.6	0.65	0.61
Free fatty acids (mmol per dm^3)	$\bar{x}$	—	0.75[a]	0.58	0.25	0.58	0.30
	$\pm s$		0.18	0.16	0.05	0.06	0.03

Significant differences:
[a] II —I, III, IV, V, VI $P < 0.05$
[b] III—I, II, VI $P < 0.05$
[c] VI—I, II, III, IV, V $P < 0.05$
[d] III—IV, VI $P < 0.05$
[e] VI—III, IV, V $P < 0.05$

In relation to the group fed on normal diet the liver mass of the group fed on lipogenic diet increased significantly. Liver mass relative to body weight increased less in the groups given coconut oil or fat mixture than upon the consumption of the same amount of sunflower seed oil or lard. Table 6 shows the serum lipid values.

As an effect of lipogenic diet, in comparison with the normal diet, the increase in total lipid and total cholesterol (*d*) level in the serum was higher when the fat was provided in the form of lard or mixed fat instead of sunflower seed oil. The mixed fat caused a lower triglyceride level (*e*) but a higher total cholesterol level (*c*) than the other fats.

Liver lipid content are summed up in Table 7.

Table 7

Lipids in the liver

		Groups					
		I	II	III	IV	V	VI
Total lipids (mg per g)	$\bar{x}$	67[a]	170[d]	355[b]	287	264	271
	$\pm s$	0.14	31	68	83	74	52
Triglyceride (mg per g)	$\bar{x}$	17.6[a]	30.4[d]	64[b]	52	40	47.9
	$\pm s$	7.0	6.2	13	9.1	11	12
Total cholesterol (mmol per g)	$\bar{x}$	6.5[a]	22.3[d]	107[b]	81	32[c]	92
	$\pm s$	1.2	5	12	11	7.5	13
Free fatty acid (mmol per g)	$\bar{x}$	14.8[a]	22[d]	8.5	11.5	9.8	10
	$\pm s$	1.8	4.8	1.6	4.2	3.6	1.1

Significant differences:
[a] I, II—III, IV, V, VI $P < 0.05$
[b] III—IV, V, VI $P < 0.05$
[c] V—III, IV, VI $P < 0.05$
[d] II—I, III, IV, V, VI $P < 0.05$

The majority of the liver lipid indices was significantly increased by the consumption of lipogenic diet, while the free fatty acid content was reduced. The total lipid and triglyceride content was lower upon consumption of coconut oil and mixed fat than upon consumption of the other fats (*b*). Total cholesterol content, on the other hand, was lower upon the consumption of lard and coconut oil (*c*). All results were higher upon the consumption of normal synthetic diet containing 20% sunflower seed oil than these obtained with normal (LATI) chow but significantly lower than the results of feeding on lipogenic diet with the same (20%) sunflower seed oil content (*d*). The histological findings were "accumulation of tiny doplets of fat" and "nutritive steatose" of the liver.

3. Conclusion

The aim of our experiments was to develop in rats a lipid metabolism disturbance provoked by dietary means which can be defined by the changes of lipid-indices, both in the serum and the liver. In such a way the role of fats

and other dietary factors (or medicaments) influencing lipid metabolism can be well documented. The advantage of our method is reflected by the facts that it results in pronounced lipid disturbance, without the death of the animals, by much smaller investments of material and in a less time-consuming way than it was used with traditional feeding experiments. Instead of the usual 2% cholesterol content of atherogen diets, our diet contains 1% cholesterol; its protein content corresponds to the upper limit of the adequate level, attaining this level by adding only casein to it without vegetable protein. We changed the proportions of mineral salts in it towards a pathologic direction. It has a diminished A and E vitamin content. Its carbohydrate content is composed partly by sugar. At the same time, in order to avoid digestive disorders it also contains fibers (sawdust).

As an effect of isoenergetical feeding of the synthetic lipogenic diet (formulated by the authors) for 6 weeks developed the known metabolic disorders: all the serum and liver indices studied and the relative liver weights increased as compared to the effect of normal rat chow and synthetic normal diet containing 20% sunflower-seed oil. The total lipid and total cholesterol levels in the serum of rats consuming sunflower oil were significantly lower than on the other fats. This result supports the known serum cholesterol reducing effect of vegetable oils rich in unsaturated fatty acids.

The liver lipids were generally higher, the total cholesterol content significantly higher in the group fed with a diet rich in sunflower oil in comparison to the groups fed on other fats. It was found in earlier experiments that 20% sunflower oil consumed in synthetic normal diet substantially decreased the total cholesterol level in the serum while increasing about two-fold the liver lipid indices in comparison to the same amount of lard or coconut oil. This phenomenon was attributed to the triglyceride and cholesterol accumulating effect of a large amount of mono- and mainly di-unsaturated fatty acids present in sunflower oil (J. N. ZSINKA et al., 1987a) and excessive cholesterol intake. Similar observations were made by CASTER (1977) who observed the increase of the cholesterol pool as an effect of monounsaturated oleic acid or linoleic acid as compared to results obtained with saturated fatty acids. STEWARD (1978) found a high cholesterol depot in calves fed on soya oil in comparison to those fed on animal fats or medium chain triglyceride (MCT) oil. The unadvantageous dietary factors of our diets increased the total lipid content of the liver about five-fold and the total cholesterol nearly twenty-fold. Upon the consumption of lard the increase in total liver cholesterol was less high, while upon the consumption of MCT-oil it was significantly lower, only about five-fold of the control.

The correlation between unsaturated fatty acid- and cholesterol metabolism is not completely clarified, although the serum cholesterol reducing effect of unsaturated fatty acids is well kown. BOCHENEK (1978) found that

cholesterol synthesis in the liver was decreased by the feed-back inhibition of cholesterol consumption particularly if cholesterol was accompanied by linolic acid consumption. HUANG and co-workers (1984) found that the introduction of 1% cholesterol inhibited the transformation of linoleic acid into arachidic acid in the liver and stimulated the cholesterol transport from the serum into the liver. An excessive cholesterol and fat intake reduce the low density lipoprotein (LDL)-receptors of the liver (GOLDSTEIN et al., 1983). These receptors are known to eliminate cholesterol of the liver. In consequence, the cholesterol content of the liver increases and this inhibits the unsaturation-reactions e.g. the transformation of linoleic acid into arachidic acid.

In the present experiments sunflower oil in lipogenic diet increased the total serum cholesterol level slightly, while the total liver cholesterol level was higher than after the consumption of other fats. It is a sign of the lipogenic effect that the free fatty acid content of the liver decreased. The fatty acid oxidation disturbance also led to the accumulation of the triglyceride content in the liver. Beside the above-mentioned factors changes in fat absorption, in the entero-hepatic circulation and in the voiding of fats accompanied the observed results and further experiments are needed to clarify the role of different fats in the in vivo lipid peroxidation, too.

Our data on fats rich in medium chain fatty acids correspond to the findings of LEVEILLE (1967) who observed that upon the simultaneous consumption of oils rich in MCT and cholesterol, the cholesterol content of the liver was lower than upon consuming corn oil. In the present case as discussed earlier (J. N. ZSINKA et al., 1987a) in consequence of the rapid oxidation of medium chain fatty acids (GELIEBTER, 1980) the lipid accumulation in the liver is substantially lower than with longer chain fatty acids. MCT-fatty acids break down to acetyl-coenzyme A and the lipid accumulation decreases (LEVACEV et al., 1982).

The fats-mixture representing the general Hungarian fat-consumption was not advantageous, the total serum cholesterol and the lipids of the liver were substantially higher than the control values. It seems that these proportional distribution of the consumed fats is not wholesome and it is highly indicated to reexamine the quantitative and qualitative factors and formulate a more adequate rate of fat consumption.

As shown by the results, under the present experimental conditions, upon feeding rats on our lipogenic diet a higher serum lipid level and a fatty liver, characteristic of fat metabolism disturbance, developed in six weeks. This lesion of the liver histologically demonstrated as a "nutritive steatosis". Our rat experimental model proved suitable for the detection of the effect of different dietary factors including fats.

Literature

BOCHENEK, W. (1978): Effects of saturated and unsaturated fats given with and without dietary cholesterol on hepatic cholesterol synthesis and hepatic lipid metabolism. *Biochim. biophys. Acta, 16,* 5281–87.

CASTER, W. O. (1976): Effects of dietary palmitate, stearate, oleate and linoleate on the tissue lipids. *J. Nutr. Res., 46,* 480–487.

DOLE, V. P. & MEINERTZ, H. J. (1960): Microdetermination of long chain fatty acids in plasma and tissues. *J. Biochem., 235,* 2595–2598.

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

GELIEBTER, A. (1980): Overfeeding with diet containing MCT impedes accumulation of body fat. *Am. J. clin. Nutr., 33,* 921–929.

GOLDSTEIN, J. L., KITA, T. & BROWN, M. S. (1983): LDL receptors and cholesterol metabolism. *N. Engl. J. Med. 309,* 288–296.

HUANG, Y. S., MANKU, M. S. & HORROBIN, D. F. (1984): The effects of dietary cholesterol on blood and liver PUFA on plasma cholesterol in rats fed various types of fatty acid diet. *Lipids, 19,* 664–672.

J. N. ZSINKA, Á., FÖLDES, V. & PERÉDI, J. (1987a): Certain serum and liver lipid parameters as affected by edible fats of different quantity and quality. *Acta Alimentaria, 16,* 217–223.

J. N. ZSINKA, Á. FÖLDES, V. & PERÉDI, J. (1987/b): Certain serum and liver lipid parameters as affected by different fat mixtures. *Acta Alimentaria, 16,* 225–230.

LEVACEV, M. M., AUST, L., KULAKOVA, S. K. PONOMAROVA, L. G., NOADE, D. & ZAHN, L. (1982): Die Wirkung einer langfristiger Zufuhr mittelkettiger Triglyceride auf den postprandialen Lipidstoffwechsel. *Nahrung, 26,* 77–87.

LEVEILLE, G. A. (1967): Influence of medium chain triglycerides on lipid metabolism in the rat. *Lipids, 2,* 287–295.

SACHS, L. (1984): *Applied statistics.* A handbook of techniques. Springer Verlag, Berlin.

Sós & SZELÉNYI, I. (1974): *Animal diets.* Akadémiai Kiadó, Budapest

STEWARD, J. W. (1978): Effect of various triglycerides on blood and tissue cholesterol of calves. *J. Nutr., 108,* 561–570.

ZÖLLNER, N. & KRISCH, K. (1962): Über die quantitative Bestimmung von Lipiden (Micromethode) mittels der vielen natürlichen Lipoiden. *Z. Ges. Exp. Med., 135,* 545–562.

Acta Alimentaria, Vol. 17 (1), pp. 53—65 (1988)

SOME PROPERTIES OF TOMATO LIPOXYGENASE

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(Received: 4 August 1986; accepted: 30 January 1987)

Lipoxygenase (E. C. I. 13. II. 12) was extracted from tomato fruits *(Lycopersicon esculentum* var. *ventura)* by a modified procedure. The enzyme activity increased proportionally with the progress of ripening at the last stages. The crude and partially purified enzyme was found to be stable at 40 °C and pH 7 for a week without any measurable loss in activity. The enzyme resisted heat treatment at 60 °C for 2 min. Kinetic properties, effects of EDTA and reaction products of the enzyme confirm that a true lipoxygenase exist in tomato extract, and no other lipid oxidizing agents. The enzyme activity had an optimum at pH 4.0–4.5 and $K_m = 0.37 \times 10^{-5}$ mol with linoleic acid substrate. The enzyme activity in the supernatant of 0.6 saturation with (NH_4/SO_4 was 3 times higher than that found in the crude extract. This indicates the removal of some inhibitor during the fractionation process. The fraction of 0.3–0.6 saturation was found to have an inhibitory effect on the partially purified enzyme.

Keywords: lipoxygenase, tomato, enzyme activity

It is well known that lipoxygenase (E. C. I. 13. II. 12) takes part in off-flavor formation and discoloration in foodstuffs through oxidation of fatty acids containing a methylene interrupted system of double bonds such as linoleic, linolenic and arachidonic acids. KAZENIAC and HALL (1970) concluded that the action of a tomato lipoxygenase is possible in the formation of hexanal and hexanals from linoleic and linolenic acids. JADHAV and co-workers (1972) observed the biogenesis of hexanal from linoleic and linolenic acid using slices of tomato fruit. PINSKY and co-workers (1971) investigated lipoxygenase content and antioxidant activity of some fruits and vegetables including tomato. They found that tomato fruit extract contains 320 unit per 10 g and has no antioxidant activity. BONNET and CROUZET (1977) proved the presence of true lipoxygenase in tomato fruits of several varieties. They purified and studied some biochemical properties of this enzyme from the variety ACE55 VF in which activity was the most abundant.

It was very interesting to study the different characteristics of lipoxygenase in the economically most important tomato variety in Hungary (ventura) which is used in production of tomato paste. We tried to extract the enzyme by a modified procedure, partially purify it and study its behavior in relation to various environmental factors.

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1. Materials and methods

1.1. Materials

Tomato fruits *(Lycopersicon esculentum* var. *ventura)* were obtained from the Department of Chemistry, University of Horticulture, Budapest. Pure linoleic acid was from Sigma (USA), other chemicals were from Reanal (Budapest). All solutions were prepared using redestilled water.

1.2. Methods

1.2.1. Enzyme extraction and purification. The method described by BONNET and CROUZET (1977) was modified by avoiding the addition of ascorbic acid and EDTA to the extracting media (both had an inhibitory effect) and reducing the time of extraction by the help of ultrasonication.

Four fruits of tomato were cut into small pieces with a knife and mixed well. Twenty-five g were randomly taken in duplicate and homogenized with quartz sand in a mortar. The macerate was then stirred with 25 cm^3 of TRIS-HCl buffer pH 7 for 15 min. The mixture was exposed to ultrasonic vibration in the ultrasonic bath, Tesla (Czechoslovakia) for 3 min, then strained through a double-layer cheese cloth and centrifuged at 15 000 r.p.m. for 20 min at 2 °C in a Beckman model V-20 refrigerated centrifuge. The supernatant was placed in small vials, frozen and stored at −20 °C until use. For $(NH_4)_2SO_4$ fractionation 75 cm^3 of the crude extract in a small beaker were put into an ice bath and crystalline $(NH_4)_2SO_4$ was gradually added under continuous stirring using a magnetic stirrer at minimum speed. $(NH_4)_2SO_4$ concentration was raised from 0–0.3 and 0.3–0.6 saturation with centrifugation at 15 000 r.p.m. at 2 °C after each step of fractionation. The precipitates were redissolved in small volumes of TRIS-HCl buffer pH 7 and kept at −20 °C when not in use. The supernatant after 0.6 saturation with $(NH_4)_2SO_4$ was found to contain higher activity than the other fractions. This supernatant was dialyzed overnight against 2 l of redestilled water at 4 °C under continuous stirring to remove $(NH_4)_2O_5$.

1.2.2 Reaction mixture. One-tenth cm^3 of pure linoleic acid was suspended in 50 cm^3 of 0.2 mol sodium tetraborate solution containing 0.1 cm^3 Tween 20 using the Tesla ultrasonic device to dissolve linoleic acid as substrate for the enzyme. The reaction mixture consists of 0.1 cm^3 of linoleic acid substrate, 2.9 cm^3 of 0.1 mol phosphate buffer pH 4 and 0.1–0.2 cm^3 of enzyme extract.

1.2.3. Enzyme assay. The method of AL-OBAIDY and SIDDIQI (1981/a), which involves initiation of the reaction in the spectrophotometer cell, was followed. 0.1 cm^3 of substrate were added to 2.9 cm^3 of phosphate buffer pH 4, vigorously shaken and allowed to stand for one minute. The reaction was initiated by adding 0.1 cm^3 of enzyme extract and the increase in absorbance at 234 nm

was automatically measured and recorded using a Spectrod M-40 (Carl Zeiss, Jena) spectrophotometer. The values in the chapter "Results" represent the average of at least 3 measurements with negligible variation between replications. The blank experiment was done by the same method with the exception that the enzyme extract was previously inactivated by boiling for 2 min in a water bath. A unit of enzyme was defined as that amount which produces a change in absorbance of 0.001 per min at 234 nm.

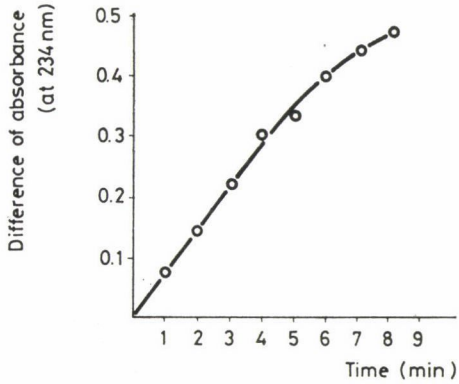

Fig. 1. Rate of tomato fruit lipoxygenase-catalyzed linoleic acid oxidation. The reaction mixture consists of 0.025 cm^3 substrate, 2.9 cm^3 phosphate buffer (pH 4) and 0.3 cm^3 enzyme extract

1.2.4. Protein determination. The spectrophotometric method for protein determination was used for purity measurements during purification studies (KLACKER, 1947). Three cm^3 of enzyme extract were pipetted into the spectrophotometer cell and absorbance at 280 and 260 nm read against a blank containing 0.1 mol phosphate buffer pH 7. The following formula was used to calculate the protein content.

$$\text{Protein (mg cm}^{-3}) = 1.45\ OD_{280} - 0.76\ OD_{260}$$

2. Results and discussion

2.1. Rate of the enzyme reaction

The increase in absorbance at 234 nm was proportional to time for the first 4 min of the reaction (Fig. 1). This allowed the determination of reaction rates in the initial phase of the reaction.

Care must be taken to avoid turbidity of the solution as a result of protein or linoleic acid insolubility at low pH values.

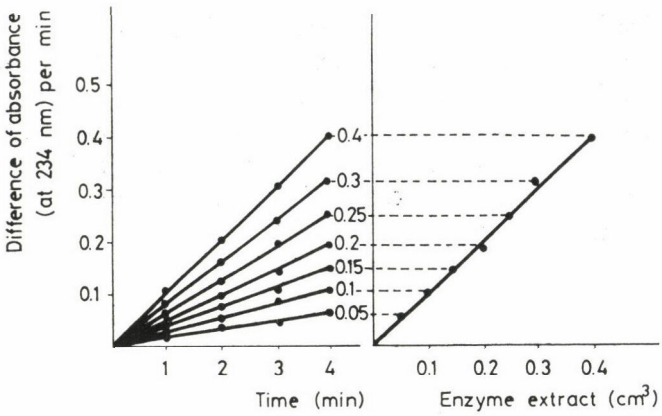

Fig. 2. Effect of various tomato lipoxygenase concentration on the rate of linoleic acid oxidation

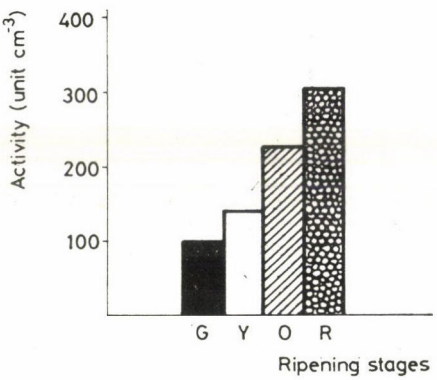

Fig. 3. Development of lipoxygenase activity during the last stages of ripening. G: mature green; Y: mature yellow; O: mature orange; R: mature red; 1 unit = 0.01 increase in absorbance at 234 nm per minute

A linear relationship between the initial rate of reaction and enzyme concentration was maintained over a broad range of enzyme concentrations (Fig.2).

2.2. Enzyme activity during ripening

We have determined activity variations during the last four stages of ripening. The enzyme activity increased proportionally to the progressive development in ripening (Fig. 3). Similar observations were reported for tomato lipoxygenase by JADHAV and co-workers (1972) and BONNET and CROUZET (1977). The increase in lipoxygenase activity during ripening is of great importance from the technological point of view since the enzyme takes part in

the formation of ethylene and the onset of the climacteric process leading to flavor deterioration and discoloration in the ripe fruit at the technologically important stage of ripening (ESKIN & PINSKY, 1977).

2.3. Partial purification of the enzyme

Before attempting purification, studies were made on the factors affecting the extractability of the enzyme, like effect of EDTA and ascorbic acid, effect of Triton X-100 and effect of time of the extraction. It was found that

Table 1

Fractionation of tomato lipoxygenase by ammonium sulfate

Ammonium sulfate concentration	Total unit	Total protein (mg)	Yield (%)	Sp. activity	Purification rate
Crude extract	5600	460	100	12.2	1
Pellet					
0–30%	850	43.5	15	19.5	1.6
30–60%	200	62	3.5	3.2	0.26
Supernatant before dialysis	9900	181	176	55	4.5
Supernatant after dialysis	4400	132	78.5	34	2.8

Sp. activity = Unit cm^{-3} per mg protein cm^{-3}

the presence of EDTA and ascorbic acid at 1% concentration in the extracting media as reported by BONNET and CROUZET (1977) and CAYREL and co-workers (1983) was not convenient and no activity was obtained. Addition of 0.25% and 0.5% of Triton X-100 in the extracting media did not cause any significant increase in the extractability of the enzyme. Stirring the macerate for 15 min at 4–6 °C was sufficient to extract the enzyme without any loss in activity.

Table 1 gives the results of $(MH_4)_2SO_4$ fractionation. The first fraction of 0–0.3 saturation contained some of the enzyme activity with about 2 fold purification. Because of the higher pectin content of this fraction, the redissolved precipitate gave a viscous solution, therefore some of the enzyme protein was entrapped in the jelly-like material of this fraction during centrifugation. The second fraction of 0.3–0.6 saturation was a greenish-yellow precipitate after centrifugation, the solution of this precipitate had the lowest enzyme activity. The richest fraction was the supernatant of 0.6 saturation after removal of the salted-out proteins by centrifugation. The supernatant had no capac-

ity to dissolve more than 0.6 saturation of ammonium sulfate under the experimental conditions. About 5 fold purification with 17.6% yield has been achieved by this process. The increase in the enzyme activity in the supernatant of 0.6 saturation was due to the removal of a natural inhibitor during the fractionation process as will be confirmed in the following part of this paper. Lipoxygenase from tomato fruit (ACE 55 VF variety) was precipitated between 0.45 and 0.7 saturation by BONNET and CROUZET (1977). A deviation was expected since the different varieties often show different characteristics, morever, the growth conditions (mineral content of soil, environmental conditions, etc.) cause some alteration in the biochemical properties of biologically active compounds like enzymes.

2.4. Separation of natural inhibitor and activator

In order to support our suggestion that a natural inhibitor has been separated from the enzyme during fractionation with $(NH_4)_2SO_4$ pellets of the first and second fraction were dissolved in TRIS-HCl buffer pH 7 and different concentrations of these solutions were added to the reaction mixture containing the active supernatant of 0.6 saturation with $(NH_4)_2SO_4$. The pellet of 0.3–0.6 saturation had an inhibitory effect on lipoxygenase. This fraction was spectrophotometrically detected to contain two major components of maximum absorbance at 273 nm and 310–315 nm, respectively (Fig. 4). The protein

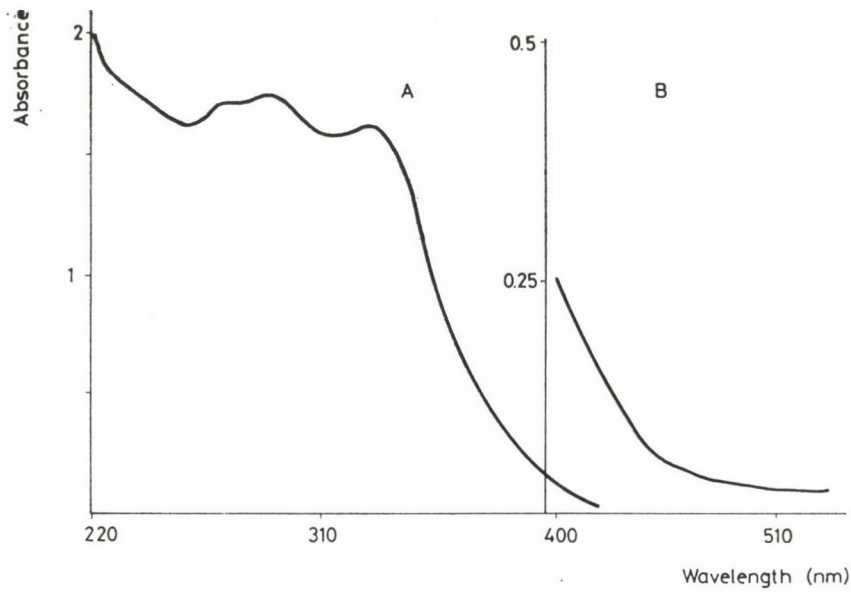

Fig. 4. Spectrum of redissolved pellet of 0.3–0.6 saturation with ammonium sulfate. A: at extension 2 between 220 and 400 nm; B: at extension 0.5 between 400 and 510 nm

components of the fraction were preliminarily separated (not completely) by gradual addition of aceton until the solution became turbid. The precipitated protein was separated either by filtration through filter paper or centrifugation. Aceton was evaporated from the clear supernatant in a rotary evaporator under vacuum. The protein pellet was spread on filter paper to remove the acetone. The greenish-yellow thick liquor remaining after complete removal of acetone and protein pellets were resuspended in a minimum volume of TRIS-

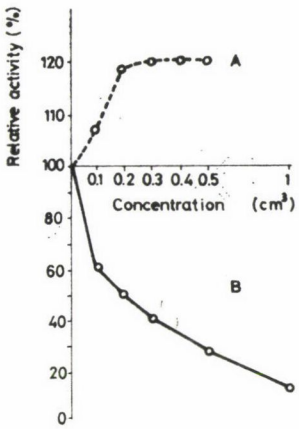

Fig. 5. Effect of protein fraction (A) and colored liquor (B) of 0.3–0.6 saturation with ammonium sulfate on the activity of partially purified tomato lipoxygenase. The fractions were separated by gradual addition of acetone to the redissolved pellets

HCl buffer pH 7 and added to the reaction mixture containing active lipoxygenase in different concentrations as shown in Fig. 5. The aceton-precipitated protein of this fraction caused about 20% increase in enzyme activity. The highly water soluble colored compounds caused 90% inhibition when 1 cm^3 of diluted solution was added. The elucidation of the nature of these components will be the aim of our next work.

2.5. Effect of some chemicals on the enzyme activity

Figure 6 shows the rate of linoleic acid peroxidation with different fractions of partial fractionation with $(NH_4)_2SO_4$. The increase in absorbance at 234 nm was fast and proportional to the time with the most active fraction (supernatant of 0.6 saturation without dialysis) at the initial phase of reaction up to 1.5 minute. In the second phase the reaction rate was lower than in the first one but still higher than with the two other fractions. This phenomenon was suggested to be due to the presence of some trace element activators. The loss of about 50% in the enzyme activity of the same fraction after dialysis

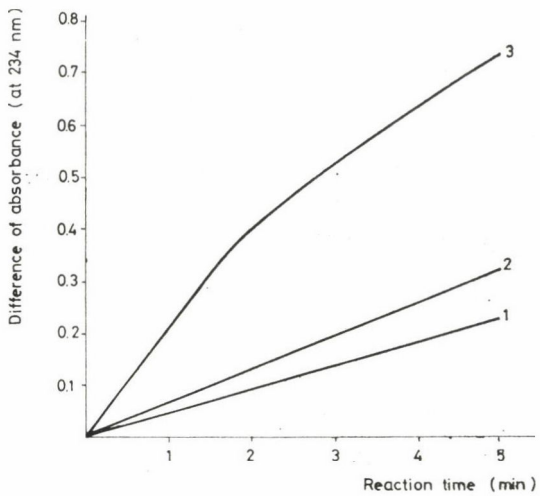

Fig. 6. Rate of linoleic acid oxidation with lipoxygenase from different fractions of ammonium sulfate fractionation. 1: supernatant of 0.6 saturation after dialysis; 2: redissolved pellet of 0.3 saturation; 3: supernatant of 0.6 saturation before dialysis. 0.1 cm^3 of each fraction was used for enzyme assay

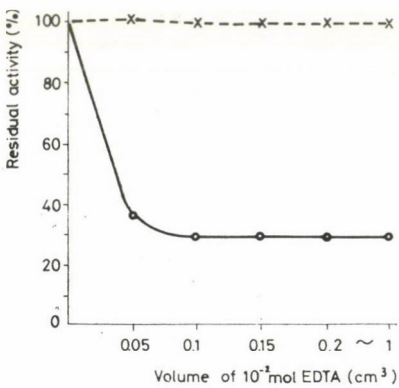

Fig. 7. Effect of EDTA on the activity of lipoxygenase of the active fractions obtained by ammonium sulfate fractionation. x————x: redissolved pellet of 0.3 saturation; o————o: supernatant of 0.6 saturation without dialysis

against distilled water for one day and the linearity of the progress curve of both dialyzed supernatant and 0.3 saturation for longer time supported our suggestion.

EDTA is well known as a metal binding agent. Its effect on different fractions is shown in Fig. 7. It is noted that further concentration of EDTA caused complete inhibition of the enzyme in the undialyzed supernatant. This means that EDTA removed the activating metal which was supposed to be found beside the enzyme in this fraction and the metal of the prosthetic group of the original enzyme is strongly bound and could not be removed by EDTA

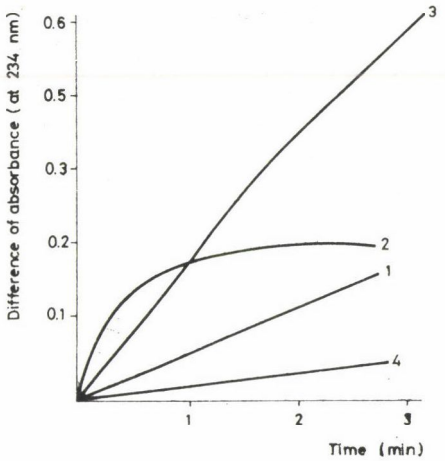

Fig. 8. Effect of Fe^{2+} and Mn^{2+} on linoleic acid oxidation in the presence of partially purified tomato lipoxygenase. 1: enzyme + substrate. 2: substrate + 10^{-6} mol Fe^{2}; 3: enzyme + substrate + 10^{-6} mol Fe^{2+}; 4: enzyme + substrate + 10^{-3} mol Mn^{2+}

at the concentrations used. On the other hand the resistance of the enzyme fraction, precipitated with 0.3 saturation to high concentrations of EDTA, is in favour of a true lipoxygenase and not a hematin compound.

These results confirm those obtained with the enzyme from various plant products such as egg-plant, rice bran, and potato tubers (GROSSMAN et al., 1972; SHASTRY & RAO, 1975; GALLIARD & PHILIPS, 1971), even though AL-OBAIDY and SIDDIQI (1981b) and KIM and GROSCH (1982) reported that lipoxygenases (Lox-es) from broad bean and apple were inhibited by EDTA indicating the involvement of metal ions in the catalysis of fatty acid peroxidation.

Figure 8 shows the results obtained from reactivation studies with some metal ions (Fe^{2+} and Mn^{2+}) on the dialyzed enzyme preparation. Fe^{2+} at 10^{-6} mol concentration activated the enzyme to a high level indicating the essentiality of iron to lipoxygenase of tomato fruit. This result is in agreement with the results reported by PISTORIUS and AXELROD (1974) and SHASTRY and RAO (1975). In the same experiment Mn^{2+} ion at 1×10^{-3} mol and 2×10^{-3} mol caused remarkable decrease in the reaction rate of the enzyme. These concentrations of Mn^{2+} were found to inhibit the enzyme from broad bean (AL-OBAIDY & SIDDIQI, 1981b).

2.6. Heat stability, optimum pH and effect of substrate concentration

Two experiments were designed for studying the heat stability of the partially purified enzyme. Effects of heating the enzyme preparation at several temperatures for a given period (2 min) and for several periods are shown in

Fig. 9. The enzyme resisted 60 °C for 4 min with about 12% loss of the original activity, while it lost all of the original activity at 80 °C in 2 minutes. These results indicate that the enzyme is moderately resistant to the heat treatment under the conditions of the experiment in comparison with the same enzyme from other sources like lipoxygenase-1 of pea seed (REYNOLDS & KLEIN, 1982). The enzyme from broad bean and tomato fruit ACE 55 VF showed similar heat stability to our preparation (AL-OBAIDY & SIDDIQI, 1981a; BONNET & CROUZET, 1977). Results with heated preparations suggest no participation of hematin compounds since these are heat stable.

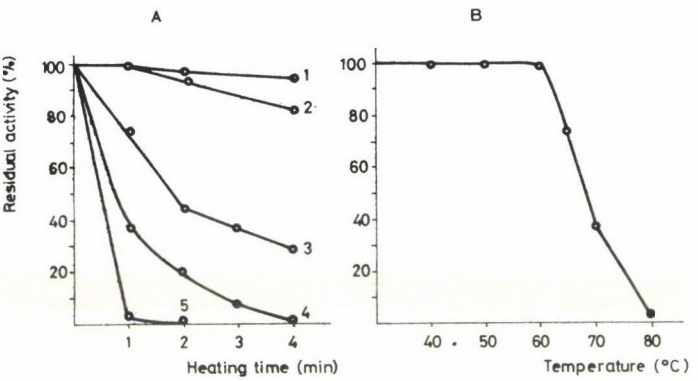

Fig. 9. Heat stability of tomato lipoxygenase. A: heating for several periods at various temperatures; B: heating for 1 min at various temperatures; 1: 40 °C; 2: 50 °C; 3: 60 °C; 4: 70 °C; 5: 80 °C

The crude extract and the partially purified enzyme were very stable at 40 °C for a week. After 10 days the crude extract lost 90% of the original activity, while the partially purified enzyme lost about 50% of its activity. At 4 °C the fractions, L_1, L_2 and L_3 isoenzymes of wheat germ lipoxygenase purified by gel filtration were stable up to 10 days and lost 50% of the their activity after that (NICOLAS et al., 1982). WALLACE and WHEELER (1975) indicated an activity loss of 60% for their L_1 enzyme from wheat on storage for 28 days at 2 °C at pH 4.8 in phosphate buffer.

The pH-response curve (Fig. 10.) shows an optimum at 4.5 and little activity at alkaline pH values. The enzyme seems to have the lowest pH optimum in comparison with the enzymes from other sources. WALLACE and WHEELER (1975), in their study on the isoenzymes from wheat germ, noticed a shift to lower pH-values at lower substrate concentrations. HSIEH and MCDONALD (1984) indicated an optimum at pH 4.8, but lower peaks of activity were observed at pH 6 and 7 for durum wheat lipoxygenase. The enzyme from the expressed juice of alfalfa leaves had an optimum value between pH 6 and 7

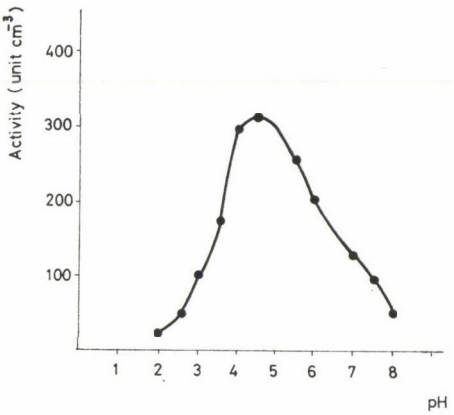

Fig. 10. Effect of pH on the activity of tomato lipoxygenase. 1 unit = 0.01 increase in absorbance at 234 nm per minute

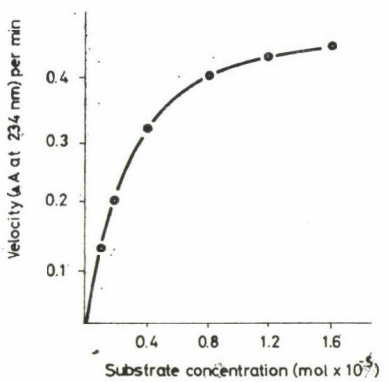

Fig.11. Effect of linoleic acid substrate on the rate of peroxidation with tomato lipoxigenase

(WHEELER & FINLEY, 1981). Alkaline pH-optima of activity were found with the enzymes from rice bran (SHASTRY & RAO, 1975) and pea seeds (REYNOLDS & KLEIN, 1982).

It was observed (Fig. 11) that the reaction followed Michaelis kinetics, when substrate concentrations (S) was plotted versus velocity (v). The Lineweaver-Burk plot gave K_m value of 0.37×10^{-5} mol (Fig. 12). This value is lower than the value reported by AL-OBAIDY and SIDDIQI (1981a) for partially purified broad bean lipoxygenase (2.8×10^{-3} mol) and higher than the value of 0.015 mol for purified lipoxygenase of tomato fruit ACE 55 VF variety (BONNET & CROUZET, 1977). The enzyme from pea seeds and rice bran showed a value of 0.18–0.2 mmol and 0.35 mmol, respectively (REYNOLDS & KLEIN, 1982; SHASTRY & RAO, 1975).

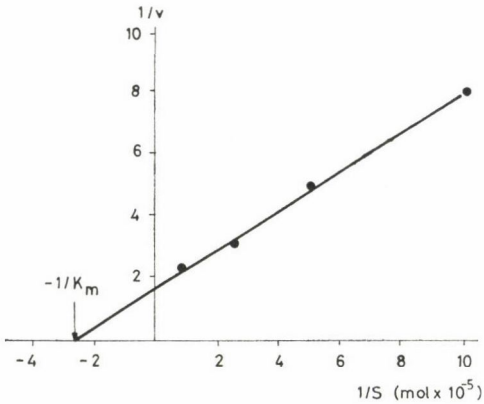

Fig. 12. Lineweaver-Burk plot with partially purified tomato lipoxygenase. S: substrate concentration; v: velocity; $y = 0.65x + 1.67$; $r = 0.99$; $K_m = 0.37 \times 10^{-5}$ mol

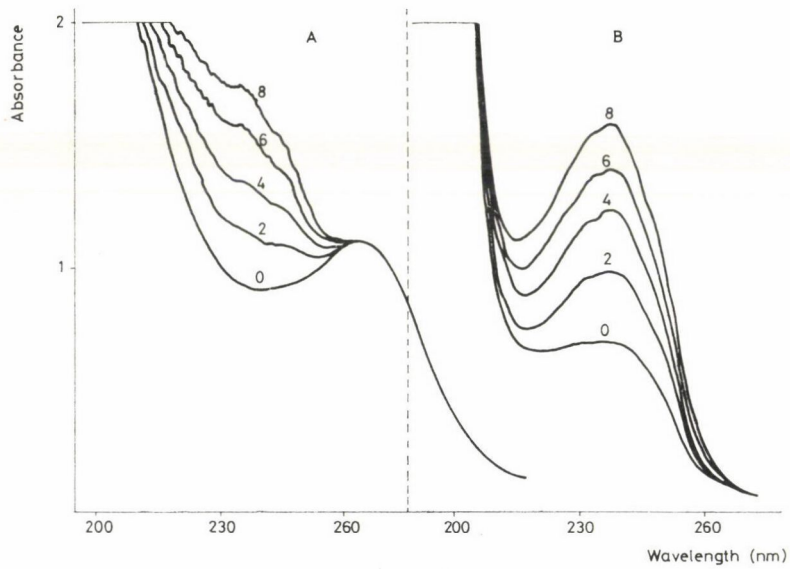

Fig. 13. Spectrum of reaction product of tomato lipoxygenase. A: crude enzyme extract B: partially purified enzyme. 0–8: reaction time in minutes

2.7. Detection of reaction products

The absorption spectrum of the reaction was automatically determined in Carl Zeiss Jena Spectrod M-40 between 200–300 nm. Figure 13 shows the spectrum of the reaction product for different enzyme preparations. It is well seen that 234 nm is the maximum absorbance for the reaction products in the case of the partially purified enzyme. As the crude extract contains different

impurities the peak at 234 nm was small and a long time was required for its appearance. This indicates the interaction between some impurities with the hydroperoxide produced. The appearance of the peak at 234 nm within a short time gives an index for the development in the purification processes.

It is well known that maximum absorbance at 232–234 nm is due to the formation of unstable conjugated dienes, which were transformed into more stable hydroperoxides having absorbance maxima at 235 nm. The results are similar to those noted for lipoxygenase from broad bean and grapes (AL-OBAIDY & SIDDIQI, 1981a; CAYREL et al., 1983).

Literature

AL-OBAIDY, H. M. & SIDDIQI, A. M. (1981a): Properties of broad bean lipoxygenase. *J. Fd Sci., 46*, 622–625, 629.

AL-OBAIDY, H. M. & SIDDIQI, A. M. (1981b): Inhibition of broad bean lipoxygenase. *J. Fd Sci., 46*, 597–600, 605.

BONNET, J. L. & CROUZET, J. (1977): Lipoxygenase from tomato fruit: Partial purification and study of some properties. *J. Fd Sci.*, 42, 625–628.

CAYREL, A., CROUZET, J., CHAN, W. S. & PRICE, K. R. (1983): Evidence for the occurrence of lipoxygenase activity in grapes (variety Carignane). *Am. J. Enol. Vitic., 34*, 77–82.

ESKIN, N. A. M. & PINSKY, S. G. A. (1977): Biochemistry of lipoxygenase in relation to food quality (Review). *CRC Crit. Rev. Fd Sci. Nutr. 9*, 1–40.

GALLIARD, T. & PHILIPS, D. R. (1971): Partial purification and properties of an enzyme that specifically oxygenates the 9-position of linoleic acid. *Biochem. J. 124*, 431–438.

GROSSMAN, S., TROP, M., AUTALION, R. & PINSKY, A. (1972): Egg plant lipoxygenase: Isolation and partial purification. *Lipids, 7*, 467–473.

HSIEH, C. C. & MCDONALD, C. E. (1984): Isolation of lipoxygenase isoenzymes from flour of durum wheat endosperm. *Cereal Chem., 61*, 392–398.

JADHAV, S., SINGH, B. & SALUNKHE, D. K. (1972): Metabolism of unsaturated fatty acids in tomato fruit: linoleic and linolenic acids as precursors of hexanal. *Pl. Cell Physiol., 13*, 449–453.

KAZENIAC, S. J. & HALL, R. M. (1970): Flavor chemistry of tomato volatiles. *J. Fd Sci., 35*, 519–522.

KIM, I. S. & GROSCH, W. (1979): Partial purification of lipoxygenase from apples. *J. Agric. Fd Chem., 27*, 243–246.

KLACKER, H. M. (1947): Differential spectrophotometry of purine compounds by means of specific enzyme. — Part 3: Studies of the enzymes of purine metabolism. *J. biol. Chem., 167*, 461–466.

NICOLAS, J., AUTRAN, M. & DRAPRON, R. (1982): Purification and some properties of wheat germ lipoxygenase. *J. Sci. Fd Agric., 33*, 365–372.

PINSKY, A., GROSSMAN, S. & TROP, M. (1971): Lipoxygenase content and antioxidant activity of some fruits and vegetables. *J. Fd Sci., 36*, 571–572.

PISTORIUS, E. K. & AXELROD, B. (1974): Iron an essential component of lipoxygenase. *J. biol. Chem., 249*, 3183–3186.

REYNOLDS, P. A. & KLEIN, B. P. (1982): Purification and characterization of a type-1 lipoxygenase from pea seeds. *J. agric. Fd Chem., 30*, 1157–1163.

SHASTRY, B. S. & RAO, M. R. R. (1975): Studies on lipoxygenase from rice bran. *Cereal Chem., 52*, 597–603.

WALLACE, J. M. & WHEELER, E. L. (1975): Lipoxygenase from wheat: An examination of its reaction characteristics. *J. agric. Fd Chem., 23*, 146–150.

WHEELER, E. L. & FINLEY, J. W. (1981): Lipoxygenase in the expressed juice of alfalfa leaves. *J. agric. Fd Chem., 29*, 912–914.

Acta Alimentaria, Vol. 17 (1), pp. 67—76 (1988)

HIGH AMYLASE FLOUR IN BAKING WITH VERY HIGH FREQUENCY (VHF) ENERGY

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(Received: 16 January 1987; accepted: 15 February 1987)

Doughs prepared from wheat flour of a moderate and of a high alfa-amylase activity (falling numbers 197 and 94, respectively were baked with very high frequency (17.7 MHz) energy in order to study the possibilities of breadmaking from sprout-damaged wheat. In a conventional baking procedure, high amylase flour produced loaves with doughy and sticky bread crumb properties. These defects were avoided by a two-phase process of high frequency baking for 6 min followed by supplementary high temperature conventional heating (5 min at 350 °C), which was required for crust formation. The positive results were further confirmed and shown to be stable by means of a series of experiments in which malt was progressively increased in the dough. Excessive malt additions to flour (falling number 62), however, caused doughy and sticky crumb in spite of the high frequency treatment. The two-phase baking procedure (high frequency treatment followed by high temperature treatment) took approximately 10 min compared to the 30 min required for the conventional baking procedure. Baking losses due to extensive water evaporation, however, were as high as 21%, indicating that problems may arise from an improperly controlled two-phase baking procedure.

Keywords: amylase, baking, electromagnetic energy

Electromagnetic energy of appropriate high frequency effects molecular movement, friction and temperature rise in materials of suitable dielectric properties. Many food materials are considered ideal for heating by means of high frequency or microwave energy because of their poor thermal conductivity and their high dielectric loss factor. Electromagnetic energy can heat materials rapidly and uniformely throughout with smaller heat gradients than those used in conventional heating techniques (DECAREAU, 1985; DEMECZKY, 1985). Thus, electromagnetic heating has a potential use in commercial baking technology for a reduction of baking time.

Portions of bread dough should be very suitable for heating in an electromagnetic field, since dough has a poor indigenous thermal conductivity, and possesses a sufficiently high dielectric loss factor due to its relatively high

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water content (approximately 50%). A supplementary conventional baking system must be applied, however, for browning and crust formation, since these are lacking in loaves baked merely with electromagnetic energy. Thus, in spite of its hypothetical advantages high frequency heating has not so far had a breakthrough in commercial breadmaking technology. The subject has been studied by various workers: e.g., LORENZ and co-workers (1973), RUNTÁG and DEMECZKY (1973), RUNTÁG (1974), SEILER (1978) and PEI (1982). Recent reviews on microwaves and very high frequency in food technology was presented by DECAREAU (1958; 1986) and by DEMECZKY (1985).

One suggested application of electromagnetic heating in which its advantages are especially favourable is baking from sprout damaged wheat. This application should be of special interest in areas where sprout damage, which produces high amylase activity in wheat, leads to considerable harvest losses. Sprout damage is a major defect of domestic wheat in Finland, e.g. where sprouting and its concomitant high amylase activity makes approximately one fifth of a yearly spring wheat crop unsuitable for baking.

The use of dielectric heating in baking bread from sprouted grain is based on the hypothesis that the detrimental effects of high enzyme activity manifest themselves mainly in the baking phase. Here the gelatinating starch is attacked by alpha-amylase in the critical temperature range of 65 to 85 °C during a period of a few min prior to the thermal inactivation of the enzyme. Rapid heating with electromagnetic energy should then reduce the critical period of amylase attack on starch.

CHAMBERLAIN (1973) was the first to study this hypothesis in practical work. He succesfully used microwave (896 MHz) heating for thermal inactivation of alpha-amylase in bread dough made of sprouted wheat. WESTERMARCK-ROSENDAHL (1981) successfully baked bread from sprouted wheat by a simultaneous use of microwave (2450 MHz) heating and conventional baking of bread from sprouted wheat. These workers used the UHF (ultra high frequency) area of electromagnetic energy.

The purpose of the present study was to collect further evidence on the dielectric baking process in the VHF (very high frequency) area using the equipment available to the authors at the Central Food Research Institute (KÉKI) in Budapest. This apparatus works at 17.7 MHz. An advantage of a lower frequency (VHF) compared to the ones used in microwave, or UHF technology is a better penetration of the energy into the product. A disadvantage of a lower frequency is that the physical shape and position of the material to be heated in the electromagnetic field become more critical. RUNTÁG and DEMECZKY (1973) and RUNTÁG (1974) reported on early experiments with an apparatus working at 13.56 MHz. The effects of dielectric heating treatments on loaves baked from two lots of wheat flour of Finnish origin, one of moderate and the other of high amylase activity, are reported here.

1. Materials and methods

1.1. Wheat flours

Two lots of wheat flour (A and B) differing in enzymatic activity were used throughout the study. The flours A and B were milled from commercial lots of wheat with falling numbers 140 and 67, respectively. Data of the properties of the two flour lots are given in Table 1.

Table 1

Quality characteristics of the two lots of flours (A and B) used in the study

Quality and dimension	Flour	
	A	B
Falling number (s)	197	94
Alpha-amylase activity (arbitrary U/g)	200	1500
Protein content (dm×5.7N)	10.5	11.4
Ash (dm%)	0.95	0.87
Moisture (%)	14.3	15.8
Dough development time (min)	0.5	0.5
Stability (min)	1.5	1.0

dm: dry matter

1.2. Breadmaking procedure

A simple straight dough breadmaking procedure was used. The baking formula included flour, compressed yeast (3%, flour weight basis), salt (1.5%) and water (59.4% for flour A and 57.6% for flour B, on flour weight basis). Vital wheat gluten (Raisio, Finland) was added (3% of flour weight) in some experiments. The total dough weight was approximately 800 g. The scale weight was 700 g. The calculated water contents of doughs A and B were 45.0% and 45.2%, respectively. Dough temperature was adjusted to 30 °C. The dough was mixed for 3 min in an electric mixer (AKA, GDR) and fermented at 30 °C (75–80% R.H.) for 60 min. It was then moulded and rolled by hand. The moulded dough was placed in a specially designed pan made of polytetrafluoroethylene (Teflon®). The dimensions of the pan were as follows: bottom 69 mm×238 mm, top opening 91 mm×259 mm, height 119 mm. The walls of the pan were removable. Proof time in the pan before treatment was 60 minutes at 30 °C and 75–80 R.H.

Baking with electromagnetic energy was performed between two electrodes of the dimensions 170 mm×360 mm. The distance between the electrodes was 92 mm. Very high frequency (VHF) energy (17.7 MHz) was induced between the electrodes with a UHF-generator (G.U.G., Czechoslovakia).

Treatment times were 4, 5, 6, 7 or 8 min. Immediately after high frequency treatment the loaf was removed from the pan and the pale loaf was baked free-standing for another 4–5 min at high temperature (350 °C). The purpose of the supplementary baking procedure was to produce a brown bread crust.

The same formula and basic procedure prior to the baking stage were used to produce loaves for reference. These loaves were baked in a conventional oven. Scale weight was 400 g and baking time was 30 min at 220–230 °C.

The baked loaves were allowed to cool on a rack for 1 h and then were weighed and wrapped. The volumes of the loaves were then measured by a rapeseed displacement procedure.

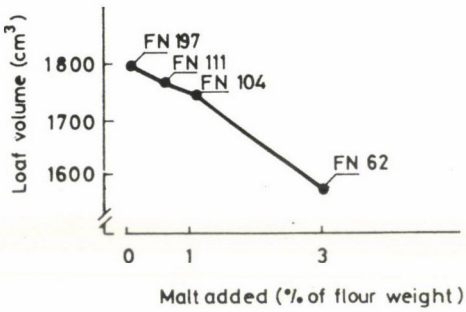

Fig. 1. The effect of malt addition on loaf volume in conventional baking of bread. Alfa-amylase activities of flour–malt mixtures are indicated in falling numbers (FN)

1.2. Crumb compression measurement

Crumb compressiveness of the variously baked loaves was measured with a penetrometer (Labor QB-204, Budapest) 4, 24 and 48 h after baking. The penetrometer had a 20 mm ∅ hemispheric sensor head weighing 200 g. Compressing time was 7 sec. Ten replicative measurements were made on different sites of a slice. The highest and the lowest values were rejected, and the means of the remaining values were given as measures of compressiveness.

1.3. Sensory evaluation

The triangle test (AMERINE et al., 1965) was used to detect differences caused by various treatments. The ranking method (AMERINE et al., 1965) was used in the evaluation of the preference of certain treatments. Verbal comments on the samples were collected during the sensory panels. The subjects were members of the laboratory personnel having experience in sensory evaluation.

1.4. Analytical procedures

The falling number (FN) and the moisture content of the flour were determined by using standard procedures of the ICC (1978). AACC (1962) procedures were used to determine protein content (46—10), ash content (08—01), and farinograph characteristics of flour (54—21). Alpha-amylase activity was measured using the procedure described by PÁRKÁNY-GYÁRFÁS and VÁMOS-VIGYÁZÓ (1979). It was the initial purpose of the study to monitor changes in alpha-amylase activities found in the loaves after various treatments. Unexpected problems arose affecting the accuracy of measure-

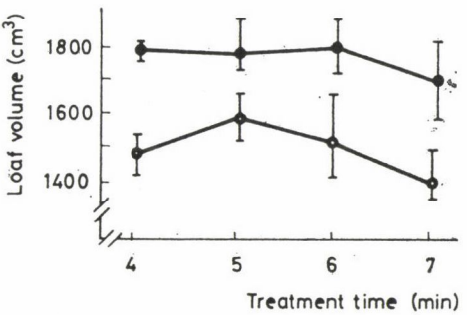

Fig. 2. The volumes of loaves baked from the two flours, shown as treatment times in very high frequency (VHF) heating increase. ●———●: Flour A (FN 197) ○———○: Flour B (FN 94)

ments in this procedure, however, due possibly to an amylase inhibitor assumed to be present in the two flours. Therefore, only arbitrary values of the activities present in both flours are given. These were calculated by a comparison of alpha-amylase activity and FN values obtained in other flour samples.

2. Results

2.1. Effects of enzyme activity on loaf volume

Loaves baked from flour A (FN 197) were significantly larger in volume than those made from the high amylase flour B (FN 94). The low volume of the loaves baked from flour B was probably a consequence of the high enzyme activity of this flour. This supposition was substantiated by means of an experiment in which malt was progressively added to flour A in each baking. The loaf volume decreased as increasing amounts of malt were added (Fig. 1). Treatment time in the high energy field did not significantly affect loaf volume (Fig. 2).

2.2 Sensory crumb properties vs. baking procedure

As expected, flour A, which has a moderate amylase activity (FN 197), and flour B, which has a high one (FN 94), produced different bread crumb properties in the conventional baking procedure. The crumb of the loaf baked conventionally from the high amylase flour (FN 94) was described as doughy and sticky. The difference between the two flours was apparent in the discriminating sensory test of the corresponding loaves ($P < 0.001$).

A 6 minute treatment with VHF energy supplemented with a 5 min heating at high temperature had no effect on crumb properties as judged by the triangle test when the flour with only moderate amylase activity

Table 2

Sensory detection of difference in bread crumb properties as affected by the UHF treatment compared to a conventional baking procedure
(Number of correct judgements divided by total in triangle tests)

Test material[a] (FN)	Treatment time (min)	
	5	6
Flour A		
FN 197	—	6/10 NS
FN 111	—	7/10*
Flour B		
FN 94	21/21***	9/10***
FN 63	—	9/11**

[a]: Amylase activity of flour–malt mixtures indicated as falling numbers
FN: Falling number
—: test not performed
NS: non-significant
*, ** and *** significant at $P = 0.05$, $P = 0.01$ and $P = 0.001$ probability level, respectively
Significant differences were always in flavour of VHF-treatment

(FN 197) was used (Table 2). Some of the panelists' comments suggested, however, that the crumb of the VHF-treated loaves felt drier than that of the conventionally baked loaves. The test baker also made this observation; and it is a logical one, since moisture losses in the loaves baked with the two-phase procedure were higher than in the conventionally baked ones (Cf., below).

The results above indicate that bread with acceptable crumb properties could be baked with the VHF procedure. On the other hand the VHF treatment had no detectable positive effects on bread quality when flour of moderate amylase activity was used.

When high amylase flour (FN 94) was used, however, crumb properties of the loaves baked with high frequency treatment were different from the

loaves baked conventionally. While the conventionally baked loaves had a doughy and sticky crumb, the loaves baked with the high frequency treatment had an acceptable and drier crumb.

A very similar positive result from high frequency treatment on crumb properties was obtained when the effects of malt additions were studied (Tables 2 and 3). Six min of high frequency treatment followed by a rapid high temperature baking for crust formation produced loaves with a drier crumb, even though a considerable amylase activity was present in the dough. Excessive malt additions (FN 62—63 in flour–malt mixture), however, produced sticky and doughy crumb properties even when a treatment of 5 or 6 min of high frequency heating was applied (Table 3).

Addition of 3% of vital wheat gluten (flour weight basis) did not improve crumb properties of flour–malt mixtures; furthermore, the sensory panel detected an odd off-flavour in VHF treated breads (Table 3). Both of the

Table 3

Preferred treatments in three sets of experiments. Results of rank tests and comments of the assessors concerning crumb properties

Test	Malt added (%)	Treatment time in UHF baking (min)	Rank sum	Comments on crumb structure, other comments
1. *Rank test* (n = 10)				
FN 197 (R)	0	5	15*	normal
FN 111	0.5	5	21	doughy
FN 104	1.0	5	24	doughy
2. *Rank test* (n = 7)				
FN 197 (R)	0	5	7***	normal
FN 62	3.0	5	22	doughy, fermented flavour
FN 62	3.0	5.5	22	doughy, fermented flavour
FN 62	3.0	6	19	doughy, fermented flavour
3. *Rank test* (n = 7)				
FN 94 (R)	0	6	12**	normal
FN 94[a]	0	6	24	normal, odd flavour
FN 64[a]	1.0	6	29	odd flavour
FN 63[a]	1.0	6	27	odd flavour
FN 62	3.0	6	49**	very doughy

FN: Amylase activity of flour–malt mixtures indicated in falling numbers
n: number of measurements
R: reference sample
[a]: 3.0% vital wheat gluten added(flour weight basis)
*, ** and ***: significantat P = 0.05, P = 0.01, and P = 0.001 probability levels, respectively

above phenomena might be related to proteolytic enzyme activities of malt. The proteolytic enzymes might degrade part of the flour or yeast proteins within a short time and give rise to an off-flavour in Maillard-type reactions.

2.3. Baking losses

Baking losses by weight varied between 12 % to 21% in the loaves baked by the two-phase baking procedure involving high frequency treatment combined with subsequent heating at high temperature (350 °C). The baking loss heavily increased with increasing VHF treatment, whereas conventional baking (350 °C) did not produce such a marked relationship between heat and

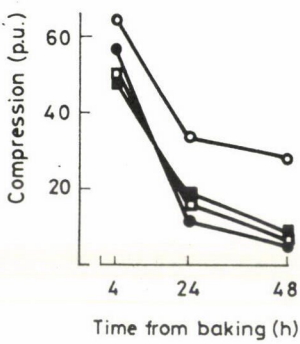

Fig. 3. Example of crumb compression in penetrometer unit (p.u.) of loaves baked with increasing malt additions. Treatment time in very high frequency (VHF) heating was 5 minutes. ●———●: 0% malt added, FN 197; ○———○: 0.5% malt added, FN 111; ■———■; 1.0% malt added, FN 104; □———□: 3.0% malt added, FN 62

baking losses. The total baking losses exceeded the baking loss range of 8 to 10% observed in the conventional process. Malt addition did not affect baking loss in these experiments.

2.4. Physical crumb properties

Crumb softness was related to loaf volume. In general, loaves with the highest volume proved also to be the softest ones as measured by a penetrometer. Addition of malt to flour A (FN 197) resulted in a significant decrease both in loaf volume (see Fig. 1) and in crumb softness, as illustrated in Fig. 3.

3. Conclusions

Application of high frequency with a subsequent high temperature treatment reduced the time needed for baking a single loaf from about 30 min to approximately 10 min. As a result of the two-phase baking process the prop-

erties of bread crumb were altered and the doughy character of the bread crumb made of high amylase flour was eliminated. This was probably not merely a consequence of reduced amylolytic starch degradation during rapid high frequency heating but also a function of reduced moisture content in the crumb, which resulted from the two-phase baking procedure. Higher than normal baking losses in UHF or microwave heating treatments were also reported in previous studies (RUNTÁG & DEMECZKY 1973; RUNTÁG, 1974; WESTERMARCK-ROSENDAHL, 1981).

Bread baked from a high amylase flour using the high frequency — high temperature procedure resulted in a product of acceptable quality whereas a sticky and doughy crumb was obtained in loaves baked with a conventional procedure. These results confirm the studies of CHAMBERLAIN (1973) and WESTERMARCK-ROSENDAHL (1981), who reported on favourable effects in baking with microwaves from high amylase flour.

The present study indicates that the application of electromagnetic energy has a potential in baking from high amylase flours. Much technical development is needed, however, to make the process technologically more feasible. Other problems arising from a possible use of sprout damaged wheat will not be discussed here.

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The work was carried out under a research agreement between the Central Food Research Institute, Budapest, and the Department of Food Chemistry and Technology (EKT), University of Helsinki.

The paper is based on a M. Sc. thesis (Food Science) carried out at the Central Food Research Institute, Budapest, and presented by Mr. HEIKKI KEROJOKI in 1983 to the Department of Food Chemistry and Technology at the University of Helsinki, Finland.

Literature

AACC (1962): *Approved methods of the AACC.* 7th ed., American Association of Cereal Chemists, Inc., St. Paul, MN.

AMERINE, M. A., PANGBORNE, R. M. & ROESSLER, E. B. (1965): *Principles of sensory evaluation of food.* Academic Press, New York.

CHAMBERLAIN, N. (1973): Microwave energy in the baking of bread. *FMBRA Bull.*, (3), 76–87.

DECAREAU, R. V. (1985): *Microwaves in the food processing industry.* Academic Press, New York.

DECAREAU, R. V. (1986): Microwave food processing equipment throughout the world. *Fd Technol., 40,* (6), 99–105.

DEMECZKY, M. (1985): Application of dielectric techniques in food production and preservation. in: THORN, S. (Ed.) *Developments in food preservation.* Elsevier Applied Science Publishers, London. Vol. 3, pp. 127–181.

ICC (1978): *Standard methods of the ICC.* International Association for Cereal Chemistry. Verlag Moritz Schäfer, Detmold.

LORENZ, K., CHARMAN, E. & DILSAVER, W. (1973): Baking with microwave energy. *Fd Technol.*, 27 (12), 28–30, 32, 34, 36.

PÁRKÁNY-GYÁRFÁS, A. & VÁMOS-VIGYÁZÓ, L. (1979): Determination of amylase activity in corn using a chromatogenic substrate. *Starch, 31,* 328–332.

PEI, D. C. I. (1982): Microwave baking — New developments. *Bakers' Dig., 56,* (1), 8, 10, 32.

RUNTÁG, T. & DEMECZKY, M. (1973): Investigations into the possibilities of application of high frequency electric fields to the baking of dough. *Acta Alimentaria 2,* 3–16.

RUNTÁG, T. (1974): Continuous high frequency dielectric equipment and technology for baking bread. *Acta Alimentaria, 3,* 349–356.

SEILER, K. (1978): Backen mit Hochfrequenz — aus der Sicht der Gegenwart. *Zuck.-u. SüsswarWirt., 31,* 100, 109–113.

WESTERMARCK-ROSENDAHL, C. (1981): Försök att förbättra bakningsegenskaperna hus mältningskadat vete genom gräddning med mikrovågor och varmluft. *Nordisk Cerealistförbunds 21. Kongress,* Espoo, Finland pp. 127–136.

Acta Alimentaria, Vol. 17 (1), pp. 77—94 (1988)

COMPARATIVE ANALYSIS OF SPICES DECONTAMINATED BY ETHYLENE OXIDE OR GAMMA RADIATION

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(Received: 11 February 1987; accepted: 17 March 1987)

A comparison was made between the microbiological efficacy of ethylene oxide treatment (800 g T gas per m^3 at 22 °C for 6 h) and irradiation with 4 or 8 kGy gamma radiation and between their respective effects on the volatile oil content of black pepper, the sensory characteristics of ground black pepper, ground paprika, onion powder and garlic powder and the water uptake of the latter two seasonings. The analyses were carried out during storage at room temperature within three weeks after the cell count reducing treatment and a second time after 5–8 months storage.

The results of the experiments can be summed up as follows:

— 4 kGy was sufficient to achieve reduction of the viable cell count equivalent at least to the effect of ethylene oxide. The residual cell count in the samples treated with 8 kGy radiation dose was 1–3 orders of magnitude lower than that of the ethylene oxide treated samples.

— Gamma radiation reduced sufficiently the cell count in the spices without affecting their sensory quality or other quality characteristics.

— Ethylene-oxide treatment, when carried out according to industrial practice, caused a substantial reduction of the volatile oils in black pepper.

— The ethylene oxide-treated black pepper showed in the initial phase of after-treatment storage a reduced seasoning capacity in comparison to the untreated samples, while in garlic powder the same treatment caused reduction in the aroma intensity.

— Flavour dilution profile analysis showed more change in the taste components of the seasoning after ethylene oxide treatment than after radiation treatment. However, the effect of storage on the flavor profile was more intense than that of cell count reducing treatments.

— According to the flavor dilution profile analysis the intensity of the sweet component of onion flavor was increased by both storage and radiation treatment. Radiation treatment significantly increased also the water uptake of onion powder.

The study has shown radiation treatment to be competitive with ethylene oxide treatment from the point of view of microbiological efficacy as well as in maintaining the quality characteristics of seasoning.

Keywords: decontamination by gamma radiation, decontamination by ethylene oxide, spices

Most of the spices become thoroughly contaminated with microorganisms, mainly aerobic species, during cultivation, harvesting and processing for utilization. Although the contamination is caused mostly by saprophytic

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organisms, pathogens, mostly salmonellae are detected with increasing frequency in spices (BOCKEMÜHL & WOHLERS, 1984). In the last 15 years in a few cases, thus, for instance in Canada and Norway occurred outbreaks of salmonellosis originating from white and black pepper as described in the literature (LAIDLEY et al., 1974; WHO, 1974, 1982; C.D.C., 1982; GUSTAFSEN & BREEN, 1984). *Bacillus cereus* and *Clostridium perfringens* are also frequently detected in spices, although with low cell counts (POWER et al., 1976; NIKOLAEVA, 1976; BAXTER & HOLZAPFEL, 1982; BOER & BOOT, 1983). Since the spores of these microorganisms may survive the cooking temperatures of foods, and may proliferate in these foods at temperatures between 20 and 50 °C, spices containing spores of this kind must be considered sources of infection. Contamination with moulds has also to be taken into account (I.C.M.S.F., 1980). Generally the mould count does not correlate with the total viable aerobic cell count as determined by plating (ITO et al., 1985). Toxigenic fungi occur relatively frequently in spices, so are aflatoxins, too, although the mycotoxin concentration is mostly low (SCOTT & KENNEDY, 1975; FLANNIGAN & HUI, 1976).

From the point of view of the food processing industry, of the microflora occurring in spices, bacterial spores cause most trouble which are known to be of high heat resistance. The total aerobic cell count in some spices may reach or surpass the order of magnitude of one million per gram and the proportion of bacterial spores in the total cell count may amount to 50–70% (FÁBRI et al., 1985; FARKAS, 1987), thus, concerning the heat tolerant microorganisms, spices imply an important source of microbial contamination and thereby form a component of decisive significance in determining the heat treatment requirement of many products, even if the amount of spice in the product does not exceed 0.1–0.5%.

Because of the above in certain branches of the processing industry preference has been given to the use of spices of reduced cell count for a long time. The most frequently used means of reducing cell count has been ethylene oxide fumigation. Ethylene oxide of alkylating effect and certain of its reaction products may form chemical residues in the fumigated produce of which the persistent halohydrins are of special importance (WESLEY et al., 1965; STIJVE et al., 1978). In the last decade the suspicion of mutagenicity, teratogenicity and carcinogenicity of ethylene oxide, and ethylene chlorohydrin has been proven and the results of certain investigations have drawn the attention to the hazard from the aspect of occupational health (HOGSTEDT et al., 1979; EHRENBERG & HUSSAIN, 1981). Thus the use of ethylene oxide is being more and more restricted (O.S.M.A., 1984) while the necessity of another cell count reducing method arises. Of the alternatives the treatment with ionizing radiations gains increasing attention. Gamma or electron radiation does not involve heat effect, practically does not form chemical residue but it is very effective

in reducing the cell count, thus, it is an ideal process for cell count reduction in various heat sensitive, dry ingredients, for instance in spices (FARKAS, 1985, 1987). In spite of this few investigations were carried out in order to compare systematically the microbiological efficacy of radiation treatment and ethylene oxide treatment and their effect on the quality of spices (VAJDI & PEREIRA, 1973; SZABAD & KISS, 1979; FARKAS & ANDRÁSSY, 1983; TOOFANIAN et al., 1985; BUY THI YEN et al., 1986). As regards the effect of radiation treatment on the quality of spices the pertinent publications are rather contradictory (FARKAS & EL-NAWAVY, 1973; GALETTO et al., 1979; NEUMAYR et al., 1983; UCHMAN et al., 1983; WEBER, 1983; FUNKE et al., 1984; GRÜNEWALD, 1984; FARKAS, 1987). Therefore, we set ourselves the target to carry out comparative tests with two important spices (black pepper and ground paprika) and two important seasonings of vegetable origin (onion powder and garlic powder) on the microbiological efficacy of radiation treatment and ethylene oxide treatment and the effect of these treatments on the volatile oil content, on the sensory quality and some other characteristics. The microbiological tests were carried out three months after the cell count reducing treatment. The other comparative tests were carried out within three weeks after treatment and a second time after 5–8 months storage of the spices, at room temperature.

1. Materials and methods

1.1. Materials tested, their water content and water activity

Black pepper was obtained from COMPACK Commercial Packaging Enterprise, Budapest, ground paprika, onion powder and garlic powder from the Szeged Paprika Processing Plant, Szeged.

Prior to cell count reduction the moisture content of the samples was as follows: black pepper 10.6%; paprika 7.3%; garlic powder 7.5%; onion powder 11.2%. The moisture content of black pepper was determined by the toluene distilling method (HUNGARIAN STANDARD, 1977b), that of paprika by the HUNGARIAN STANDARD (1977a) method applying a temperature of 95 °C and drying for 4.5 h. The moisture content of onion and garlic powder was determined by the HUNGARIAN STANDARD (1980) method from the loss of mass. The samples were weighed in the weighing dish dried with quartz sand and afterwards moistened with 5–10 cm^3 ethyl-alcohol. Ethyl-alcohol was evaporated at room temperature overnight, than the samples were dried at 103 $\pm$ 2 °C till constant weight. Water activity values as measured with a Novasina apparatus: black pepper 0.58; paprika 0.38; onion powder 0.38; garlic powder 0.39.

1.2. Packaging and storage of the samples

The spices were packed into enamelled cans, generally used for tea packaging, cube shaped of 10 cm edge length with snapping lid. Eight samples each of the seasonings were filled in so as to fill completely the volume of the can. After treatment the samples were stored at room temperature.

1.3. Ethylene oxide treatment

The samples were filled into double-layer paper bags for fumigation with ethylene oxide. After treatment the samples were replaced into the cans according to para. 1.2. Fumigation was carried out according to industrial practice in the Szeged Paprika Processing Plant in a Degesch type equipment under vacuum at 22 °C throughout 6 h. T-gas was used for fumigation (90% ethylene oxide + 10% CO_2) at 800 g per m^3 volume of the fumigation chamber.

1.4. Irradiation

The spice samples were prepared for storage in the cans (see 1.2.). After closing down the cans were exposed to 4 or 8 kGy radiation dose in a ^{60}Co radiation source at 9.6 kGy dose rate at room temperature.

1.5. Microbiological tests

The tests served to determine the mesophile and thermophile aerobic viable cell count, the *Bacillus cereus* count, the number of bacteria belonging to the Enterobacteriaceae family and of the mould propagules.

Ten g of each spice were suspended in 90 cm^3 diluting solution and this stock solution was used to make a decimal dilution series. One cm^3 or 0.1 cm^3 of the stock solution and of each dilution were mixed into the layer or spread on the surface of the media for plating. The diluent liquid contained 0.1% pepton, 0.9% sodium chloride and 0.01% Tween 80 in distilled water. The diluting liquid used to suspend onion powder and garlic powder contained only 0.5% NaCl and to neutralize the possible microbicid effect of the powders 0.5% $Na_2S_2O_3$ was added (WILSON & ANDREWS, 1976).

For the determination of mesophilic aerobic viable cell count (MACC) the TSBYA nutrient agar was used. This was prepared by dissolving Oxoid TSB (Tryptone soya broth, marked CM 129) and complement it with 3 g yeast extract and 15 g agar per litre. The pH of the nutrient was 7.3. The plates inoculated with 1 cm^3 inoculum were incubated at 30 °C and the number of colonies was established after 5 days.

To determine the thermophilic aerobic viable cell count (TACC) TSBYA nutrient agar plates were used, too. The inoculated Petri dishes were wrapped in PVC foil and incubated at 55 °C for 48 h prior to counting the colonies.

To determine Enterobacteriaceae (EC) Oxoid CM 485 VRBG agar (violet red bile glucose) of pH 7.4 was used. The medium containing 1 cm^3 inoculum was plated in thin layers. After solidification a second layer was poured. Plates were incubated for 24 h at 37 °C and then the characteristic colonies were counted.

To determine the mould count (MC/OGY/oxitetracycline-glucose-yeast extract) nutrient medium (Oxoid CM) of pH 7.0 was used complemented with 150 mg per dm^3 Bengal red. Mixed with 1 cm^3 inoculum thin layers were poured and these were evaluated after 5 days incubation period at room temperature.

To count *Bacillus cereus* (BCC) 0.1 cm^3 inoculum was streaked on the surface of blood agar nutrient medium (Oxoid CM 55, pH 7.3) plates. The plates incubated for 24 h at 37 °C were evaluated for characteristic colonies by a hemolytic zone.

1.6. Volatile oil determination

The volatile oil content in black pepper was determined by a steam distillation method according to the pertinent HUNGARIAN STANDARD (1978). Twenty g spice were suspended in 500 cm^3 distilled water and this was then distilled for 5 h. The volatile oil content was expressed as percentage dry matter. The other three seasonings were not tested for their volatile oil content because in preliminary experiments their volatile oil content proved to be very low in comparison to that of black pepper.

1.7. Analysis of the spice extracts by spectrophotometry

The lipid-soluble extract of ground paprika (to establish probable changes in the coloring capacity) was studied by spectrophotometry . Five g of the spice were suspended in 50 cm^3 sunflower oil and blended in a magnetic blender for half an hour keeping the suspension in continuous motion but avoiding mixing in air. The oil extract was then filtered through cottonwool and the filtrate and its 50-fold oil dilutions analysed in a Perkin-Elmer UV/137 type spectrophotometer in the visible wavelength range ($d = 1$ cm).

Ground paprika, onion powder and garlic powder were extracted also with 10% NaCl solution. One g of the samples were suspended in 50 cm^3 solution and the extraction was carried out as with oil. These suspensions were filtered on MN 615 1/4 type folded filterpaper prior to spectrophotometry. The extracts and their dilutions were studied in the visible and the UV range.

1.8. Water uptake capacity of onion and garlic powder

Water uptake capacity was measured according to HUNGARIAN STANDARD (1971). Two grams of the samples were suspended in distilled water, allowed to stand for 24 h then filtered through a MN 615 1/4 folded filterpaper of 15 cm diameter. Judging by the volume of the filtered solution and in the knowledge of the water imbibed by the filterpaper during 90 min filtering period the amount of water absorbed in 24 h by 10 g onion powder was calculated and given in cm^3 as the character of water uptake capacity. Three parallel measurements were carried with each sample.

1.9. Sensory tests

The test panel consisted of 10–12 members. To establish the seasoning capacity of the spices amounts determined in preliminary experiments were dispersed in whipped cream and used for tasting. The spice samples were added to 200 cm^3 cream and whipped to a hard foam during appr. 2 min in a Girmi Z020 type household whipping machine at maximum speed. The concentration of the individual spices in the whipped cream was as follows: black pepper 0.5%; ground paprika 1.0%; onion powder 0.5%; garlic powder 0.3%.

The test papers used to judge the flavor intensity characteristic of the given spice displayed 10 cm long lines. The starting point marked total lack of flavor, the end point the maximum flavor intensity. The task of the panel members was to mark on the line the point corresponding to the flavor intensity as judged by them. The length of the line between the start and the marked point was exactly measured, the values thus obtained were evaluated by variance analysis, taking 20 mm length as unit flavor intensity.

To test odour intensity the samples to be tested were filled into 100 cm^3 glass bottles with ground stopper. To prevent the panel members to be influenced by the color of the samples, the bottles were wrapped in black paper. The panel members were expected to mark the intensity of smell as judged by them on lines similar to those used for flavor intensity measurement. The line length were statistically evaluated.

The flavor dilution profiles were analyzed by the modified methods of TILGNER (1965) and of MIHÁLYI and ZUKÁL (1972). Twenty g spice were homogenized with 400 cm^3 tap water in a Girmi Z020 type immersed blade homogenizer for 1 min. This suspension was then further diluted with tap water in the proportion of 1 : 3. Thus, the fifth dilution level contained only 49 mg per dm^3 spice. Panel members tested the samples containing plain water with the suspensions in the order of increasing spice concentration. The dilution series were kept in dark bottles. Panel members were expected

Table 1

Flavor components as found in flavor profile dilution analyses and determined in preliminary experiments

Black pepper	Ground paprika	Onion powder	Garlic powder
any black pepper	any paprika	any onion	any garlic
pungent	sweet	raw onion	raw garlic
tannic, astringent	roasted	cooked, roasted	cooked, roasted
hot	nauseating	sweet	sweet
terpenic, aromatic	tannic, astringent	pungent	pungent
bitter	pungent, harsh	tannic, astringent	tannic, astringent
	bitter	bitter	bitter

to mark on the test paper which of the flavor components selected in the preliminary experiments and listed in Table 1 could be detected in the individual level of dilution.

2. Results and discussion

2.1. Microbiological efficacy

The results of microbiological tests carried out three months after treatment on the samples in storage, are summarized in Table 2.

It can be seen that the most contaminated sample of the four was black pepper. This corresponds to the results of many other investigations (ICMSF, 1980; FARKAS, 1987). Compared to the current microbiological criteria in Hungary (MINISTRY OF PUBLIC HEALTH, 1978) the mesophilic aerobic total viable cell count, the Enterobacteriaceae count and mould count of the untreated black pepper was found objectionable, while objection could be raised on the basis of the Enterobacteriaceae count and mould count against the onion powder.

According to Table 2 even a treatment with 4 kGy was more efficient in some cases than ethylene oxide treatment as carried out according to the industrial practice. The residual viable cell count of the samples treated with 8 kGy was lower by 1–3 orders of magnitude that of the ethylene oxide-treated samples.

In the practice of international spice trade a 10^3–10^4 total mesophilic aerobic viable cell count is a permitted maximum for spices treated to reduce cell count (GERHARDT, 1969; WEBER, 1980). According to the results of our experiments to achieve this a treatment of 4 kGy would have been necessary

Table 2

Microbial contamination of spices as affected by ethylene oxide treatment and gamma radiation doses

Spice	Treatment	Logarithm of viable cell counts[a]				
		MACC	TACC	EC	MC	BCC
Black pepper	untreated	6.83±0.06	5.54±0.03	3.20±0.10	4.45±0.04	4.43±0.08
	4 kGy	4.53±0.07	2.32±0.11	1.11±0.55	<0.7	3.52±0.08
	8 kGy	2.41±0.08	1.18±0.5	<1.0	<0.7	<2.0
	EtO	5.30±0.02	3.20±0.03	<1.0	0.7	3.38±0.09
Paprika	untreated	4.74±0.06	3.23±0.10	2.74±0.17	2.23±0.42	3.18±0.12
	4 kGy	2.38±0.09	1.00±0.02	<1.0	1.18±0.35	<2.0
	8 kGy	1.40±0.3	<1.0	<1.0	<1.0	<2.0
	EtO	2.04±0.04	1.88±0.18	<1.0	1.68±0.25	2.18±0.4
Onion powder	untreated	5.20±0.03	2.85±0.17	3.80±0.03	4.20±0.03	2.68±0.19
	4 kGy	3.60±0.07	<1.0	<1.0	1.78±0.20	<2.0
	8 kGy	3.00±0.05	<1.0	<1.0	<1.0	<2.0
	EtO	4.60±0.02	<1.0	1.30<0.35	2.40±0.13	2.30±0.2
Garlic powder	untreated	5.89±0.05	4.20±0.03	<1.0	2.78±0.15	3.43±0.08
	4 kGy	4.48±0.03	3.26±0.10	<1.0	1.48±0.3	2.30±0.4
	8 kGy	2.08±0.15	1.30±0.4	<1.0	<1.0	<2.0
	EtO	5.32±0.02	3.76±0.06	<1.0	2.2 ±0.11	3.04±0.12

[a] average ± confidence interval ($P \leq 0.05$)
MACC: mesophilic aerobic cell count
TACC: thermophilic aerobic cell count
EC: Enterobacteriaceae count
MC: Mould propagule count
BCC: *Bacillus cereus* viable cell count

for paprika, and radiation doses between 4 and 8 kGy for black pepper, onion powder and garlic powder. Except with paprika, the ethylene oxide treatment did not bring about the desirable effect.

The results of these microbiological tests correspond to those described in related literature (SZABAD & KISS, 1979; FARKAS, 1987). In earlier experiments it was found also that the residual microflora of the radiation treated spices was more sensitive to antimicrobial influences administrated during further processing of the food products than the microflora of untreated spices (FARKAS, 1973; FARKAS et al., 1973; FARKAS & ANDRÁSSY, 1983).

2.2 Effect of cell count reducing treatments on the volatile oil content of black pepper

Volatile oil determinations carried out one month and six months after treatment are summed up in Table 3.

One month after radiation treatment the volatile oil content of the samples was found to be equal to that of the untreated samples, whereas that of

Table 3

Yield of volatile oil from black pepper in cm³ related to 100 g dry matter

Treatment	1 month after treatment	6 months after treatment
Untreated	2.83±0.03 (a)	2.82±0.11 (a)
4 kGy	2.84±0.07 (a)	2.85±0.10 (a)
8 kGy	2.83±0.10 (a)	2.60±0.05 (b)
EtO	1.99±0.07 (c)	2.00±0.07 (c)

The reported values are averages and ± standard deviations of 4 measurements
Average values not differing significantly from one another are marked with the same letter
($LSD_{0.05} = 0.15$ cm³)

the ethylene oxide-treated samples was significantly lower, about 70% of that of the untreated samples. After six months storage the significant difference in the volatile oil content of the samples given two different treatments remained unchanged. The volatile oil content of the untreated and the 4 kGy-treated samples remained unchanged during the whole storage period, of those treated with 8 kGy was slightly reduced, with about 8% of the initial. The results correspond to those published by VAJDI and PEREIRA (1973) who found, too, a significant loss of volatile oil content in black pepper given cell count reducing ethylene oxide treatment, while the radiation treatment did not cause any change.

2.3. Effects of cell count reducing treatments and storage upon the absorbancy of spice extracts

To characterize the coloring capacity of ground paprika samples one month after treatment, the absorption spectra of their sunflower seed oil extracts were compared with the variance analyses of their absorbancies as measured at 465 nm and 668 nm absorption maxima. Significant differences were not found between the absorption spectra of the oil extracts of treated and untreated samples. These results correspond to earlier observation according to which there was no significant difference in the benzene or hexane-soluble pigment content of untreated and radiation treated paprika samples, applying radiation doses of the same order of magnitude (TÖRÖK & FARKAS, 1961; FARKAS et al., 1973; VAJDI & PEREIRA, 1973; NEUMAYR et al., 1983).

Photometric investigation of the sodium chloride extract of the spice samples was carried out in order to compare the amount of color substances formed during possible browning. Ground paprika was studied after 1 month storage, onion powder and garlic powder after 2 and 8 months storage, taking absorbancies measured at 410 nm as a basis. The absorbancy of ground paprika

extract in salt solution treated with 8 kGy was statistically significantly higher than that of the untreated sample. On the other hand the absorbancy of the extract of ethylene oxide-treated paprika was significantly higher than that of the 8 kGy-treated paprika. The onion and garlic powder samples, when studied after 2 months storage, did not show significant differences as a function of treatment. In comparing the samples after 8 months storage with the samples stored for 2 months the advance in browning appeared in a signif-

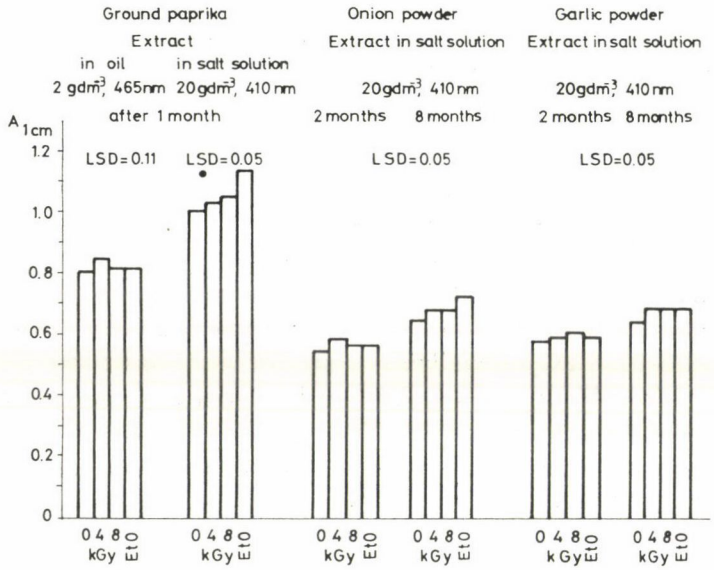

Fig. 1. Absorbancy of the spice extracts as affected by cell count reducing treatments and storage. LSD = least significant difference ($P \leq 0.05$), EtO = sample treated with ethylene oxide

icant increase of absorbancy. The ethylene oxide-treated onion samples changed significantly in comparison with the untreated sample. Results are illustrated in Fig. 1.

The results of spectrophotometry are in agreement with those of JOSIMOVIC (1983) who studied the aqueous extract of seven different irradiated spices and did not find qualitative changes in comparison to the untreated samples. The observations related to irradiation of onion powder support the findings of GALETTO and co-workers (1979).

2.4. Effect of cell count reducing treatments upon the water uptake capacity of onion and garlic powder

The results of water absorption tests, carried out 2 and 6 months after treatment are shown in Table 4.

Table 4

Water absorbing capacity of the untreated and cell count reduced onion and garlic powder samples 2 months and 6 months after treatment

Treatment	Onion powder		Garlic powder	
	after 2 months	after 6 months	after 2 months	after 6 months
Untreated	53.3±5.2 (b)	55.0±2.0 (b)	35.8±1.4 (d)	34.7±0.5 (d)
4 kGy	68.3±2.9 (a)	71.7±2.4 (a)	35.8±1.4 (d)	40.8±3.1 (c)
8 kGy	71.7±2.9 (a)	73.3±3.1 (a)	39.2±1.4 (cd)	38.3±2.4 (cd)
EtO	52.5±2.5 (b)	54.2±1.2 (b)	35.0±0 (b)	37.5±2.0 (cd)

Average values not differing significantly are marked with the same letter
Onion powder: $LSD_{0.05} = 7.0$ cm³; garlic powder: $LSD_{0.05} = 4.8$ cm³
Water absorbing capacity: amount of water absorbed by 10 g sample during 24 h in cm³
Values in the Table are averages and ± standard deviations of 3 parallel measurements

In the case of onion powder both radiation doses caused significant increase in water absorption already after 2 months storage. For garlic powder the effect of irradiation was similar, however, the increase of water absorption capacity was less extensive.

This effect of irradiation seems to be connected with the degradation to some extent of the structural polysaccharides, particularly that of pectin and is probably analogous to the tenderizing, cooking time reducing radiation effect, that has been earlier described in relation to irradiated dried vegetables (SCHROEDER, 1962) and was observed by the author, too. (FARKAS et al., 1970).

2.5. Sensory evaluation

The results of the sensory evaluation of the seasoning capacity (flavor intensity of the whipped cream suspension) and the directly perceptible odour intensity are illustrated in Figs. 2 and 3.

The figures show the averages of the arbitrary units calculated from the intensity values as marked by the 10–12 panel members and the least significant difference (LSD) values calculated by variance analysis. Based on the latter, the confidence interval of the average intensity values of the untreated samples after a storage period of 10–20 days, was marked.

As it can be seen in Fig. 2. statistically significant differences between the untreated samples and the treated black pepper samples became apparent only at the first analysis, after 10 days storage. The average flavor intensity, expressed in the arbitrary units, of the 4 kGy-treated samples was significantly higher, and that of the ethylene oxide-treated samples significantly lower than that of the untreated samples. During a five months storage period

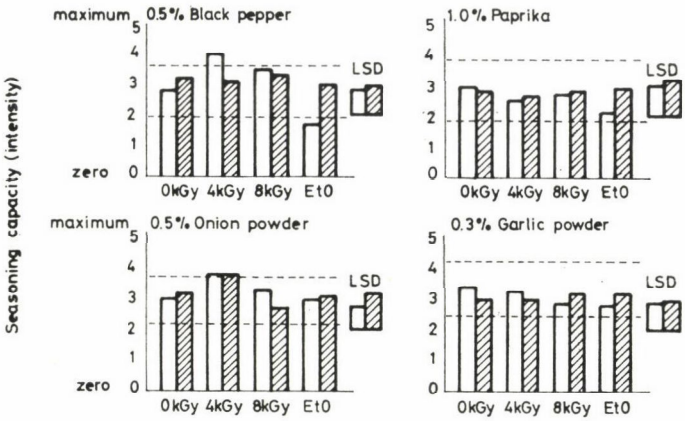

Fig. 2. Average flavor intensity values of the whipped cream suspensions of spices and the least significant differences (LSD) expressed in the arbitrary units as described in para. 2. Dashed lines mark the confidence intervals belonging to the average values of the first analyses of the untreated samples. □: 10–20 days storage; ////: 5–6 months storage For other legends see Fig. 1

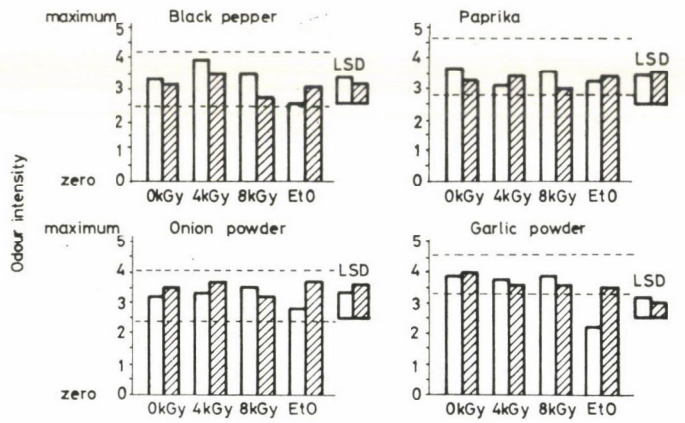

Fig. 3. Odour intensity of spice samples in the arbitrary units as described in the text as a function of cell count reducing treatments and storage time. □: 10–20 days storage; ////: 5–6 months storage. For legends see Fig. 2

these differences disappeared, too. The flavoring capacity of the 4 kGy-treated onion powder tended to be higher at the time of both control analyses than that of the other samples.

The flavoring capacity of the samples stored in cans at room temperature, as measured by the given method, remained practically unchanged during the about six months storage period.

As it is shown in Fig. 3 the averages of odour intensity in black pepper revealed similar tendency, however, the differences compared to the untreated samples did not reach a statistically significant level.

Supposing, that by analysis of the global flavor or odour intensity slight changes cannot be discovered, were the flavor profile analyses of the tap water suspensions and of their dilutions carried out. Figures 4 and 5 show the profilograms of the 20-fold dilutions.

In preparing these Figures the characteristic flavor components as determined in preliminary experiments, were represented as radii and the center of the circle represented 0, the point on the circle 100% observation

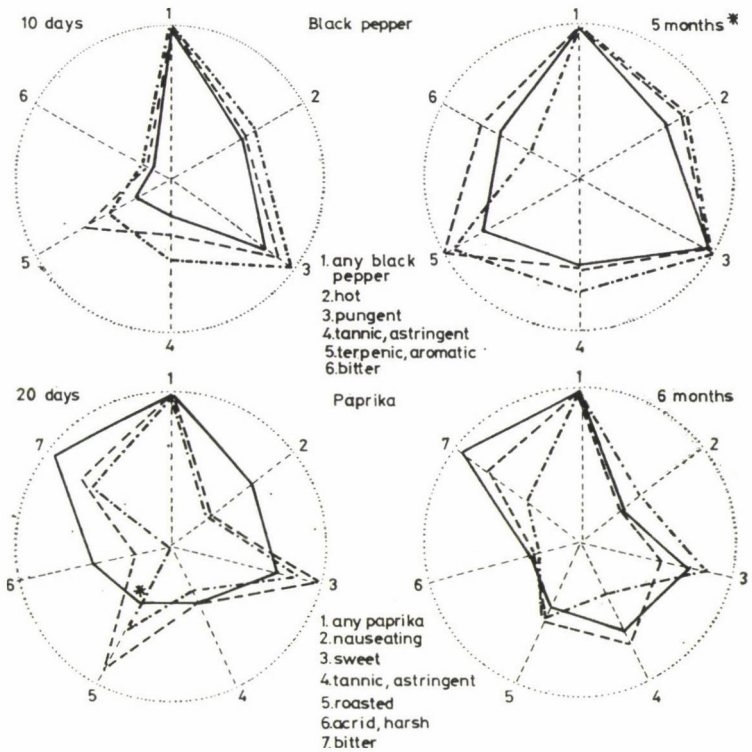

Fig. 4. Flavor profilograms of the 20-fold aqueous dilution of black pepper and paprika samples. For detailed explanation see para. 2.4. ----: OkGy; —·—·—: 8 kGy; ——: EtO; *: statistically significant difference compared with the control samples ($P \leq 0.05$)

frequency. The lengths proportional to the observation frequencies were marked on the flavor component radii and by connecting these points, the flavor profilogram belonging to a given treatment, was obtained. To achieve a better lucidity of arrangement only the profilograms belonging to the higher radiation dose, 8 kGy was presented. The differences in the observation frequencies of the individual flavor components were statistically evaluated by the $\varkappa^2$ test as a function of treatments or of storage periods. Although the flavor profile analyses resulted in more or less differing flavor profilograms,

taking into consideration identical storage periods, gave only rarely observation frequencies statistically significantly differing ($P = 0.05$) from the observation frequencies as obtained in relation to individual flavor components of the untreated samples. The ethylene oxide treatment tended to distort more frequently the profilograms related to the untreated samples than radiation treatment. The about six months storage period caused greater change in the flavor profile of the samples than the coll count reducing treatments.

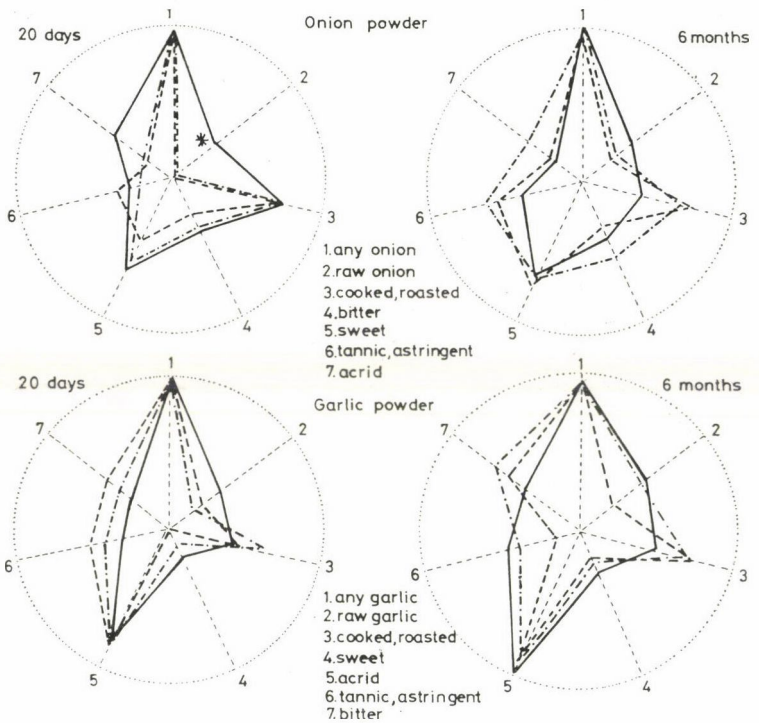

Fig. 5. Flavor profilograms of the 20-fold aqueous dilutions of onion and garlic powder. For detailed explanation see para 2.4. For symbols see Fig. 4

In the case of those main flavor components, where the observation frequency unambiguously diminished with increasing rate of dilution a probit analysis of observation frequency was carried out. Here, namely, observation frequency as function of dilution rate could be described with a more or less descending sigmoid curve and the connection between the probit of observation frequency and the logarithm of dilution could be approximated with straight lines. By means of probit analysis the logarithms of the dilutions belonging to 50% observation frequency were established and these values characteristic of the intensity of flavor components, were presented in Figs. 6 and 7.

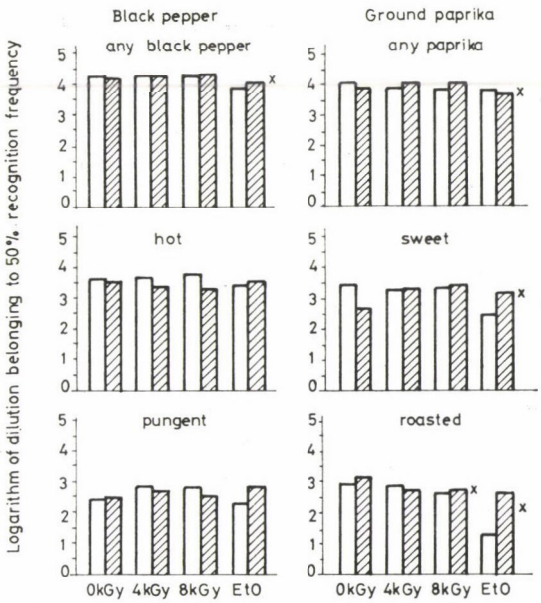

Fig. 6. Logarithms of the dilutions belonging to the observation frequencies of the main flavor components in the aqueous dilutions of black pepper and ground paprika samples as functions of cell count reducing treatments and storage time. □: 10–20 days storage; ////: 5–6 months storage. x: for explanations see para. 2.5

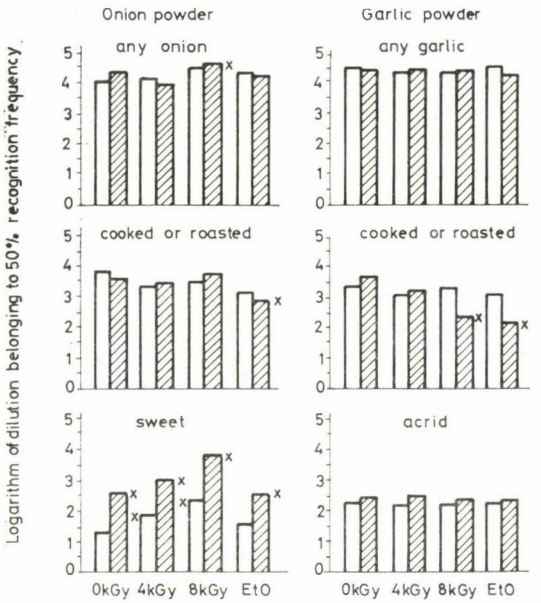

Fig. 7. Logarithms of the dilutions belonging to the 50% observation frequencies of the main flavor components of the aqueous onion and garlic powder dilution as a function of cell reducing treatments and storage time. □: 20 days storage; ////: 6 months storage. *: for explanation see para. 2.5

This analysis has shown, too, that apart from a few exceptions (marked with "x"), about the same dilution was required to reduce the flavor components of the treated samples to the 50% observation frequency as with the untreated samples. Taking into account the exeptions it seems that the intensity of some flavor components weekened mainly upon treatment with ethylene oxide (the necessary dilution levels were lower than with the untreated samples). It is interesting that the intensity of "sweet" component of onion substantially increased as an effect of both irradiation and storage. An analogy seems to exist between the irradiation affected water absorption capacity and the intensification of the sweet component in onions. In the experiments here described was not observed a slight caramelisation of the onion powder upon treatment with 8 kGy as it was found in earlier experiments carried out with Egyptian onions (FARKAS & EL-NAWAVY, 1973). The present results are in agreement with those obtained in the USA (SILBERSTEIN et al., 1979: GALETTO et al., 1979) which did not show any change of taste in onion upon irradiation to reduce cell count.

3. Conclusions

The experiences gained in this study support findings of researchers in the spice processing industry (e.g. WEBER, 1983; EISS, 1984) according to which it is possible to achieve reduction of cell count by treatment with gamma radiation without impairment of the sensory quality or other quality characters of the spices. To sum up, it can be concluded that to reduce the cell count in spices irradiation is competitive with ethylene oxide treatment from the point of view of both microbiological efficacy and maintenance of quality.

Literature

BAXTER, R. & HOLZAPFEL, W. H. (1982): A microbial investigation of selected spices, herbs and additives in South Africa. *J. Fd Sci., 47*, 570–573.

BOCKEMÜHL, J. & WOHLERS, B. (1984): Zur Problematik der Kontamination unbehandelter Trockenprodukt der Lebensmittelindustrie mit Salmonella. *Zentbl. Bakt. Hyg., Orig., B 178*, 535–541.

BOER, E. DE & BOOT, E. M. (1983): Comparison of methods for isolation and confirmation of *Clostridium perfringens* from spices and herbs. *J. Fd Protection, 46*, 533.

BUI THI YEN, STEGEMAN, H. & STREUTJENS, E. P. M. (1986): Effect of irradiation and fumigation on the microbiological and chemical quality of ginger and red pepper. IFFIT Report No. 65. International Facility for Food Irradiation Technology, Wageningen, The Netherlands (Unpublished).

C. D. C. (CENTERS FOR DISEASE CONTROL) (1982): Outbreak of *Salmonella oranienburg* infection — Norway. *Morb. Mort. Wkly Rep., 31*, 655–656.

EHRENBERG, L. & HUSSAIN, S. (1981): Genetic toxicity of some important epoxides. *Mutation Res., 86*, 1–113.

EISS, M. I. (1984): Irradiation of spices and herbs. *Fd Technol. Aust., 36*, 362, 363, 366, 370.

FARKAS,J. (1973): Radurization and radicidation of spices. in: *Aspects of the introduction of food irradiation in developing countries*, IAEA. Vienna, pp. 43–59.

FARKAS,J. (1985): Sugárkezelés, mint at etilénoxid alternativája (Radiation treatment as an alternative of ethylene oxide fumigation). *Élelm. Ipar, 39*, 1–8.

FARKAS, J. (1987): *Irradiation of dry food ingredients*. CRC Press, Inc., Boca Raton, Fla, USA (in press)

FARKAS, J. & ANDRÁSSY, É. (1983): Besugárzott fűszerek túlélő mikroflórájának megnövekedett érzékenysége. (Increased sensitivity of the surviving microflora of irradiated spices). *Húsipar, 32*, 113–118.

FARKAS, J., BECZNER, J. & INCZE, K. (1973): Feasibility of irradiation of spices with special reference to paprika. — in: *Radiation preservation of food*. IAEA, Vienna, pp. 389–402.

FARKAS, J. & EL-NAWAVY, A. S. (1973): Effect of gamma radiation upon the viable cell count and some other quality characteristics of dried onions. *Acta Alimentaria, 2*, 437–445.

FARKAS, J., KÁLMÁN, B., BENCZE-BÖCS, J. & JAUERNIG, A. (1970): Kísérletek a zöldségszárítmányok minőségének javítására ionizáló sugárzással. (Experiments to improve the quality of dried vegetable lots by means of ionizing radiation). *Kísérl. Közl.* XIII/E. *Élelmiszeripar*, (1–3), 21–40.

FÁBRI, I., NAGEL, V., TABAJDI-PINTÉR, V., ZALAVÁRI, ZS., SZABAD, J. & DEÁK, T. (1985): Qualitative and quantitative analysis of aerobic sporeforming bacteria in Hungarian paprika. — in: DRING, G. J., ELLAR, D. J. & GOULD, G. W. (Eds) *Fundamental and applied aspects of bacterial spores*. Academic Press, London, pp. 445–462.

FLANNIGAN, B. & HUI, S. C. (1976): The occurrence of aflatoxin-producing strains of *Aspergillus flavus* in the mold floras of ground spices. *J. appl. Bact., 41*, 411–418.

FUNKE, D., KREY, P. & JANTZ, A. (1984): Microbiological and sensory evaluation of several gamma-irradiated spices. — in: *ZFI-Mitteilungen No. 98*. Zentralinstitut für Isotopen- und Strahlenforschung, Leipzig, pp 642–647.

GALETTO, W., KAHAN, J., EISS, M., WELBOURN, J., BEDNARCZYK, A. K. & SILBERSTEIN, D. (1979): Irradiation treatment of onion powder: effects on chemical constituents. *J. Fd Sci., 44*, 591–595 + 605.

GERHARDT, U. (1969): Entkeimung von Gewürzen. *Gordian, 69*, 427–432.

GRÜNEWALD, TH. (1984): *Untersuchungen zur Bestrahlung von Trockenprodukten*. BFE-R-84–02, Berichte der Bundesforschungsanstalt für Ernährung, Karlsruhe.

GUSTAFSEN, S. & BREEN, O. (1984): Investigation of an outbreak of *Salmonella oranienburg* infection in Norway caused by contaminated black pepper. *Am. J. Epidemiol.*, 806–812.

HOGSTEDT, G., MALMQUIST, C. N. & VADMAN, D. (1979): Leukemia in workers exposed to ethylene oxide. *J. Am. med. Ass., 241*, 1132–1133.

HUNGARIAN STANDARD (1971): *Tartósított élelmiszerek. Vízfelvevőképesség és főzési idő meghatározása*. (Conserved foods. Determination of water absorbing capacity and of the cooking time.) MSz 3607/2–71.

HUNGARIAN STANDARD (1977a): *Fűszerpaprika őrlemény vizsgálata. Víztartalom meghatározása*. (Ground spice paprika — Tests. Moisture content determination.) MSz 9681/3–77.

HUNGARIAN STANDARD (1977b): *Fűszerek vizsgálata. Víztartalommeghatározás*. (Spices-tests. Determination of the moisture content.) MSz 20622/2–77.

HUNGARIAN STANDARD (1978): *Fűszerek vizsgálata. Illóolajtartalom meghatározása*. (Spices-tests. Determination of the volatile oil content.) MSz 20622/5–78.

HUNGARIAN STANDARD (1980): *Tartósított élelmiszerek nedvességtartalmának meghatározása*. (Preserved foodstuffs determination of moisture content.) MSz KGST 2788–80.

ICMSF (1980): Spices. — in: SILLIKER, J. H., ELLIOTT, R. P., BAIRD-PARKER, A. C., BRYAN, F. L., CHRISTIAN, J. H. B., CLARK, D. S., OLSON, J. C., Jr. & ROBERTS, T. A. (Eds) *Microbial ecology of foods*. Vol. 2. International Committee on Microbiological Specifications for Foods. Academic Press, New York, pp. 731–751.

ITO, H., WATANABE, H., BAGIAWATI, S., MUHAMED, L. J. & TAMURA, N. (1985): Distribution of microorganisms in spices and their decontamination by gamma irradiation. Paper IAEA-SM-271 at IAEA/FAO Internat. Symp. on Food Irradiation Processing, Washington, D. C., U.S.A. p. 110.

JOSIMOVIC, L. (1983): Radiation treatment of spices and some protein rich food. IAEA Research Contract No. 3277/RB. Boris Kidric Institute of Nuclear Sciences, Vinca, Beograd, Yugoslavia.

LAIDLEY, R., HANDZEL, S., SEVERS, D. & BUTLER, R. (1974): *Salmonella weltevreden* outbreak associated with contaminated pepper. *Epidem. Bull.*, (Dep. Natl. Health Welfare, Ottawa/*18*, (4), 62.

MIHÁLYI, V. & ZUKÁL, E. (1972): Az izprofil-analízis használata fűszerek és fűszerkivonatok vizsgálatára. (Using the flavour profile analysis in the examination of spices and spice extracts.) *Húsipar, 21*, 127–132.

MINISTRY OF PUBLIC HEALTH (1978): Az egészségügyi miniszter 6/1978/VII. 14./EüM számú rendelete az élelmiszerek élelmezésegészségügyi mikrobiológiai szennyeződésének elhárításáról. (Regulation No. 6/1978/VII. 14) of the Ministry of Public Health on the elimination of microbial contamination in foods.) *Mezőgazdasági és Élelmezésügyi Értesítő*, 29, (22), 626–641.

NEUMAYR, L., PROMEUSCHEL, S., ARNOLD, I. & LEISTNER, L. (1983): *Gewürzentkeimung, Verfahren und Notwendigkeit.* Abschlussbericht für die Adalbert-Raps-Stiftung zum Forschungsvorhaben. Institut für Mikrobiologie, Toxikologie und Histologie der Bundesanstalt für Fleischforschung, Kulmbach.

NIKOLAEVA, S. A. (1976): *Cl. perfringens* in foodstuffs and in semimanufactured products in the canning industry (In Russian). *Gig. Sanit., 32*, (5) 30–34.

O. S. H. A. (1984): Occupational exposure to ethylene oxide. Safety and Health Administration. *Fed. Regist., 49*, (122), 25734.

POWERS, E. M., LATT, T. G. & PROWN, T. (1976): Incidence and levels of *Bacillus cereus* in processed spices. *J. Milk Fd Technol., 39*, 668–670.

SCOTT, P. M. & KENNEDY, B. P. C. (1975): The analysis of spices and herbs for aflatoxins. *Can. Inst. Fd Technol. J., 8*, 124–125.

SCHROEDER, C. W. (1962): *Dehydrating vegetables.* U. S. Patent No. 3025171.

SILBERSTEIN, O., GALETTO, W. & HENZI, W. (1979): Irradiation of onion powder: effect on microbiology. *J. Fd Sci., 44*, 975.

STIJVE, T., KALSBACH, R. & EYRING, G. (1978): Determination and occurrence of ethylene chlorohydrin residues in foodstuffs fumigated with ethylene oxide. *Trav. Chim. aliment. Hyg., 67*, 504–428.

SZABAD, J. & KISS, I. (1979): Comparative studies on the sanitising effects of ethylene oxide and of gamma radiation in ground paprika. *Acta Alimentaria, 8*, 383–395.

TILGNER, D. J. (1965): Flavor dilution profilograms. *Fd Technol., 19*, (1), 25–29.

TOOFANIAN, F., STEGEMAN, H. & STREUTJENS, E. (1985): *Comparative effect of ethylene oxide and gamma irradiation on the chemical, sensory and microbial quality of ginger, cinnamon, fennel and fenugreek.* IFFIT Report No. 60, International Facility for Food Irradiation Technology. Wageningen, The Netherlands.

TÖRÖK, G. & FARKAS, J. (1961): Kísérletek fűszerpaprika őrlemények ionizáló sugárzásos csiraszámcsökkentésére. (Investigations into the reduction of viable cell count of paprika powder by ionizing radiation.) *Közp. Élelmiszeripari Kutatóintéz. Közl.* (3), 1–7.

UCHMAN, W., FISZER, N., MROZ, I. & PAWLIK, A. (1983): The influence of radappertization upon some sensory properties of black pepper. *Nahrung, 27*, 461–468.

VAJDI, M. & PEREIRA, N. N. (1973): Comparative effects of ethylene oxide, gamma irradiation and microwave treatment on selected spices. *J. Fd Sci., 38*, 893–895.

WEBER, F. E. (1980): Controlling microorganisms in spices. *Cereal Foods Wld, 25*, 319.

WEBER, H. (1983): Gewürzentkeimung, Einflüsse von Elektronen und Gammastrahlen auf die Qualität verschiedener Gewürze. *Fleischwirtschaft, 63*, 1069–1071.

WESLEY, F., ROURKE, B. & DARBISHIRE, O. (1965): The formation of persistent toxic chlorohydrins in foodstuffs by fumigation with ethylene oxide and with propylene oxyde. *J. Fd Sci., 30*, 1037–1042.

WHO (1974): *Salmonella* survei llance. World Health Organization, *Geneva. Wkly epidem. Rec., 42*, 351.

WHO (1982): Foodborne disease surveillance. Outbreak of *Salmonella oranienburg* infection. World Health Organization, Geneva. *Wkly epidem., Rec., 57*, 329–330.

WILSON, C. R. & ANDREWS, W. H. (1976): Sulfite compounds as neutralizers of spice toxicity for Salmonella. *J. Milk Fd Technol., 39*, 464–466.

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ACTA ALIMENTARIA

VOLUME 17 No. 1 — 1988

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Index: 26.039

ACTA

ALIMENTARIA

An International Journal of Food Science

Editor

J. HOLLÓ

Volume 17 **June 1988** **Number 2**

Akadémiai Kiadó
Budapest

Kluwer Academic Publishers
Dordrecht/Boston/London

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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Kluwer Academic Publishers
P.O. Box 17, 3300 AA Dordrecht, Holland
and 101 Philip Drive, Norwel, MA 02061 U. S. A

Publication programme, 1988: Volume 17 (4 issues)
Subscription prices per volume: Dfl. 276,—/$ 130.00 including postage.

Acta Alimentaria is published 4 times per annum: March, June, September and December

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.

ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

VOLUME 17

1988

AKADÉMIAI KIADÓ
BUDAPEST

KLUWER ACADEMIC PUBLISHERS
DORDRECHT/BOSTON/LONDON

Acta Alimentaria, Vol. 17 (2), pp. 95–101 (1988)

GAS-LIQUID-CHROMATOGRAPHIC ANALYSIS OF THE LIPID COMPOSITION OF *ELETTARIA CARDAMOMUM* SEEDS

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(Received: 25 June 1986; revision received: 6 April 1987; accepted: 13 April 1987)

Lipid fractions of *Elettaria cardamomum* seeds were obtained by successive soaking in petroleum ether, acetone, diethylether and ethanol, respectively. All the lipid samples were analysed for fatty acids, hydrocarbons, tocopherol and sterols. The composition of the lipid fractions showed significant differences; palmitic and oleic were the dominant fatty acids, while smaller amounts of other fatty acids were also detected. The inherent hydrocarbons: C_{22}, C_{24}, C_{25}, C_{26}, C_{28}, C_{29}, C_{30} and C_{33} were detected (or not) among the extracts with different percentages.

α-Tocopherol was found in all extracts, with maximum concentration in the ethanol extract. Ether extract had slightly higher sterol content than the other fractions and included campesterol, desmosterol, β-sitosterol, stigmasterol, $\Delta^{5,7,22}$ cholestatrienol and traces of cholesterol.

Keywords: Lipid composition, *Elettaria cardamomum*, gas-liquid chromatography

Elettaria cardamomum seeds (PARRY, 1921) are widely used in flavouring of food due to their high volatile oil content. Furthermore, extracts of *Elettaria cardamomum* seeds reveal antimicrobial and antifungal activities (MARUZZELLA & FREUNDLICH, 1959; MARUZZELLA & LICHTENSTEIN, 1956; MARUZZELLA & LIGUORI, 1958) as well as a therapeutic action as RES stimulators.

Several studies (e.g. JELLUM, 1971) on cotton seed, peanut and soyabean oils showed that the rate, amount, color and quality of total lipids were influenced by the solvent used in the extraction procedure. TSEN and coworkers (1962) found that the amount of lipids extracted from wheat products by a mixture of ethyl alcohol–water–chloroform was greater than obtained with petroleum ether alone.

The present study was concentrated on the composition of the lipid fractions obtained by successive soaking in different solvents (petroleum ether, acetone, diethylether, ethanol).

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Table 1

Retention times and percentage of the separated fatty acid methyl esters of Elettaria cardamomum *seeds, using different solvents on 15% diethylene glycol succinate packed column*

Fatty acids	Number of C-atoms	R_t (min)	Petroleum ether (%)	Acetone (%)	Ether (%)	Ethanol (%)
Lauric	12 : 0	5.4	—	2.85	—	0.83
Myristic	14 : 0	8.4	—	0.16	1.05	0.28
Myristoleic	14 : 1	9.8	—	2.74	—	—
Palmitic	16 : 0	11.6	37.50	33.98	47.12	12.89
Palmitoleic	16 : 1	12.3	1.95	2.96	5.76	—
Heptadecanoic	17 : 0	13.2	—	4.79	—	—
Phytanic	17 : 1	15.4	—	0.91	—	—
Stearic	18 : 0	16.4	1.56	6.84	1.57	—
Oleic	18 : 1	17.6	35.16	32.84	44.50	77.67
Linoleic	18 : 2	20.4	0.39	9.12	—	—
Linolenic	18 : 3	21.6	23.44	2.85	—	8.32

Table 2

Retention times and percentage of the unsaponifiable components of Elettaria cardamomum *seeds, using different solvents on 5% OV-101 packed column*

Contents	Compound	R_t (min)	Petroleum ether extract (%)	Acetone extract (%)	Diethyl ether extract (%)	Ethanol extract (%)
Hydrocarbons	C_{22}	0.25	—	—	45.23	—
	C_{24}	0.60	4.07	—	—	—
	C_{25}	0.70	—	—	10.37	0.58
	C_{26}	0.75	0.68	6.14	—	—
	C_{28}	0.85	1.02	1.02	—	—
	C_{29}	0.95	18.98	—	—	0.58
	C_{30}	1.15	55.59	70.31	19.02	—
	C_{33}	1.35	4.75	2.05	—	—
Tocopherol	α-Tocopherol	1.7	2.71	5.46	1.44	98.27
Sterols	$\Delta^{5,7,22}$Cholestatrienol	1.95	2.71	2.73	0.58	—
	Desmosterol	2.2	5.42	5.46	0.58	—
	24-Methylene cholesterol	2.5	2.03	3.07	—	0.58
	Campesterol	2.6	—	—	19.60	—
	Cholesterol	2.7	0.68	—	—	—
	Stigmasterol	2.9	1.36	0.68	1.15	—
	β-Sitosterol	3.2	—	3.07	1.73	—

1. Materials and methods

1.1. Extraction procedure

Approximately 625 g of ground seeds of *Elettaria cardamomum* were soaked in petroleum ether, acetone, diethylether and ethanol, respectively, each for one week at room temperature with occasional shaking. Each extract was filtered off and evaporated under reduced pressure till dryness. Yields were 0.586%, 0.472%, 0.488% and 0.525%, respectively, in order of the solvents above.

1.2. Saponification and esterification procedures

A sample from each extract was saponified (JAMIESON & REID, 1965) with 0.5% alcoholic KOH, under reflux for one hour. The resulting solutions were poured into an equal volume of cold water each separately, and the unsaponifiable materials removed by successive extraction with ether. The saponifiable materials were separated, acidified releasing the free fatty acids

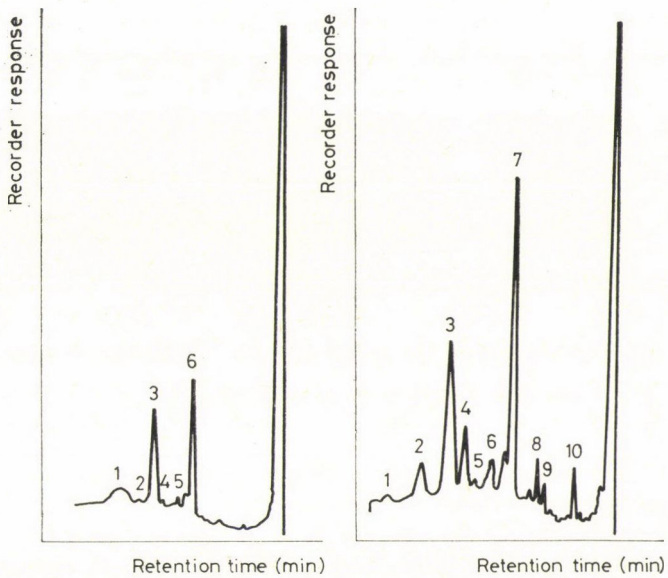

Fig. 1. Separation of fatty acid methyl esters of the lipid extracts on column packed with 15% diethylene glycol succinate polymer on Chromosorb W/Aw DMCS. Petroleum ether extract. 1: linolenic acid; 2: linoleic acid; 3: oleic acid; 4: stearic acid; 5: palmitoleic acid; 6: palmitic acid

Fig. 2. Separation of fatty acid methyl esters of the lipid extracts on column packed with 15% diethylene glycol succinate polymer on Chromosorb W/Aw DMCS. Acetone extract. 1: linolenic acid; 2: linoleic acid; 3: oleic acid; 4: stearic acid; 5: phytanic acid; 6: palmitoleic acid; 7: palmitic acid; 8: myristoleic acid; 9: myristic acid; 10: lauric acid

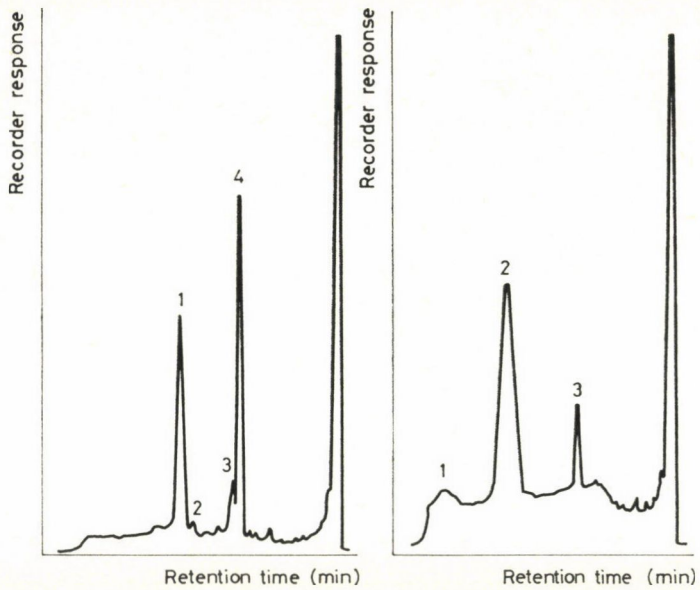

Fig. 3. Separation of fatty acid methyl esters of the lipid extracts on column packed with 15% diethylene glycol succinate polymer on Chromosorb W/Aw DMCS. Diethyl-ether extract. 1: oleic acid; 2: stearic acid; 3: palmitoleic acid; 4: palmitic acid

Fig. 4. Separation of fatty acid methyl esters of the lipid extracts on column packed with 15% diethylene glycol succinate polymer on Chromosorb W/Aw DMCS. Ethanol extract. 1: linolenic acid; 2: oleic acid; 3: palmitic acid

which were extracted with ether and the combined ether extracts were dried over anhydrous sodium sulfate. After filtration, the ether was removed from the combined extracts by distillation. The fatty acids obtained were converted into methyl esters by refluxing for five hours in a solution of 10 cm^3 of 3% sulfuric acid in anhydrous methanol. The resulting solutions were poured into an equal volume of water and the methyl esters were extracted with petroleum ether. The separated fatty acid methyl esters were dried over anhydrous sodium sulfate, followed by filtration and distillation till dryness.

1.3. Gas-liquid-chromatography

Gas-liquid-chromatographic analysis of the methyl esters was carried out on (3 m long $\times$ 4 mm i.d.) a packed column of diethylene glycol succinate (15%), on Chromosorb W/Aw DMCS, using N_2 gas as the mobile phase at a flow rate of 35 $cm^3\,min^{-1}$. The apparatus was a Perkin Elmer model 900, with flame ionization detector with H_2 flow rate of 20 $cm^3\,min^{-1}$ and air (30 BSIG). The column temperature was 120/200 °C at 6 °C min^{-1}, and the injector temperature was 250 °C. The obtained fatty acid constituents were

identified by comparing their retention times with those of the methyl esters of authentic specimens of the fatty acids.

The unsaponified compounds (ACKMAN & SEBEDIO, 1981) of each lipid extract were separated, and analyzed with a gas chromatograph of Beckman

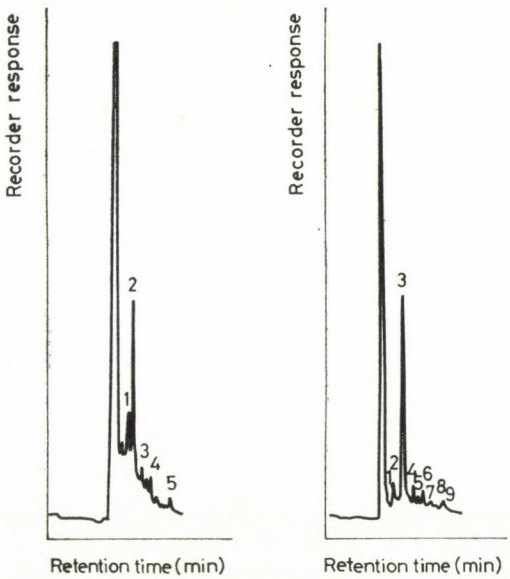

Fig. 5. Separation of unsaponifiable components of the lipid extracts on column packed with 5% OV-101. Petroleum ether extract. 1: C_{29}; 2: C_{30}; 3: α-tocopherol; 4: desmosterol; 5: stigmasterol

Fig. 6. Separation of unsaponifiable components of the lipid extracts on column packed with 5% OV-101. Acetone extract. 1: C_{26}; 2: C_{28}; 3: C_{30}; 4: C_{33}; 5: α-tocopherol; 6: desmosterol; 7: 24-methylene-cholesterol; 8: stigmasterol; 9: β-sitosterol

type equipped with flame ionization detector at a H_2 flow rate of 15 $cm^3\ min^{-1}$ and air of 200 $cm^3\ min^{-1}$. The S.S. column packed with 5% OV-101 on Chromosorb G-HP (100/120), with N_2 carrier gas flow rate of 12 $cm^3\ min^{-1}$. The column temperature was 190–275 °C and injector temperature 290 °C. The unsaponifiable materials obtained were characterized in comparison with authentic standards as cholesterol, stigmasterol, β-sitosterol, as well as by comparing their retention times with data in the literature (PATTERSON, 1971; EL SHAHAWY, 1984). The areas under the peaks were obtained by multiplying the peak height by the width at half peak height, and the percentage area obtained by the internal normalisation technique. The results obtained for the relative amounts of the faity acids, hydrocarbons, α-tocopherol and sterol constituents of *Elettaria cardamomum* seed lipids are shown in Tables 1 and 2 and Figs. 1–8.

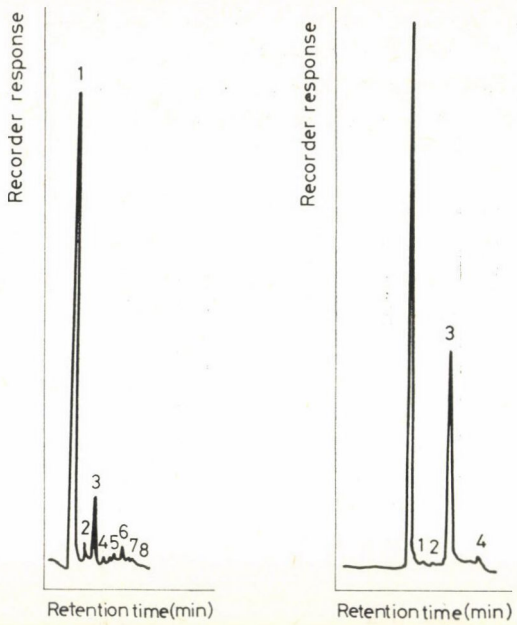

Fig. 7. Separation of unsaponifiable components of the lipid extracts on column packed with 5% OV-101. Diethylether extract. 1: C_{22}; 2: C_{25}; 3: C_{30}; 4: α-tocopherol; 5: desmosterol; 6: campesterol; 7: stigmasterol; 8: β-sitosterol

Fig. 8. Separation of unsaponifiable components of the lipid extracts on column packed with 5% OV-101. Ethanol extract. 1: C_{25}; 2: C_{30}; 3: α-tocopherol; 4: 24-methylene-cholesterol

2. Results

Gas-liquid-chromatographic analysis of the fatty acid content of *Elettaria cardamomum* seeds revealed palmitic and oleic acids, which were mainly extracted with ether and ethanol, respectively.

Linolenic acid was mainly extracted with petroleum ether. Other fatty acids were found in lower amounts: stearic, linoleic, myristic and lauric. Myristoleic, heptadecanoic and phytanic acids were also identified (Table 1).

In addition, it was concluded from the results of the analysis of the unsaponified components obtained from the lipid fractions, that hydrocarbon C_{22} was the main component of the ether extract. The C_{26}, C_{28} and C_{30} were mainly extracted by petroleum ether and acetone.

α-Tocopherol was the only tocopherol found in the four extracts, and represented the main component in ethanol extract.

On the other hand, the sterol contents of the four extracts were also different, of which $\Delta^{5,7,22}$ cholestatrienol, desmosterol, stigmasterol were found in petroleum ether, acetone and diethylether extracts. Campesterol was ex-

tracted only by diethylether, while cholesterol was identified in petroleum ether only. Furthermore, β-sitosterol was found in the acetone and diethylether extracts in smaller amounts.

To sum up *Elettaria cardamomum* seeds contain only two essential fatty acids (linoleic and linolenic acids), one tocopherol (α-tocopherol) and two main sterols (desmosterol and campesterol).

3. Conclusions

Extraction of lipids by successive soaking in petroleum ether, acetone, diethylether and ethanol, respectively, showed optional order of solvent extraction sequence.

GLC studies of *Elettaria cardamomum* seeds of fatty acids content revealed palmitic and oleic acids which mainly extracted by diethylether and ethanol, while linolenic acid was mainly extracted by petroleum ether.

α-Tocopherol was the only tocopherol found in *Elettaria cardamomum* seeds.

Desmosterol, stigmasterol and $\Delta^{5,7,22}$ cholestatrienol of *Elettaria cardamomum* seeds were completely extracted by petroleum ether, acetone and diethylether.

β-Sitosterol was extracted only by acetone and diethylether from *Elettaria cardamomum* seeds in a small amount.

Literature

ACKMAN, R. & SEBEDIO, J. (1981): Fatty acids and sterols in oils from Canola screenings. *J. Am. Oil Chem. Soc.*, *58*, 594–598.

EL SHAHAWY, I. (1984): Doctoral thesis. Faculty of Science, Alexandria University.

JAMIESON, G. & REID, E. (1965): The analysis of oils and fats by gas chromatography. *J. Chromat.*, *1*, 230–237.

JELLUM, M. (1971): Fatty acid composition of corn endosperm germ oils as influenced by different extraction procedures. *J. Am. Oil Chem. Soc.*, *48*, 355–357.

MARUZZELLA, J. & FREUNDLICH, M. (1959): Antimicrobial substances from seeds. *J. Am. pharm. Ass.*, *44*, 356–358.

MARUZZELLA, J. & LICHTENSTEIN, M. (1956): The in vitro antibacterial activity of oils. *J. Am. pharm. Ass.*, *44*, 378–381.

MARUZZELLA, J. & LIGUORI, L. (1958): The in vitro antifungal activity of essential oils. *J. Am. pharm. Ass.*, *46*, 250–254.

PARRY, J. (1921): *The chemistry of essential oils and artificial perfumes.* Vol. 1. pp. 101–106.

PATTERSON, G. (1971): Relation between structure and retention time of sterols in gas chromatography. *Analyt. Chem.*, *43*, 1165–1170.

TSEN, C., LEVI, I. & HLYNKA, I. (1962): A rapid method for the extraction of lipids from wheat products. *Cereal Chem.*, *39*, 195–203.

Acta Alimentaria, Vol. 17 (2), pp. 103–111 (1988)

APPLICATION OF CHROMATIC PARAMETERS TO FOLLOW TIME-DEPENDENT SPOILAGE OF WINES

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(Received: 11 December 1986; accepted: 15 June 1987)

Time elapsed before the chromatic analysis is undertaken has an outstanding influence on the color of red wines. To examine this influence several chromatic systems have been applied to four sets of red and rose Andalusian wines. Methods from the O.I.V. (Office Internationale de la Vigne et du Vin) and Color Spaces recommended by the C.I.E. (Commission Internationale de l'Eclairage) were used. Saturation appears to be the most useful chromatic parameter to assess the preservation status of red wines.

Keywords: Andalusian red wines, chromatic parameters, spoilage

It is well-known that red wines undergo color changes during aging chiefly due to reactions of their polyphenolic constituents (SOMERS & EVANS, 1977; MARECA, 1981; RIBEREAU-GAYON et al., 1983; GLORIES, 1984). In the course of research on the chromatic qualities of Andalusian red wines, the influence of the time elapsed between taking of samples and chromatic measurements on color quality, was examined. Application of new colorimetric systems including Uniform Color Spaces permitted the determination of several chromatic parameters, by means of which the aging processes were followed.

1. Materials and methods

1.1. Materials

Twenty-four Andalusian red and rose wine samples from Cencibel grape variety were studied, all from the same region in Eastern Andalusia and 1984 vintage. Industrial fermentation in 7 m^3 cement tanks and skin-must maceration during the fermentation process (approx. 12 days), were carried out. The samples were taken during the vinification process directly from the fermentation tanks. The wines were allowed to stand during different periods of time before analysis of color (from some hours to several weeks). Non-hermetic containers and ambient temperatures were utilized. So, wines were grouped into four sets each having six samples from a common origin

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Table 1

Time elapsed. Characteristics of the absorption spectrum of wines

Set	Sample No.	Time elapsed	Maximum wavelength (nm)	Absorbance[a]
I	1	Less than 24 h	527.4	5.730
	2	Less than 24 h	529.4	5.310
	3	Two days	527.4	5.630
	4	One week	528.2	6.150
	5	Between two and three weeks	520.2	5.490
	6	Three weeks	526.2	5.180
II	7	Less than 24 h	529.0	5.370
	8	Less than 24 h	527.4	4.770
	9	Two days	527.4	5.550
	10	Between two and three weeks	522.2	5.420
	11	Four weeks	525.8	6.810
	12	Five weeks	524.2	7.020
III	13	Less than 24 h	525.0	1.940
	14	Less than 24 h	525.0	1.760
	15	One week	524.2	1.922
	16	Between two and three weeks	527.0	3.050
	17	Four weeks	521.8	2.815
	18	Five weeks	517.8	2.955
IV	19	Less than 24 h	523.3	0.936
	20	24 h	522.6	0.726
	21	One week	522.2	0.958
	22	Between two and three weeks	517.0	1.220
	23	Four weeks	517.4	1.273
	24	Five weeks	515.0	1.413

[a] Absorbance measured in 10 mm glass cells

as Table 1 shows. The first set (1 to 6) and the second set (7 to 12) were made up of similar red wines. The third set (13 to 18) was formed by red wines with a lower color intensity. The fourth set (19 to 24) consisted of rose wines.

1.2. Methods

1.2.1. O.I.V. Methods (SAMBUC & NAUDET, 1965; STELLA, 1966). These methods are based on Hardy's selected-ordinate method (HARDY, 1936). They involve measurements of transmittances at certain specified wavelengths that are then applied to obtain the tristimulus values and other important parameters for the chromatic analysis of wines. The samples of wine are placed into glass cells with various pathlengths. Transmittances at 445, 495, 550 and 625 nm, with reference to distilled water as a blank, are measured (O.I.V. 1979).

The apparatuses used were Bausch & Lomb spectrophotometer "Spectronic 2000", Bausch & Lomb continous X-Y recorder 333508, Hellma precision glass cells with 1, 2, 5, 10 mm pathlength.

The so-called Rapid Method (SUDRAUD, 1958) is only applied to red and rose wines. Color intensity and tonality are determined by this method. Color intensity is the sum of absorbances at two characteristic wavelengths ($E_{420} + E_{520}$). Tonality is expressed by the arc tangent of the difference between these absorbances.

1.2.2. C.I.E. Colorimetric systems. Usually the weighted-ordinate method is applied (BERG & MARSH, 1956; BERG et al., 1964; WYSZECKI & STILES, 1982). Tristimulus values (X, Y, Z) are calculated from transmittances (T) obtained at constant wavelength intervals (10 nm) through the visible spectrum (380–770 nm). The following equations were applied:

$$X = k\ \Sigma T(\lambda)\ S(\lambda)\ \bar{x}(\lambda)\ \Delta\lambda$$

$$Y = k\ \Sigma T(\lambda)\ S(\lambda)\ \bar{y}(\lambda)\ \Delta\lambda$$

$$Z = k\ \Sigma T(\lambda)\ S(\lambda)\ \bar{z}(\lambda)\ \Delta\lambda$$

where $S(\lambda)$ is the spectral concentration of the radiant power of the source illuminating the object at wavelength λ, and $\bar{x}(\lambda)$, $\bar{y}(\lambda)$, $\bar{z}(\lambda)$ are the standard color-matching functions The $S(\lambda)$ is assumed constant for all wavelengths within the wavelength interval of width $\Delta\lambda$.

In the C.I.E. 1931-x, y system, dominant wavelength and purity or saturation are considered.

The C.I.E. 1964-($U^*V^*W^*$) system (WYSZECKI, 1963) includes the U^*V^*-chromaticity indices and the W^*-lightness index. The newer C.I.E. recommendations, including the uniform spaces CIELUV and CIELAB (C.I.E., 1978), have been also applied. Likewise the latter spaces define chromaticity indices (u^*, v^* and a^*, b^*) and a common psychometric lightness index, L^*. The uniform spaces signify several new chromatic parameters, such as chroma (C^*_{uv}, C^*_{ab}), saturation (S^*_{uv}) and hue angle (h_{uv}, h_{ab}), which are defined by the equations as follows:

$$C^*_{uv} = [(u^*)^2 + (v^*)^2]^{1/2}$$

$$C^*_{ab} = [(a^*)^2 + (b^*)^2]^{1/2}$$

$$S^*_{uv} = \frac{C^*_{uv}}{L^*}$$

$$h_{uv} = \text{arc tan}\ \frac{v^*}{u^*}$$

$$h_{ab} = \text{arc tan}\ \frac{b^*}{a^*}$$

2. Results and discussion

A study of the characteristics of the visible absorption spectrum of wines considered has been done prior to the chromatic analysis. Maximum wavelengths and absorbances are given in Table 1. In a 83% of all the cases the maximum absorption wavelength of the wine samples was observed over 520 nm. This observation agreed well with observations by some authors relating to young wine samples (LEMPERLE & KERNER, 1968). Only the samples of red wines with lower color intensity (clear red and rose wines) when measured after two to five weeks showed wavelengths under 520 nm.

In Table 2, saturation, lightness and dominant wavelength are presented. A very strong correlation, above 0.99, was obtained between the O.I.V. and C.I.E.-x, y systems. Correlation between time elapsed and saturation is outstanding ($\bar{r} = 0.93$, $s_{n-1} = 0.022$) where $\bar{r}$ is the mean value of the correlation coefficients of each set (n = 4).

Table 2

Lightness, saturation and dominant wavelength (O.I.V. and C.I.E. 1931-x, y Methods)

Set	Sample No.	Lightness		Saturation		Dominant wavelength	
		O.I.V.	C.I.E. 1931	O.I.V.	C.I.E. 1931	O.I.V.	C.I.E. 1931
I	1	1.026	1.001	97.566	97.693	615.0	636.0
	2	2.072	1.987	97.361	97.422	615.0	636.0
	3	1.911	1.760	97.547	97.551	615.0	634.0
	4	1.112	1.075	98.434	98.503	615.0	636.0
	5	0.443	0.650	97.751	98.473	615.0	643.0
	6	11.511	14.102	99.125	99.265	614.5	640.0
II	7	1.434	1.288	96.932	96.859	615.5	634.0
	8	2.897	2.571	95.915	95.729	616.0	633.0
	9	2.024	1.805	97.157	97.089	615.5	633.0
	10	0.648	0.976	97.517	98.301	615.0	645.0
	11	0.438	0.662	99.338	99.556	614.5	643.0
	12	0.135	0.223	99.329	99.585	614.5	645.0
III	13	7.947	7.891	64.622	63.997	630.0	626.0
	14	10.433	10.471	60.050	59.614	636.0	629.5
	15	10.198	10.250	62.938	62.507	640.0	631.0
	16	1.863	1.761	86.356	86.160	617.0	624.0
	17	4.128	3.819	85.536	84.701	619.0	624.0
	18	3.536	3.268	88.064	87.339	617.0	622.0
IV	19	25.565	27.669	25.445	27.989	493.5[a]	429.5[a]
	20	39.392	43.186	22.714	25.980	494.0[a]	492.0[a]
	21	28.797	31.638	29.837	32.852	493.0[a]	660.0
	22	17.021	17.282	40.668	40.827	670.0	639.0
	23	16.052	16.658	43.146	43.852	645.0	628.0
	24	11.469	11.682	43.567	43.747	637.0	629.5

[a] Complementary wavelength (WISZECKI & STILES, 1982)

Table 3

Color intensity and tonality (Rapid Method, O.I.V.)

Set	Sample No.	Color	
		intensity	tonality
I	1	8.930	67.295
	2	8.110	66.225
	3	8.520	68.199
	4	9.490	69.746
	5	8.960	63.435
	6	7.560	69.109
II	7	8.340	65.158
	8	7.300	63.435
	9	8.390	68.119
	10	8.640	65.556
	11	10.710	70.017
	12	11.470	68.277
III	13	3.411	23.419
	14	3.048	23.419
	15	3.224	30.491
	16	5.500	31.383
	17	4.880	36.502
	18	5.120	33.024
IV	19	1.564	16.383
	20	1.153	15.908
	21	1.551	19.341
	22	2.076	19.965
	23	2.188	19.762
	24	2.463	19.356

Color intensity: sum of absorbances at two characteristic wavelengths ($E_{420} + E_{520}$)
Color tonality: the arctangent of the difference of the above absorbances

Both uniform and non-uniform spaces presented lightness values which underwent no time-correlated variations ($\bar{r} = -0.50$, $s_{n-1} = 0.723$). Very spoiled samples showed higher lightness values (sample No. 6) that were probably due to the precipitation of coloring matter.

Color intensity ($\bar{r} = 0.60$, $s_{n-1} = 0.611$) and tonality ($\bar{r} = 0.54$, $s_{n-1} = 0.453$) (Table 3) showed a medium correlation with the time-factor.

Samples of wines from the same origin and treatment were located on the U^*V^*-diagram (Fig. 1) making up couples having in common the evolution time. In the case of the most spoiled wines, abnormally high values for the coordinate U^* were obtained. Red wines showed an evident linearity in Fig. 1. This behaviour indicates that these samples have a similar hue.

The CIELUV space appeared as a slight modification of the preceeding $U^*V^*W^*$ space. As observed from Fig. 2, it leads to the same conclusions which have been already stated.

The CIELAB space (Fig. 3) presented some analogies and differences with regard to the previous spaces expounded. A larger dispersion of the color points on the a^*b^*-diagram than CIELUV was obtained. This fact suggests that a more selective chromatic discrimination of the samples occurs. The arrangement by pairs of samples of red wines having in common the same evolution remains. Again, the sample no. 6 stands aloof clearly from the others.

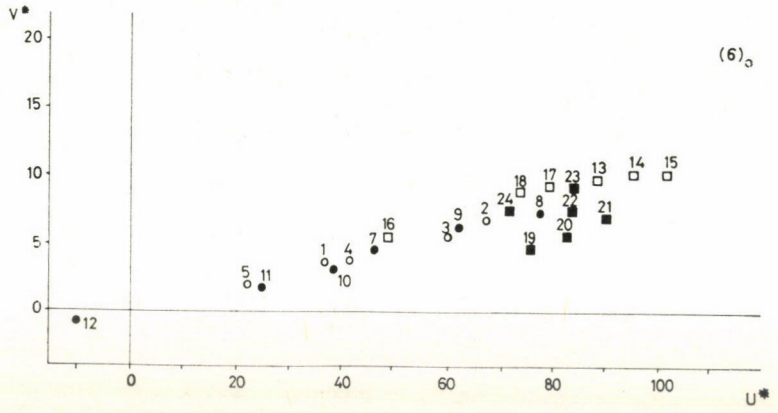

Fig. 1. Location of the samples in the U*V*-diagram. Points marked ○ represent samples in set I ($r = 0.999$, $s_r = 0.014$, n = 6). Points marked ● represent samples in set II ($r = 0.997$, $s_r = 0.037$, n = 6). Points marked □ represent samples in set III ($r = 0.96$, $s_r = 0.134$, n = 6). Points marked ■ represent samples in set IV ($r = 0.26$, $s_r = 0.482$, n = 6)

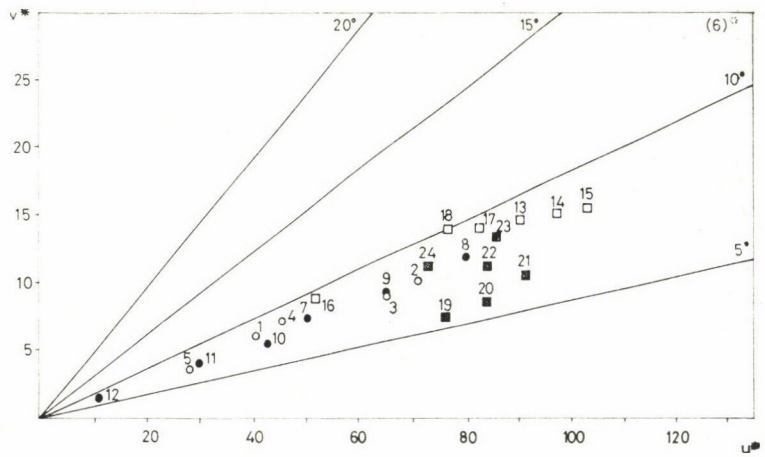

Fig. 2. Location of the samples in the U*V*-diagram. Points marked ○ represent samples in set I ($r = 0.999$, $s_r = 0.016$, n = 6). Points marked ● represent samples in set II ($r = 0.997$, $s_r = 0.039$, n = 6). Points marked □ represent samples in set III ($r = 0.96$, $s_r = 0.144$, n = 6). Points marked ■ represent samples in set IV ($r = 0.28$, $s_r = 0.480$, n = 6)

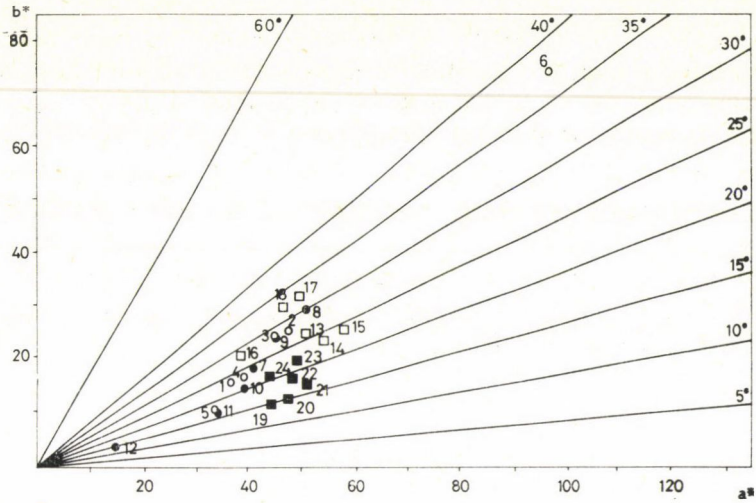

Fig. 3. Location of the samples in the a^*b^*-diagram. Points marked ○ represent samples in set I ($r = 0.999$, $s_r = 0.010$, n = 6). Points marked ● represent samples in set II ($r = 0.93$, $s_r = 0.179$, n = 6). Points marked □ represent samples in set III ($r = 0.25$, $s_r = 0.484$, n = 6). Points marked ■ represent samples in set IV ($r = 0.01$, $s_r = 0.500$, n = 6)

Table 4

Lightness, chroma, saturation and hue angle (C.I.E. Uniform Color Spaces)

Set	Sample No.	Lightness		Chroma		Saturation	Hue angle	
		W^*	L^*	C^*_{uv}	C^*_{ab}	S^*_{uv}	h_{uv}	h_{ab}
I	1	8.012	9.004	42.862	41.790	4.760	8.070	21.219
	2	14.431	15.420	72.851	55.012	4.724	8.143	27.856
	3	13.183	14.173	66.611	52.141	4.700	8.261	27.030
	4	8.612	9.603	46.113	43.286	4.802	8.096	22.091
	5	4.671	5.884	29.376	35.728	4.993	7.431	16.201
	6	43.399	44.378	220.173	122.727	4.961	7.704	37.493
II	7	10.198	11.188	51.808	45.340	4.631	8.370	24.218
	8	17.249	18.237	83.032	59.323	4.553	8.416	29.899
	9	13.439	14.430	66.920	52.150	4.638	8.396	27.380
	10	7.796	8.788	43.994	43.115	5.006	7.350	20.157
	11	4.793	5.984	30.277	36.398	5.060	7.438	16.381
	12	—1.843	2.013	10.232	14.668	5.083	7.369	13.621
III	13	32.773	33.756	91.137	55.632	2.700	9.526	25.515
	14	37.693	38.674	98.728	58.227	2.553	9.000	23.836
	15	37.306	38.288	104.550	61.253	2.731	8.686	23.984
	16	13.188	14.178	52.723	43.483	3.719	9.904	28.101
	17	22.077	23.064	84.048	57.537	3.644	9.916	31.747
	18	20.098	21.086	78.045	55.296	3.701	10.326	32.475
IV	19	58.614	59.588	76.922	45.375	1.291	5.620	16.640
	20	70.711	71.681	84.360	48.415	1.177	5.914	1.177
	21	62.069	63.042	91.900	52.431	1.458	6.823	16.681
	22	47.636	48.613	86.266	50.590	1.775	7.753	18.939
	23	46.848	47.826	86.449	50.500	1.808	9.194	21.277
	24	39.726	40.707	74.025	45.134	1.818	8.979	20.978

Among the new chromatic parameters defined by the uniform color spaces, chroma, saturation and hue angle have been chosen as most representative. They are shown in Table 4. Chroma values present a week correlation with the period of time for sample damage ($\bar{r} = 0.11$, $s_{n-1} = 0.612$). As in previous systems it was shown, saturation presents a considerable increase with regard to the time interval from one week onwards ($\bar{r} = 0.94$, $s_{n-1} = 0.025$). This means that it is a reliable index to use for the study of damage in wine samples due to an inadequate method of preservation. Hue angles, although providing a suitable discrimination of the samples, showed a poor correlation with their preservation status.

3. Conclusions

This paper shows how the chromatic characteristics of wine samples can lead to useful knowledge concerning their preservation status.

Among the several chromatic parameters, saturation appears as the most adequate index for this purpose. From an analysis of variance (2-way ANOVA method), taking saturation as representative parameter of color evolution, we can conclude (sample $F >$ tabular F, $P < 0.05$) that this evolution with time elapsed is not independent of the particular set involved.

In order to discriminate the wines, C.I.E. Uniform Color Spaces are more reliable than non-uniform ones.

*

The authors are indebted to Bodegas Villagrán (Seville) for supplying the samples.

Literature

BERG, H. W. & MARSH, G. L. (1956): Effect of heat treatment of musts on the color characteristics of red wine. *Fd Technol., 10*, 4–9.

BERG, H. W., OUGH, C. S. & CHICHESTER, C. O. (1964): The prediction of perceptibility of luminous-transmittance and dominant wavelength differences among red wines by spectrophotometric measurements. *J. Fd Sci., 29*, 661–667.

C.I.E. (1978): *Recommendations on uniform color spaces, color differences, equations, psychometric color terms.* Bureau Central de la Commission Internationale de l'Éclairage, Paris. C.I.E. publication No. 15, suppl. 2, p. 1971.

GLORIES, Y. (1984): La couleur des vins rouges. Partie 2. Mesure, origine et interprétation. *Connais. Vigne Vin, 18*, 253–271.

HARDY, A. C. (1936): *Handbook of colorimetry.* Technology Press, Cambridge, Mass.

LEMPERLE, E. & KERNER, E. (1968): Color of red wine. — Part I. Color intensity measurement. *Wein-Wiss., 23*, 281–294. — ref.: C.A., *96*, 95087 (1968).

MARECA, I. (1981): Alteraciones del color del vino. Prevención, soluciones. *Sem. vitivinic., 36*, 623–631.

O.I.V. (1979): *Recopilación de los métodos internacionales de análisis de vinos.* Publ. Ministerio de Agricultura, Madrid, pp. 20–31.

RIBEREAU-GAYON, P., PONTALLIER, P. & GLORIES, Y. (1983): Some interpretations of colour changes in young red wines during their conservation. *J. Sci. Fd Agric., 34*, 505–516.

SAMBUC, E. & NAUDET, M. (1965): Sur la couleur des huiles. *Revue fr. Cps. gras, 10*, 585–594.

SOMERS, T. C. & EVANS, M. E. (1977): Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2, "chemical age". *J. Sci. Fd Agric., 28*, 279–287.

STELLA, C. (1966): Il grado di decolorazione di un liquido espresso con un nuovo indice spettrofotometrico $-I_d-$. *Olearia. Riv. mat. grasse*, 5-6–7-8, 111–116.

SUDRAUD, P. (1958): Interpretation des courbes d'absorption des vins rouges. *Annls. Technol. agric., 2*, 203–208.

WYSZECKI, G. (1963): Proposal for a new color-difference formula. *J. opt. Soc. Am., 53*, 1318.

WYSZECKI, G. & STILES, W. S. (1982): *Color science. Concepts and formulae.* 2nd ed., Wiley, New York, pp. 130–175.

Acta Alimentaria, Vol. 17 (2), pp. 113–125 (1988)

APPLICATION OF NEAR INFRARED REFLECTANCE TECHNIQUE IN THE ANALYSIS OF SELECTED PIG FEED MIXES

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(Received: 29 December 1986; accepted 22 May 1987)

Mixed pig feeds produced in Czechoslovakia (beside feeds for piglets) were classified into 3 groups according to their composition, purpose and content of determined constituents. Seventy-five samples belonging to each group were analyzed for moisture, crude protein, fat, ash and crude fibre content by classical methods. Fifty-five analyzed samples were used for calibration of InfraAlyzer 400. Selection of filters of InfraAlyzer was performed on the basis of testing all possible combinations.

Sets of 6–9 filters are most suitable for the analysis of mixed pig feeds. The optimal combination of filters for a particular constituent was selected from 2–4 sets proposed by the computer. Statistical evaluation of the results of analysis of 20 "unknown" samples (which were not used for calibration) was utilized for this purpose.

Distribution of constituents was as follows: 8.2–13.5 mass % of moisture; 12.1–18.2 mass % of crude protein; 1.1–3.1 mass % of fat; 3.1–7.2 mass % of ash; 2.8–8.1 mass % of crude fibre. Values of standard error of calibration (RSD) were the following: 0.208–0.371 mass % for moisture; 0.278–0.354 mass % for crude protein; 0.087–0.109 mass % for fat; 0.187–0.265 mass % for ash; 0.261–0.377 mass % for crude fibre. Values of standard error of prediction (SEP) were the range of: 0.220–0.264 mass % for moisture; 0.316–0.454 mass % for crude protein; 0.072–0.146 mass % for fat; 0.226–0.350 mass % for ash; 0.188–0.386 mass % for fibre.

Influence of some ingredients (wheat bran) distorting results of NIRA is discussed. Results of NIRA are satisfactory for all constituents prediction and so this technique is able to substitute classical methods of analysis of feed mixes for pigs.

Keywords: near infrared reflectance analysis, pig feed mixes, component prediction

Near infrared reflectance analysis (NIRA) is a method widely used for animal feeding-stuffs. History and application of NIRA in analysis of foods and feeds was reviewed by POLESELLO and GIANGIACOMO (1983). First experiences of utilizing this method in mixed feed analysis were published by WILLIAMS and co-workers (1978) and WILLIAMS and STARKEY (1980).

Evaluation of the quality of feed-mixes according to their moisture, protein, fat, ash and fibre content is widely used. But determination of these constituents by classical methods is time consuming. So application of NIRA in analysis of these components is very economical, but some experimental problems must be solved.

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

1. Materials and methods

Samples of mixed pig feeds were obtained from daily production of various feed mills of Zemědělské Zásobování Co. in central Bohemia. These mixed feeds were classified into three groups according to their composition and content of predicted constituents, which are listed in Table 1. Actual composition of particular samples was also known.

Samples of mixed feeds were ground in a laboratory mill (Zempo VM 7 type, Czechoslovakia), samples of wheat bran in a Cyclotec Sample Mill (Tecator AB, Sweden).

Seventy-five samples of feed-mixes belonging to one group were analyzed by classical methods. Moisture content was determined after 4 h drying at 103 °C. Protein content was determined by Kjehldahl's method (90 min digestion in DS-20 digestor connected with programmable temperature control Autostep 1012 Controller, Tecator AB, Sweden) at temperatures increasing from 300 to 420 °C. Distillation and titration was performed on Kjeltec Auto

Table 1

Composition of analyzed mixed feeds

Group	Components	Rate (%)	Note
1. (for pigs weighing 17–65 kg)	wheat	30–70	totally 70–85%
	barley	15–55	
	soya meal	5–12	
	protein concentrate	0– 8	fish meal, bonemeat meal, soya meal
	mineral and vitamine supplement	3	
	oats, peas, wheat bran, wheat flour	0– 6	
2. (for pigs weighing more than 65 kg)	wheat	10–75	totally 75–95%
	barley	15–70	
	oats	0–10	
	soya or groundnut meal	3– 6	
	wheat bran	0–10	
	mineral and vitamine supplement	3	
	rye, dried alfalfa, peas, maize	0– 6	
3. (for swines)	wheat	15–30	
	barley	15–50	
	oats	10	
	wheat bran	10–20	
	soya or groundnut meal	4–10	
	mineral and vitamine supplement	5	
	protein concentrate, peas, dried potatoes, blood meal, bone-meat meal	0–7	

Table 2

Wavelengths of the filters used in InfraAlyzer 400

Filter number	Wavelength (nm)
2	2336
3	2348
4	2310
5	2270
6	2230
7	2208
8	2190
9	2136
10	2180
11	1982
12	1818
13	1778
14	2100
15	1759
16	1940
17	1734
18	1722
19	1445
20	1680

Analyzer (Tecator AB, Sweden). This procedure with a catalyst containing selenium and copper sulphate pentahydrate gives approx. 98.5% recovery of nitrogen (PAZOUREK, 1986). Fat content was determined after 30 min hot and 30 min cold extraction with diethylether in Soxtec HT (Tecator AB, Sweden). The result of this procedure corresponds to 4 h Soxhlet extraction according to the experiments of BERANOVÁ and PAZOUREK (1985). Ash content was determined after 4 h ashing at 550 °C. Fibre content was determined after 30 min boiling in 1.25% sulfuric acid and 30 min boiling in 1.25% potassium hydroxide solution in Fibertec M (Tecator AB, Sweden).

Samples of wheat flour, wheat bran and wheat flour complemented with wheat bran were analyzed for moisture and protein content by the above mentioned methods.

Fifty-five samples analyzed, randomly selected from above-mentioned set of 75 samples from each group of mixed feeds, were used for the calibration of InfraAlyzer 400 (Technicon G.m.b.H., Vienna) connected with the computer HP-85B including All Possible Combination Search Programme for selection of sets of filters used in analysis. Wavelengths corresponding to the filters of InfraAlyzer 400 are listed in Table 2.

Mass per cents of a particular constituent were calculated according to the multiple regression formula

$$c(\%) = F_{0c} + F_{1c} \log \frac{1}{R} + \ldots + F_{ic} \log \frac{1}{R_i} + \ldots + F_{20c} \log \frac{1}{R_{20}}$$

where:

c (%): mass per cents of predicted constituent;
F_{00c}, F_{ic}: calibration constants (F-values) for predicting constituent c;
R_i: reflectance value at each of nineteen wavelengths (filters).

Twenty analyzed samples not included in the calibration set were used for evaluation of accuracy of the obtained calibration regression equations.

The definition of Residual Standard Deviation (RSD) of calibration used in regression calculation is:

$$\mathrm{RSD} = \sqrt{\frac{\Sigma(d_i - \bar{d})^2}{n - k - 1}}$$

where:

d_i: difference between the result of the chemical determination of the constituent and the result of its calculation by the regression formula;
$\bar{d}$: average value of d_i;
n: number of samples used for calibration;
k: number of wavelengths used for calibration.

The definition of Standard Error of Prediction (SEP) used in evaluation of the accuracy of NIRA results of analysis of "unknown" samples is:

$$\mathrm{SEP} = \sqrt{\frac{\Sigma(d_i - \bar{d})^2}{n - 1}}$$

where:

d_i: difference between the result of chemical determination of the constituent and the result of its calculation by the regression formula;
$\bar{d}$: average value of d_i;
n: number of samples used in evaluation of accuracy of the calibration formula by analysis of samples not included in the calibration set.

Since the results of analyses of samples were established after 2 replicated determinations by both methods, the Pooled Standard Deviation of the Classical Method (SD_{LAB}) and NIRA (SD_{NIRA}) were utilized for evaluation of reproducibility of both methods.

The definition of the Pooled Standard Deviation (SD) is as follows:

$$\mathrm{SD}_{\mathrm{LAB(NIRA)}} = \sqrt{\frac{\Sigma d_i^2}{2n}}$$

where:

d_i: difference between results of 2 parallel determinations of a particular constituent by the traditional method or NIRA;
n: number of analyzed samples.

2. Results

Number of selected filters, values of correlation coefficients, residual standard deviations and ranges of values of predicted constituents are summarized in Table 3. Several conclusions can be drawn of this set of data.

Sets of 6–9 (exceptionally 12 filters) are successfully utilized in mixed feed analysis. Increased number of filters in InfraAlyzer 400 usually improves agreement between the results of InfraAlyzer and traditional methods. But

Table 3

Results of calibration of InfraAlyzer 400

	Moisture content	Protein content	Fat content	Ash content	Fibre content
			First group of mixed pig feeds		
Filter number	⑪, 14, 15, 16, 17, ⑲	5, 7, 9, 13, 14, ⑮, 17	2, ④, ⑤, 7, 14, 15, ⑱, 20	2, 3, 4, 9, ⑪, 12, 13, ⑰, 19	4, ⑥, 7, 10, *12*, *17*, 18, 20
r	0.989	0.950	0.945	0.878	0.936
RSD	0.208	0.354	0.087	0.189	0.275
Cal. range	8.6–13.5	13.3–18.2	1.5–2.7	3.2–5.5	2.8–6.6
			Second group of mixed pig feeds		
Filter number	2, 5, 7, ⑪, *13*, ⑲	2, 3, 4, 6, 7, ⑮, 17	3, ④, ⑤, 11, 12, ⑱	3, 5, 6, 9, 10, ⑪, 12, *14*, *16*, ⑰, 19, 20	⑥, 9, 11, *12*, 14, 15, *17*, 18, 19
r	0.971	0.962	0.972	0.798	0.935
RSD	0.359	0.309	0.090	0.187	0.261
Cal. range	9.2–13.4	12.1–17.7	1.1–3.1	3.1–4.4	3.2–6.4
			Third group of mixed pig feeds		
Filter number	4, 10, ⑪, *13*, 14, 16, ⑲, 20	*5*, 8, 10, 13, ⑮, 20	④, ⑤, 8, 9, 13, ⑱	*2*, 8, ⑪, 13, *14*, *16*, ⑰, 18	3, ⑥, 7, 9, 13, 20
r	0.968	0.980	0.890	0.899	0.944
RSD	0.371	0.278	0.109	0.265	0.377
Cal. range	8.2–12.7	12.5–17.2	1.5–2.6	4.6–7.2	3.8–8.1

Encircled numbers: filters predicting one constituent in all groups
Underlined numbers: filters predicting one constituent in two groups
r: correlation coefficient
RSD: residual standard deviation
Calibration ranges are expressed in mass %

this law is valid only with limitations as a too high number of filters can cause a noise into InfraAlyzer measurements according to the observations of MULLARD (1986). Our experience with a sufficient number of filters is also consistent with conclusions published by SHENK and co-workers (1978) about improving of NIRA results in forage analysis.

Table 4

Results of analysis of "unknown" samples

	Moisture content	Protein content	Fat content	Ash content	Fibre content
		First group of mixed pig feeds			
r	1.000	0.997	0.993	1.000	0.996
SEP	0.264	0.316	0.146	0.226	0.188
SD_{NIRA}	0.125	0.220	0.075	0.171	0.224
SD_{LAB}	0.087	0.171	0.072	0.054	0.142
		Second group of mixed pig feeds			
r	0.998	0.999	0.994	0.993	0.992
SEP	0.504	0.454	0.130	0.286	0.386
SD_{NIRA}	0.227	0.408	0.064	0.202	0.407
SD_{LAB}	0.072	0.085	0.052	0.036	0.144
		Third group of mixed pig feeds			
r	0.998	0.999	0.998	0.994	0.993
SEP	0.497	0.389	0.072	0.350	0.317
SD_{NIRA}	0.220	0.229	0.078	0.219	0.261
SD_{LAB}	0.070	0.033	0.029	0.076	0.154

r: correlation coefficient
SEP: standard error of prediction
SD_{NIRA}: standard deviation of NIRA results
SD_{LAB}: standard deviation of traditional analysis results

Selective wavelengths for particular constituent prediction can be hardly found, even though there exist filters predicting one constituent in all groups (see encircled numbers in Table 3) or two groups of mixed feeds (see underlined numbers in Table 3).

Values of the correlation coefficient did not reach 0.9 in some cases (ash in all mixed feed groups, fat in third group). This is caused most probably by the too narrow distribution range of these constituents in these mixed feeds.

Values of RSD are in these cases, nevertheless, low and so these constituents can be predicted with satisfactory results.

Results of analysis of 20 "unknown" samples (not included in the calibration set) are summarized in Table 4. Relationships between constituents determined by chemical analysis and values predicted by NIRA are shown in Figs. 1–5.

Values of correlation coefficients are high in all cases. Values of Standard Error of Prediction (SEP) for moisture and protein are only slightly higher than those published for wheat analysis by InfraAlyzer 300 (WILLIAMS et al., 1985).

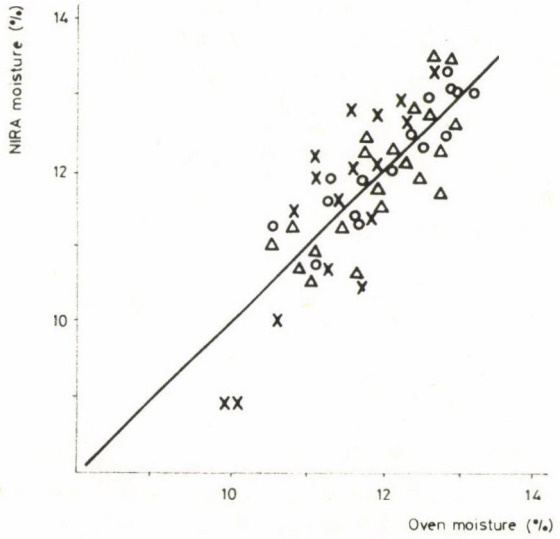

Fig. 1. Relationship between moisture content determined after oven drying and predicted value by NIRA. ○: first group of feed mixes; △: second group of feed mixes; ×: third group of feed mixes

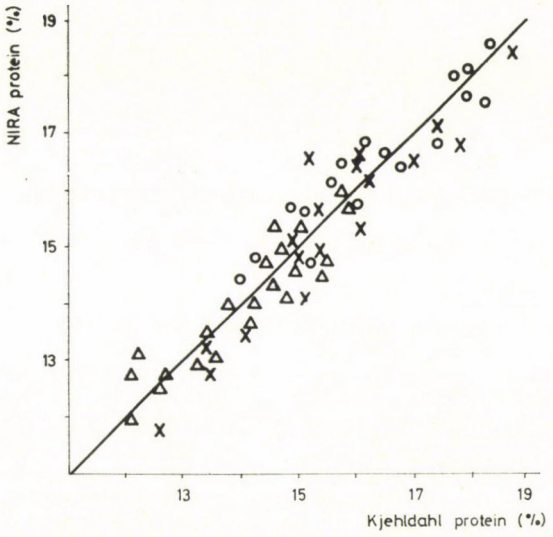

Fig. 2. Relationship between calculated protein content determined by Kjehldahl method and predicted value by NIRA. ○: first group of mixed feeds; △: second group of mixed feeds; ×: third group of mixed feeds

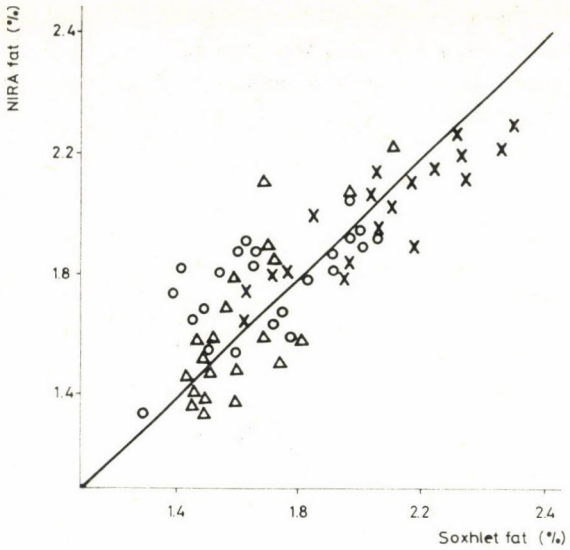

Fig. 3. Relationship between fat content determined after diethylether extraction and predicted value by NIRA. ○: first group of mixed feeds; △: second group of mixed feeds; ×: third group of mixed feeds

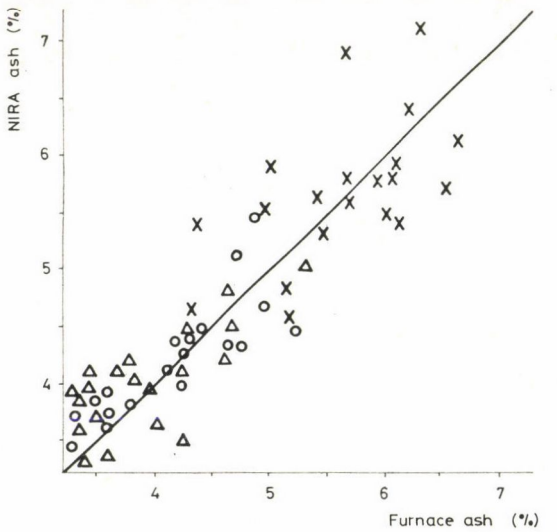

Fig. 4. Relationship between ash content determined after furnace ashing and predicted value by NIRA. ○: first group of mixed feeds; △: second group of mixed feeds; ×: third group of mixed feeds

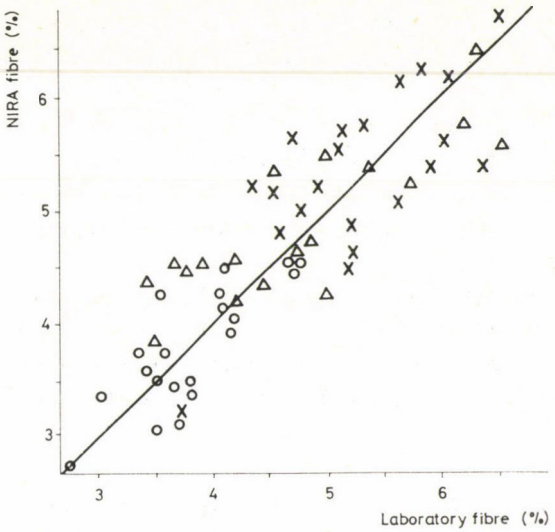

Fig. 5. Relationship between crude fibre content determined after acid and alkaline hydrolysis and predicted value by NIRA; ○: first group of mixed feeds; △: second group of mixed feeds; ×: third group of mixed feeds

Effect of various components of mixed feeds on NIRA results is discussed by WILLIAMS and STARKEY (1980). They found that NIRA results were vitiated in the presence of oats, dried alfalfa, urea and soy-bean hulls in the mixed feeds.

Mixed pig feeds included into the first group contain mainly whole grains of cereals, soya meal, supplements and rarely wheat bran. According to the evaluation of NIRA results related to the composition of particular samples it can be said that the ratio of wheat and barley in these mixed feeds does not affect the agreement of the results of InfraAlyzer 400 and traditional methods.

Wheat bran is one of the ingredients vitiating results of NIRA. Near infrared absorption spectra of wheat bran were recorded by NORRIS and HART (1965) and a study on the application of NIRA on dietary fibre of wheat bran determination was carried out by HORVÁTH and co-workers (1984).

The second group of pig feed mixes contains 0–10% and the third group 10–20% of wheat bran (the third group contains 10% of oats, too). These mixed feeds contain no alfalfa. With increasing of wheat bran in the mixtures increase the SEP values for moisture, protein, ash and fibre. Reproducibility of results of InfraAlyzer 400 is also affected. This is shown by comparison of the values of Pooled Standard Deviations (SD) (see Table 4) of both methods. Whereas the SD values of InfraAlyzer 400 and of traditional methods are comparable in the first group of feed mixes (except for ash prediction) while

the SD values of InfraAlyzer 400 are 2–4-times higher than those of the traditional methods in the second and third groups of mixed feeds. This is probably due to the composition of these feed mixes.

Therefore the influence on the results of InfraAlyzer 400 of increasing wheat bran content in wheat flour (for animal feeding) was followed up. Comparison of the results of NIRA and traditional methods is shown in Table 5 (InfraAlyzer 400 was calibrated for analysis of this sort of wheat flour). Accuracy of moisture and protein prediction was vitiated in the samples containing 10–20% (exceptionally 5%) wheat bran. Reproducibility of moisture prediction was affected in wheat flour samples containing 5–10% wheat bran. Surprising are the false results of moisture prediction in pure wheat flour. This can be accidental or caused by error in traditional laboratory analysis.

Table 5

Results of analysis of wheat flour samples containing wheat bran

Wheat bran (mass %)	Moisture content NIRA	$d_{i\,NIRA}$	Moisture content LAB	$d_{i\,LAB}$	$d_{NIRA-LAB}$
0	13.12	0.21	12.78	0.08	+0.34
5	12.72	0.67	12.54	0.07	+0.18
10	12.45	0.48	12.24	0.01	+0.21
15	12.20	0.19	11.82	0.38	+0.38
20	12.48	0.09	11.84	0.11	+0.64

Wheat bran (mass %)	Protein content NIRA	$d_{i\,NIRA}$	Protein content LAB	$d_{i\,LAB}$	$d_{NIRA-LAB}$
0	14.70	0.11	14.90	0	−0.20
5	14.83	0.14	14.92	0.09	−0.09
10	14.66	0.11	15.14	0	−0.48
15	15.10	0.10	15.18	0	−0.08
20	15.08	0.05	15.42	0.13	−0.26

Wheat bran (mass %)	Ash content NIRA	$d_{i\,NIRA}$	Ash content LABo	$d_{i\,LAB}$	$d_{NIRA-LAB}$
0	2.68	0.07	2.78	0.13	−0.10
5	2.72	0.30	2.78	0.19	−0.06
10	2.60	0.09	2.79	0.06	−0.19
15	2.73	0.16	2.86	0.01	−0.13
20	2.63	0.21	2.90	0.01	−0.27

mass % of constituent: average of 2 determinations
d_i: difference between parallel NIRA (traditional) determinations
$d_{NIRA-LAB}$: difference between NIRA and traditional determinations

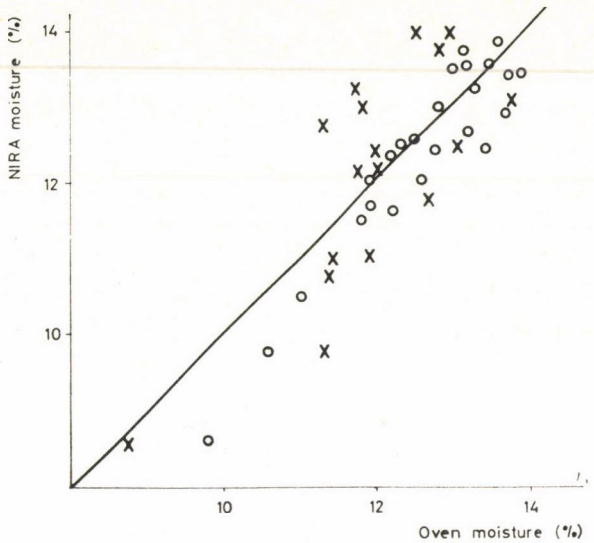

Fig. 6. Relationship between moisture content determined after oven drying and predicted value by NIRA. ○: samples containing 0–10% of wheat bran; ×: samples containing 10–20% of wheat bran

Results of ash prediction were not affected by the change of wheat bran content in analyzed samples.

These results are consistent with conclusions of evaluation of results of analysis of particular mixed pig feed samples (related to their actual composition). Namely, samples with increased wheat bran content had higher mean values of SEP and SD for the majority of constituents in Table 4. Results of moisture prediction in mixed feeds for pigs were improved after their classification into 2 subgroups, the first one containing 0–10% wheat bran, second one containing 10–20%. Improved results of moisture predic-

Table 6

Improved results of moisture prediction in mixed feeds for pigs

	Wheat bran in mixed feeds (mass %)	
	0–10	10–20
n	24	16
r	1.000	0.998
SEP	0.165	0.439

n: number of analyzed samples
r: correlation coefficient
SEP: standard error of prediction

tion in these samples are summarized in Table 6 and relationships between moisture content as determined by traditional method and values predicted by NIRA, are shown in Fig. 6. Results of moisture prediction in the subgroup of mixed feeds for pigs are similar to those for the first group of mixed feeds for piglets (see Table 5).

The described properties of wheat bran are probably a consequence of the origin of samples. The wheat bran samples incorporated into mixed feeds were produced in different mills on different milling devices. This fact probably affects their composition and other properties, which could not be changed even by subsequent fine grinding on the Cyclotec grinder. Similar problems were discussed by SCHŐGGL and GLATTES (1985) for other products of the cereal processing industry.

It can be said also that less than 5% of any of the ingredients mentioned in Table 1 does not affect results of NIRA. The results are in agreement with conclusions of WILLIAMS and STARKEY (1980).

3. Conclusion

Results of NIRA of mixed pig feeds are nevertheless satisfactory in all presented cases, in spite of the false results caused by increased wheat bran content in some mixed feeds. The success of the application of NIRA in mixed feed analysis depends on the selection of samples for calibration taking into account their composition, selection of the proper wavelengths and finding the ingredients which are prone to distort NIRA results.

Literature

BERANOVÁ, H. & PAZOUREK, K. (1985): Unpublished results.

HORVÁTH, L., NORRIS, K. H., HORVÁTH-MOSONYI, M., RIGÓ, J. & HEGEDÜS-VÖLGYESI, E. (1984): Study into determining dietary fibre of wheat bran by NIR-technique. *Acta Alimentaria, 13*, 355–382.

MULLARD, Y. (1986): Private communication.

NORRIS, K. H. & HART, J. R. (1965): Direct spectrophotometric determination of moisture content of grain and seeds. *Proc. 1963 Intern. Symp. on Humidity and Moisture*. Reinhold, New York, Vol. 4, p. 19.

PAZOUREK, K. (1986): Possibility of utilization of programmable temperature control for the digestion of fodder samples for the determination of nitrogen by Kjehldahl method. *Chem. Listy, 80*, 874–880.

POLESELLO, A. & GIANGIACOMO, R. (1983): Application of near infrared spectrophotometry to the nondestructive analysis of foods: a review of experimental results. *CRC Crit. Rev. Fd Sci. Agric., 18*, 203–230.

SCHŐGGL, G. & GLATTES, H. (1985): Infrared spectroscopy — applicability in the Austrian cereal and cereal processing industry. *Bodenkultur, 36*, 245–258.

SHENK, J. S., WESTERHOUS, M. O. & HOOVER, M. R. (1978): Infrared reflectance analysis of forages. *International Grain and Forage Harvesting Conference Proceedings*. American Society of Agricultural Engineers, St. Joseph, pp. 242–244, 252.

WILLIAMS, P. C., STEVENSON, S. G., STARKEY, P. M. & HAWTIN, G. C. (1978): The application of near infrared reflectance spectroscopy to protein testing in pulse breeding programmes. *J. Sci. Fd Agric.*, *29*, 285–292.

WILLIAMS, P. C. & STARKEY, P. M. (1980): Influence of feed ingredients upon the prediction of protein in animal feed-mixes by near-infrared reflectance spectroscopy. *J. Sci. Fd Agric.*, *31*, 1201–1213.

WILLIAMS, P. C., NORRIS, K. H. & SOBERING, D. C. (1985): Determination of protein and moisture in wheat and barley by near-infrared transmission. *J. agric. Fd Chem.*, *33*, 239–244.

Acta Alimentaria, Vol. 17 (2), pp. 127–139 (1988)

BIOCHEMICAL PROPERTIES OF SAFFLOWER LIPASE

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(Received: 29 January 1987; accepted: 16 May 1987)

True lipase was extracted, partially purified and characterized from the seed of safflower. Sucrose solution of 0.2 mol was the most efficient extracting medium of four solutions used. During the reaction period of 0–22 h, in the first twelve hours the reaction rate was linear and it was proportional to the enzyme concentration. Partial purification of 3fold was achieved by means of gel filteration using Sephadex G-150 column and sucrose solution as eluent. Effect of substrate concentration on the reaction rate followed normal Michaelis-Menten kinetics. The K_m value for the enzyme was 3.3×10^{-2} mol and 8×10^{-3} mol with Tween 80 and tributyrin as substrates, respectively. The optimum pH range was 8.5–9, whereas the stability was highest in the alkaline pH range between 8–10. The enzyme showed maximum activity at 30 °C, while the heat stability study indicated that the enzyme is heat sensitive under the conditions applied. The enzyme was found to be specific to long chain fatty acid esters of low degree of saturation. Inhibition and activation of the enzyme by some chemicals are also included in this work.

Keywords: safflower, lipase

A lipase (EC 3.1.1.3) is the enzyme catalyzing the hydrolysis of fatty acid esters of glycerol in foodstuffs causing the so-called hydrolytic rancidity. This is considered as a serious problem occurring during storage and processing of many food products like oil seeds, which are very susceptible and sensitive to the frequent oxidizing agents after hydrolysis. This susceptibility is due to the higher unsaturated fatty acid content of these seeds.

The occurrence of lipases in higher plants has been reported by DESNULLE (1972). Two of them have been widely investigated, i.e. the castor bean lipase (ORY, 1969) and rice bran lipase (FUJIKI et al., 1978). Interest was also paid to the enzymes from peanut (SANDERS & PATTEE, 1975), *Vicia faba* (DUNDAS et al., 1978), poppy seeds (HOLASOVA & BLATTNA, 1980) and potato tubers (HASSON & LATIES, 1976).

Because of many difficulties like insolubility of enzyme protein (BAI et al., 1972), its low concentration (KUMAR, 1967) and removal of an essential cofactor (ORY et al., 1964), the enzyme has not been well purified and studied in many plant products, like oil seeds, in comparison with the enzyme from other sources.

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

The purpose of this investigation was the determination of enzyme activity, partial purification and studying the biochemical properties of lipase from one of the most important oil seeds, Safflower seed.

1. Materials and methods

1.1. Materials

Safflower seeds were obtained from Agronomy Department, College of Agriculture, University of Baghdad. All chemicals used were purchased from BDH (British Drug House, London). Deionized water was used in the preparation of different solution.

1.2. Methods

1.2.1. Extraction of enzyme. Twenty grams of seeds were disintegrated in a blendor with 100 cm^3 extracting medium for 5 min at 4 °C. The mixture was strained through a double layer cheese-cloth and centrifuged at $10000 \times G$ for 30 min at 0 °C using IEC-B-20 Beckman refigerated centrifuge. The middle supernatant was carefully taken by syringe, to avoid remixing of the upper lipid pad, and used as crude enzyme preparation.

1.2.2. Partial purification. Ammonium sulfate fractionation was carried out by gradual addition of the crystalline salt to an appropriate volume of the crude extract with continuous stirring in ice bath. After complete dissolution the mixture was allowed to stand for 15 min and then centrifuged at $10000 \times G$ and 0 °C. The precipitates were redissolved in the minimum volume of 0.25 mol sucrose solution. Enzyme activity and protein concentration were determined in the different fractions in triplicate at least.

Sephadex gel filtration column (G-150) was made according to the instructions of Pharmacia Fine Chemicals under the following conditions:

Column dimensions: 2.5×42 cm;

Hydrostatic pressure: 24 cm;

Flow rate: 1 $cm^3\ min^{-1}$;

Eluent: 0.25 mol sucrose solution;

Starting preparation: crude extract.

Acetone precipitation was carried out by adding cold acetone (−20 °C) to the crude extract in the ratio of 1 : 0.5, 1 : 1 and 1 : 1.5. The precipitates were collected by centrifugation at $5000 \times G$ for 10 min at −20 °C. Remaining acetone was removed from the precipitate under vacuum at ambient temperature.

1.2.3. Assay procedure. Substrates were prepared according to the method described by BIER (1955). Fifteen cm^3 of Tween-80 were added to 100 cm^3 of

phosphate buffer pH 8, magnetically stirred until complete dissolution. The emulsion of triglycerides and commercial oils were prepared by mixing oil samples with emulsifying media [5% poly(vinyl alcohol), "PVA", or 5% Gum Arabic] using high speed blendor for 2 min. Stock solutions for both soluble and insoluble substrates were prepared and stored in refrigerator when not in use.

Assay method used depended on titration of liberated fatty acids with alkaline solution to the end point (starting pH value). Ten cm^3 of substrate emulsion were mixed with 5 cm^3 of phosphate buffer at desired pH and 5 cm^3 of deionized water in a conical flask. The reaction was then initiated by adding 5 cm^3 of enzyme extract and incubating at 30 °C for 12 h. After the incubation period 5 cm^3 of absolute alcohol were added to stop the reaction and the mixture was titrated to the end point with 0.1 *N* NaOH. The blank was prepared in the same way, but, with heat inactivated enzyme extract. One enzyme unit equals 0.1 cm^3 of 0.1 *N* NaOH required to neutralize the liberated fatty acids per one hour.

1.2.4. Protein determination. The spectrophotometric method for protein determination was used for purity measurements during extraction and purification studies. Three cm^3 of the diluted enzyme extract were put in the spectrophotometric cell (Beckmann-DB-spectrophotometer) and the absorbance read at 260 nm and 280 nm against the blank containing extracting medium. The concentration of protein was calculated according to the following formula:

$$\text{Protein (mg cm}^{-3}) = 1.55 \times \text{O.D.}_{280\text{ nm}} - 0.76 \times \text{O.D.}_{260\text{ nm}}$$

2. Results and discussion

2.1. Extraction and purification of the enzyme

Of four extracting solutions used 0.25 mol sucrose was the most efficient one, since the specific activity obtained with it was 1.8, 2.7, and 3.6 times higher than with sodium chloride, distilled water and phosphate buffer, respectively (Table 1). Use of sucrose solution for extraction of some enzymes from adipose tissues was advised by KUMAR (1967) and MORTON (1954). The ability of sucrose solution to extract the enzymes of tissue rich in lipids may be due to the protecting effect against mechanical injuries taking place during extraction processes and/or to the role of sucrose in the dissociation of lipid aggregates, accumulating around enzyme protein providing an opportunity for the enzyme to disperse in the water phase.

Fractionation with ammonium sulfate gave no satisfactory and reproducible results, since not more than 1.37fold of purification was obtained with

Table 1

Extraction of safflower lipase by different extracting media

Extracting media	Activity (U cm^{-3})	Protein (mg cm^{-1})	Specific activity (U cm^{-3} per mg protein cm^{-3})
0.25 mol sucrose	10.6	53.6	0.20
Iced distilled water	4.2	57.0	0.073
0.1 mol phosphate buffer (pH 7)	4.0	74.0	0.056
0.1 mol sodium chloride	5.0	45.0	0.11

The values represent the average of 3 or 4 measurements with negligible deviation

high loss in the yield (Table 2). This may be due to the removal of an essential cofactor during the fractionation process, or to the effect of remaining lipids on the normal salting out of enzyme protein. In order to remove lipids of the crude extract, cold acetone (−20 °C) was gradually added alone and with barium acetate at −20 °C. Acetone powder obtained after complete removal of acetone showed no activity. Disappearance of the activity of the enzyme can be explained either by the denaturation effect of acetone even at −20 °C (WHITAKER 1972) or by separation of an essential lipid cofactor. The

Table 2

Ammonium sulfate fractionation of crude safflower lipase under different conditions

Ammonium sulfate concentration (% w/v)	Activity (U cm^{-3})	Total units	Yield (%)	Protein (mg cm^{-3})	Specific activity (U cm^{-3} per mg protein cm^{-3})	Purification rate
		A				
Crude extract	20	3000	100	62	0.3	1.0
0–15	11	1100	36	29	0.4	1.3
15–30	8.4	840	28	35	0.16	0.53
30–45	6.4	640	21	21	0.3	1.0
		B				
Crude extract	18	2700	100	61	0.13	1.0
0–17	7.2	540	20	40	0.18	1.35
17–34	3.6	270	10	30	0.12	0.9
		C				
Crude extract	18	2700	100	56	0.32	1.0
0–30	8	400	14.8	84	0.095	0.3
30–45	10.4	520	20	45.8	0.23	0.8

essentiality of a lipid cofactor for castor bean lipase was estimated by ORY and co-workers (1964). The factor was found to be the cyclic polymer of ricinoleic acid. The other probable reason for the loss in enzyme activity is the separation of some protein activator of different molecular characteristics such as molecular weight and solubility. ORY and co-workers (1967) separated

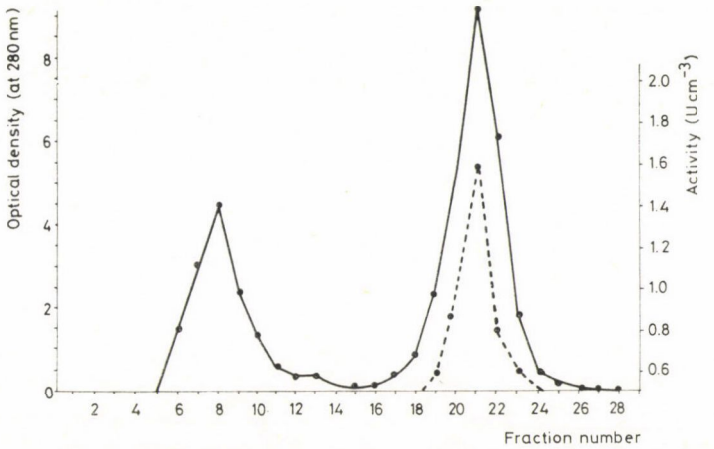

Fig. 1. Sephadex Gel Filtration G-150: Equilibrated and eluted with 0.25 mol dm^{-3} sucrose solution. Flow rate 10 cm^3 per 10 min. ●——● Optical density; ●– – –●: enzyme activity

and partly purified a heat stable protein activator from the crude extract of castor bean lipase.

Gel filtration on Sephadex G-150 column resulted in two protein peaks (Fig. 1). The activity of the enzyme appeared with the larger one. Only 3fold of purification with 21% yield was achieved by this method. These results support our suggestion that some factors disturb the normal separation and fractionation of safflower lipase.

2.2. Reaction rate

Figure 2 shows the results obtained when the volume of the alkaline required to neutralize the liberated fatty acids was plotted versus the time of reaction. The sigmoid shape of the curve obtained indicates that there is a lag period of about 6 h, which is probably due to the presence of impurities or inhibitors especially when the enzyme is present at a low concentration. Carboxy esterase of rat liver needed more than one hour to hydrolyse impure ethyl mondelate, this period could be shortened by redistillation and recrystallization of the substrate (WILLSTÄTTER et al., 1927).

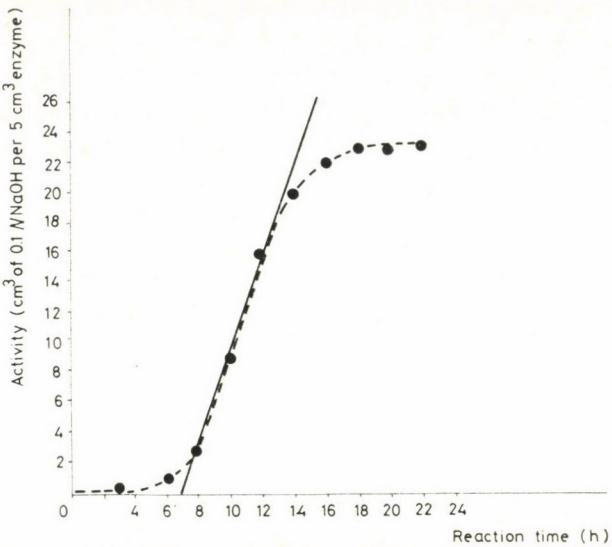

Fig. 2. Reaction rate of safflower lipase-catalyzed tributyrin hydrolysis. $y = 2.75x - 18.75$; $r = 0.995$

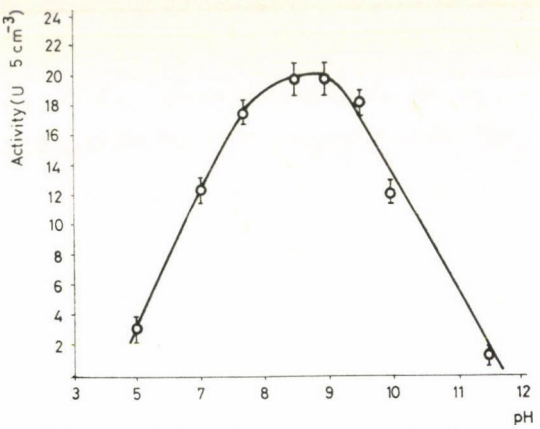

Fig. 3. Effect of pH on safflower lipase-catalyzed tributyrin hydrolysis

t-Values of Student's test

pH	7.5	8.5	9
8.5	2.8*		
9	3.13*	0.12	
9.5	0.41	2.4	2.91*

* Significant at P < 95% probability level
FG = 4

The linear relationship between time and enzyme activity has manifested itself after 6 h up to 12 h. The rate of reaction could be calculated from the slope of the straight line obtained between 6–12 h. Reaction rate of safflower lipase revealed that the enzyme exists in low concentration with some complexity in its biochemical system. KUMAR (1967) found that sesame lipase requires 22 h to hydrolyse its endogeneous substrate, morever, poppy seed lipase was found to be slower than both sesame and safflower seed lipase (HOLASOVA & BLATTNA, 1980). The most active lipase of castor bean required 24 h to show an observable activity after irradiation with γ-rays (THANKI et al., 1970).

2.3. Effect of pH on enzyme activity

Bell-shaped curve was obtained when the rate of reaction was plotted versus different pH values (Fig. 3). This shape is familiar for most enzymes.

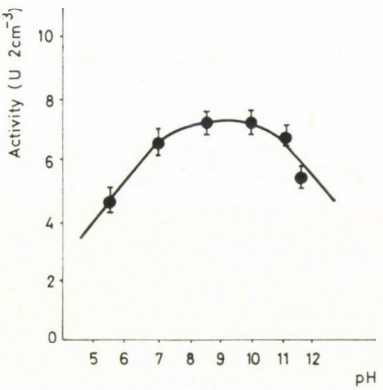

Fig. 4. Effect of pH on the stability of safflower lipase at ambient temperature. Enzyme extracts were diluted with phosphate buffer and pH values maintained with concentrated HCl or NaOH

t-Values of Student's test

pH	5.5	7	8.5	10	11
7	7.8**				
8.5	11.9***	2.18*			
10	11.3***	1.6	0.63		
11	10.25***	0.12	3.6*	1.4	
11.5	4.10*	5.25**	9.4***	8.15	7.3**

* Significant at P $\geq$ 95% probability level
** Highly significant at P $\geq$ 99% probability level
*** Very highly significant at P $\geq$ 99.9% probability level
FG = 4

The pH optimum of safflower lipase was at 8.5–9 since significant differences were not obtained in the activity of the enzyme in this pH range. Similar value was reported for the alkaline lipase of cotton seeds (MADYAROV et al., 1975), peanut seeds (SANDERS & PATTEE, 1975) and apple (RHODES & WOOSTORON, 1967).

The enzyme showed high stability at pH 8–10 while significant decrease was obtained below and over this range (Fig. 4). At this range of pH no denaturation occurred during an incubation period of 3 h at room temperature. Both pH optima and stability were in the alkaline range indicating that the enzyme is very sensitive to acid pH values.

2.4. Effect of temperature on reaction rate and stability

To test the effect of temperature on the rate of safflower lipase catalyzed tributyrin hydrolysis, reaction rates were measured at different temperatures between 20 °C and 50 °C (Fig. 5). Enzyme activity increased with the rise of temperature from 20 °C to 30 °C and then declined. The optimum temperature was near 30 °C. Temperature optima for sesame lipase, oat lipase and rice bran lipase-1 were reported to be 37 °C (KUMAR, 1967; MARTIN & PEERS, 1953; AIZONO et al., 1976). HOLASOVA and BLATTNA (1980) reported 20–25 °C for poppy seed lipase.

As evident from Fig. 6, the enzyme showed no activity after heat treatment at 70 °C for 1 min. The loss in enzyme activity was 25%, 60%, 92% and 98% of the original when it was heat treated at 45 °C, 52 °C, 60 °C and 70 °C temperature, respectively. These results show that the enzyme from safflower

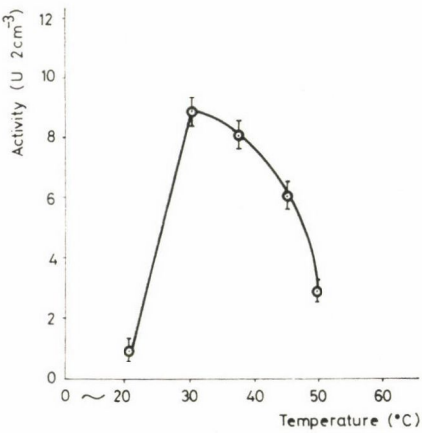

Fig. 5. Effect of incubation temperature on the activity of safflower lipase. Student's *t*-value is 3.21*, between the activity at 30 °C and 35 °C, significant at P $\geq$ 95% probability level (FG = 4)

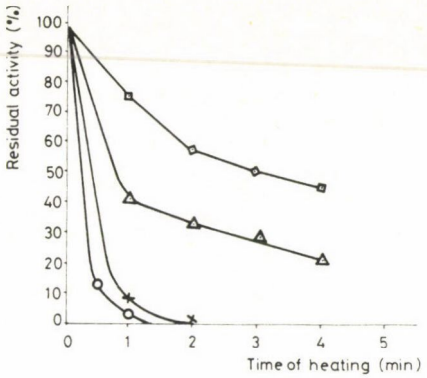

Fig. 6. Effect of different heat treatments on the stability of safflower lipase itself. □——□: 45 °C; △——△: 52 °C; ×——×: 60 °C; ○——○: 70 °C

seeds is very sensitive to heat treatment over 40 °C in comparison with the enzyme from other sources like peanut seed lipase, which resisted 60 °C for 5 min without any noticeable inactivation (SANDERS & PATTEE, 1975).

2.5. Effect of substrate concentration

As shown in Fig. 7 the reaction rate increased with increase in tributyrin concentration until it reached a constant value. This was in conformity with the general pattern of enzyme reactions.

Two substrates were used to test the effect of substrate concentration on the rate of enzyme reaction. The first was tributyrin emulsified with PVA, whereas the second was water soluble Tween-80. The results were expressed as Linewaever-Burk plot of 1/s vs 1/v. With both substrates straight lines were obtained and K_m values were calculated to be 3.3×10^{-4} mol and 8×10^{-3} mol

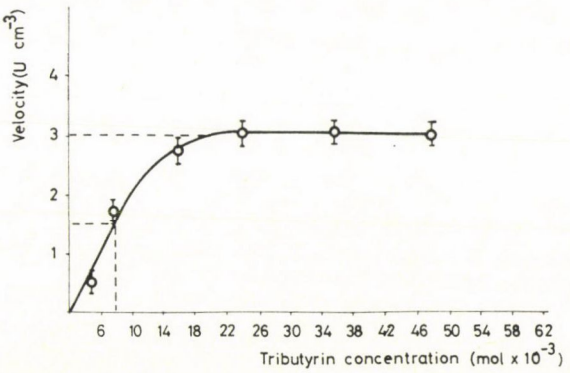

Fig. 7. Effect of substrate concentration on safflower lipase-catalyzed tributyrin hydrolysis

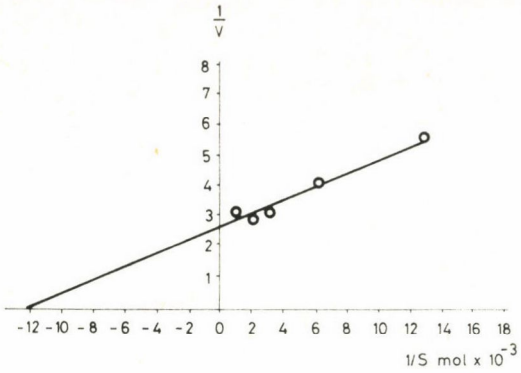

Fig. 8. Linewaever-Burk plot safflower lipase-catalyzed tributyrin hydrolysis.
$y = 2.63 + 0.22x$; $r = 0.98$

with Tween-80 and tributyrin, respectively (Fig. 8). Because lipase acts only on the interface between lipid and water, the concentration of water soluble substrate was necessarily higher than of water insoluble one to reach at first saturation level and then to be in contact with the enzyme, therefore the value of K_m with Tween-80 was higher than with emulsified tributyrin. DUNDAS and co-workers (1978) reported 2.2×10^{-2} mol for *Vicia faba* lipase and SANDERS and PATTEE (1975) reported 2.6×10^{-4} mol for peanut alkaline lipase using tributyrin as substrate.

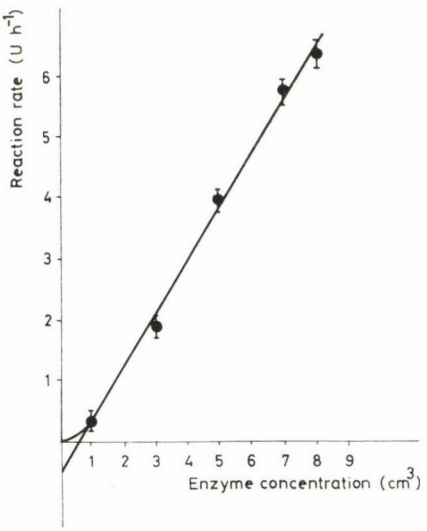

Fig. 9. Effect of enzyme concentration on the activity of safflower lipase.
$y = 0.88x - 0.58$; $r = 0.99$

2.6. Effect of enzyme concentration

A linear relationship was observed between the initial rate of reaction and enzyme concentration obtained over a broad range of enzyme concentration (Fig. 9) with very low rate at the lowest concentration of enzyme. The low rate of reaction may be due to the presence of some impurities in the substrate and/or in the emulsifier used. The linearity maintained between enzyme concentration and reaction rate illustrates that the concentration of substrate and any required cofactor were greatly in excess of enzyme concentration in the crude extract of safflower lipase.

2.7. Substrate specificity

The observation that pure triglycerides and monoglycerides containing long chain fatty acid esters were hydrolyzed by enzyme extract confirms the occurrence of true lipase in safflower seeds.

It was noticed that the rate of tributyrin hydrolysis was higher than that of unsaturated triglycerides. This reflects the preference of safflower lipase to the esters of saturated fatty acids (Table 3). The enzyme activity decreased with the increase of the degree of unsaturation of triglycerides used. This

Table 3

Effect of different lipid substrates on the activity of partially purified safflower lipase

Substrate	Concentration	Activity (U cm^{-3})
Tributyrin	2.5×10^{-3} mol	6.2 ± 0.35
	1.25×10^{-3} mol	6.0 ± 0.30
Triolein	2.5×10^{-3} mol	3.0 ± 0.225
	1.25×10^{-3} mol	2.7 ± 0.2
Trilinolein	2.5×10^{-3} mol	2.0 ± 0.2
	1.25×10^{-3} mol	1.7 ± 0.05
Corn oil	0.25 g	1.4 ± 0.02
	0.125 g	3.1 ± 0.25
Mixed oil[a]	0.25 g	2.36 ± 0.06
	0.125 g	1.8 ± 0.025
Tween-80	6.6×10^{-1} mol	10.4 ± 0.87
	3.3×10^{-1} mol	7.4 ± 0.32
Tween-20	6.6×10^{-1} mol	6.7 ± 0.2
	3.3×10^{-1} mol	6.6 ± 0.15
Monostearin	2.5×10^{-2} mol	5.0 ± 0.125
	1.25×10^{-2} mol	5.1 ± 0.12

The results are the mean value of 4 parallels, ± standard deviation

[a] Degree of saturation of mixed oil (sesame oil + cotton seed oil) is higher than of corn oil according to the analysis of the State Company of Vegetable Oils, Baghdad, Iraq

could also be noticed when two different commercial oils were used as substrates: the higher the degree of unsaturation of the oil, the lower the rate of reaction of safflower lipase. The results agree with those reported by ERLANSON and BORGSTRON (1970) and CHANDAN and SHAHANI (1963) for pancreatic and milk lipase, respectively.

On the other hand, the rate of hydrolysis of Tween-20 (monolaurate ester) was lower than that of Tween-80 (monooleate ester), though the latter is an ester of unsaturated fatty acid. This indicates that the enzyme prefers long chain fatty acid esters.

2.8. Effect of some chemicals

Several types of chemicals were tested for their effect on safflower lipase (Table 4). The SH-binding agents ($MgCl_2$, iodoacetate, and *p*-chloromercuribenzoate) inhibited lipase activity when they were added to the reaction mixture in 5×10^{-4} mol concentration. Reactivation of 38% and 70% was achieved by adding 1×10^{-2} mol of cysteine. These results confirm the existence of the SH group in the active site of the enzyme.

Addition of EDTA in 5×10^{-3} mol to the reaction mixture caused 85% inhibition. Complete reactivation was achieved when 5×10^{-3} mol $CaCl_2$ were added to the enzyme. This observation indicates that Ca^{2+} ions are necessary and required for safflower lipase-catalyzed lipid hydrolysis. The increase (7%) obtained in the enzyme activity by adding 5×10^{-3} mol $CaCl_2$ to the reaction mixture supports the above-mentioned suggestion. Moreover, it was noticed

Table 4

Inhibition and activation of partially purified safflower lipase by some chemicals

Treatment	Enzyme activity (U cm^{-3})	Residual activity (%)
Enzyme + substrate + water	6.45 ± 0.205	100
Enzyme + substrate + water + 5×10^{-4} mol PCMB	0.0	0.0
Enzyme + substrate + water + 5×10^{-4} mol PCMB + + 10^{-2} mol cysteine	4.55 ± 0.12	70.4
Enzyme + substrate + water + 5×10^{-4} mol $HgCl_2$	0.22 ± 0.01	3.4
Enzyme + substrate + water + 5×10^{-4} mol $HgCl_2$ + + 10^{-2} mol cysteine	2.5 ± 0.05	38
Enzyme + substrate + water + 5×10^{-4} mol IAA	1.27 ± 0.025	19
Enzyme + substrate + water + 5×10^{-3} mol EDTA	0.96 ± 0.02	15
Enzyme + substrate + water + 5×10^{-3} mol EDTA + + 5×10^{-3} mol $CaCl_2$	6.4 ± 0.2	99
Enzyme + substrate + water + 5×10^{-3} mol $CaCl_2$	6.9 ± 0.25	107

Water was used to complete the volume to 20 cm^3
PCMB: *p*-chloromercuribenzoate
IAA: iodoacetate
EDTA: ethylene diamino tetra acetic acid

that dialysis of the active fraction of ammonium sulfate fractionation for 24 h against 0.25 mol sucrose solution caused significant decrease in enzyme activity. The enzyme activity could be doubled by adding 3×10^{-3} mol $CaCl_2$ giving another evidence for the activating effect of Ca^{2+} ions on safflower lipase.

It is well-known that Ca^{2+} ions are required for lipase activity to remove liberated fatty acids from the interface as water soluble soap protecting the enzyme against the shift in pH-occurring as a result of fatty acid accumulation (BROCKERHOFF & JENSEN, 1974).

Literature

AIZONO, Y., FUNATSU, M., FUJIKI, Y. & WATANABE, M. (1976): Purification and characterization of rice bran lipase-11. *Agric. & biol. Chem., 40*, 317–324.

BAI, N. J., KUMAR, K. & KRISHNAMURTHY, S. (1972): Studies on enzymes of oil seeds. Solubilization and properties of lipase from seeds of *Sesamum indicum* (Gingelly). *Enzymologia, 43*, 345–351.

BIER, M. (1955): Lipase. — in COLOWICKE, S. P. & KAPLAN, N. O. (Eds) *Methods in enzymology*. Acad. Press, New York, Vol. 1.

BROCKERHOFF, H. & JENSEN, R. (1974): *Lipolytic enzymes*. Acad. Press, New York.

CHANDAN, R. C. & SHAHANI, K. M. (1963): Purification and characterization of milk lipase. *J. Dairy Sci., 46*, 275–282.

DESNULLE, P. (1972): The lipase. — in BOYERS, P. D. (Ed.) *The enzymes*. Acad. Press, New York, p 575.

DUNDAS, D. G. A., HENDERSON, H. M. & ESKIN, N. A. M. (1978): Lipase from *Vicia faba* minor. *Fd Chem., 3*, 171–177.

ERLANSON, C. & BORGSTRON, M. (1970): Tributyrin as a substrate for determination of lipase activity of pancreatic juice and small intestinal content. *Scand. J. Gastroenterol., 5*, 293–298.

JUJIKI, Y., AIZONO, Y. & FUNATSU, M. (1978): Chemical properties of major subunit of rice bran lipase. *Agric. biol. Chem., 42*, 599–606.

HASSON, E. P. & LATIES, G. G. (1976): Separation and characterization of potato lipid acylhydrolase. *Pl. Physiol., 57*, 142–147.

HOLASOVA, M. & BLATTNA, J. (1980): Lipolytic activity of poppy seeds. *Nahrung, 24*, 607–613.

KUMAR, K. S. (1967): The lipolytic activity of oil seeds. — Part 1. *Sesanum indicum*. *Enzymologia, 33*, 243–248.

MADYAROV, SH., DZANBIAEVA, N. R., ATAULLAEV, A. KH., ABDUMALIKOV, A. KH. & RAKHIMOV, M. M. (1975): Lipoliticheskie fermenty semyan khlopchatnika i ikh stabil'nost. *Prikl. Biochem. Mikrobiol., 11*, 437–443.

MARTIN, H. F. & PEERS, F. G. (1953): Oat lipase. *Biochem. J., 55*, 523–529.

MORTON, R. K. (1954): The purification of alkaline phosphotase of animal tissues. *Biochem. J., 57*, 595–603.

ORY, R. L., BARKER, R. H. & BOUDREAUX, G. J. (1964): Nature of the cofactor for the acid lipase of *Ricinus communis*. *Biochemistry, 3*, 2013–2018.

ORY, R. L., KIRCHER, H. W. & ALSCHUL, A. M. (1967): Separation of a heat-stable protein activator for the castor bean lipase. *Biochim. biophys. Acta, 147*, 200–206.

ORY, R. L. (1969): Acid lipase of the castor bean. *Lipids, 4*, 177–185.

RHODES, M. O. C. & WOOSTORON, L. S. C. (1967): The respiration climacteric in apple fruit. The action of hydrolytic enzymes in peel tissue during the climacteric period in fruit detached from the tree. *Phytochemistry, 6*, 1–9.

SANDERS, T. H. & PATTEE, H. E. (1975): Peanut alkaline lipase. *Lipids, 10*, 50–54.

THANKI, R. J., PATEL, K. C. & PATEL, R. D. (1970): Variation in lipase activity during germination (in dark) of γ-irradiated castor bean seeds. *Lipids, 5*, 519–525.

WHITAKER, J. R. (1972): *Principle of enzymology for the food sciences*. Marcel Dekker, New York. pp. 12–54.

WILLSTÄTTER, R., KUHM, R., LIND, O. & NEUMAN, F. (1927): Über Hemmung der Leber-esterase durch Ketocarbonsäureester. *J. physiol. Chem., 167*, 304–311.

Acta Alimentaria, Vol. 17 (2), pp. 141–145 (1988)

ESTRONE IN *PHALARIS CANARIENSIS* AND *ECHINOCHLOA CRUSGALLI* GRAINS

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(Received: 3 February 1987; accepted: 30 May 1987)

HPLC was used for qualitative and quantitative determination of the hormone estrone in the acetone, diethylether and methanol extracts of *Phalaris canariensis* (I) and *Echinochloa crusgalli* (II) grains. The presence of estrone hormone was also examined by TLC in each extract separately after treating with Girard's reagent T.

Keywords: Estrone, *Phalaris canariensis*, *Echinochloa crusgalli*, HPLC

Phalaris canariensis (I) and *Echinochloa crusgalli* (II) are annual plants belonging to the family Gramineae. (I) is rare in Egypt while (II) is common on moist ground, gardens and in rice fields specially in the Nile region including the Delta, the valley of Fayoum, and Oases. The grains of these plants are utilized as human and bird foodstuffs, as well as forage for animals.

These plants were subjected to numerous investigations (FISYUNOV, 1970; FEBREL & CARHALLIDE, 1965; MALIC & WILLIAMS, 1966). However, no work has been done to detect the presence of estrone in these grains. Accordingly, in this study we try to investigate its presence.

1. Materials and methods

The grains of (I) and (II) were obtained from the department of crops, Faculty of Agriculture, Alexandria University, Egypt.

Samples (1500 g) of each finely ground grains of (I) and (II), were soaked in acetone, diethylether, and methanol, respectively, for one week each at room temperature, with occasional shaking. Each extract was filtered off and evaporated to dryness (AMIN et al., 1969), (Table 1).

A sample (2.2 g) of each extract from (I) and (II) was treated with Girard's reagent T, and the liberated hormone was separately extracted with diethylether, then evaporated to dryness (AMIN & BASSIOUNY, 1979).

The residue obtained from each extract was examined by thin-layer chromatography on silica gel-G coated plates, with benzene–acetone–chloroform (60 : 15 : 12) as solvent system and Liebermann's reagent as spray agent.

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Table 1

Mass percentage of total lipids extracted from (I) and (II) grains by different solvents

Sample	Acetone extract (%)	Ether extract (%)	Methanol extract (%)
(I)	3.81	1.60	1.24
(II)	2.06	1.21	1.20

Also the isolated hormone was subjected to HPLC analysis, using Perkin-Elmer apparatus equipped with a stainless-steel column (25 × 0.46 cm) packed with analytical silica (—HC—ODS), with a solvent system of *n*-hexane–ethanol (9 : 1), at a flow rate of 3 $cm^3\ min^{-1}$ and maximum pressure 12 MPa. The volume of the injected sample was 10 μl. The instrument was equipped with an ultraviolet detector which operated at 286 nm, with sensitivity 0.16 and Chart speed 1 cm min^{-1} (LIN & HEFTMANN, 1981).

Quantitative determination of the separated hormone was achieved using an authentic specimen of estrone in different concentrations (0.1–0.5 $\mu g\ cm^{-3}$). The recovery of estrone was found to be 73%. The concentration of estrone in each extract was estimated according to the following equation:

$$\text{Concentration } (\mu g\ cm^{-3}) = \frac{\text{area under the peak}}{\text{slope of standard curve}}$$

See Tables 2 and 3; Figs. 1 and 2.

Table 2

Peak areas of different concentrations of estrone

Concentration ($\mu g\ cm^{-3}$)	0.1	0.2	0.3	0.4	0.5
Peak area (cm^2)	0.88	1.90	2.80	3.80	5.30

Table 3

Peak areas and mass weight percentages of the obtained hormone extracted by different solvents of grain (I) and (II)

	Grain (I)			Grain (II)		
	Aceton	Ether	Methanol	Aceton	Ether	Methanol
	extract					
Peak area (cm^2)	3.78	trace	1.85	2.25	1.85	1.60
Hormone quantity (mg%)	66.57	trace	64.98	39.63	32.49	28.18

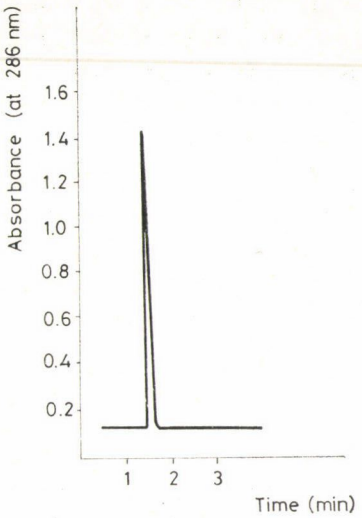

Fig. 1. HPLC of an authentic specimen of estrone

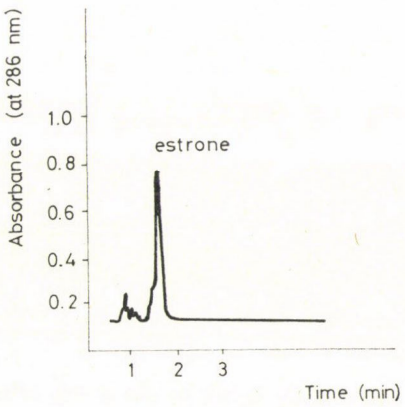

Fig. 2. HPLC of acetone extract (I) after treating with Girard's reagent T

2. Results

As a part of our program for separation of estrone from different species of plants, herein we try to isolate estrone from two different species of Gramineae, *Phalaris canariensis* (I) and *Echinochloa crusgalli* (II) grains. Total lipids were isolated from both grains by extraction in succession, separately, with acetone, ether and methanol. Each extract was treated with Girard's reagent T to remove non-ketonic compounds, while the obtained ketonic compounds in each extract were subjected to HPLC analysis.

It was found that lipid extracts of (I) and (II) contain estrone. The acetone and methanol extracts of (I) contained about 66.57 and 64.98 mg %, while acetone, ether and methanol extracts of (II) were 39.63, 32.49 and 28.18 mg %, respectively.

However, a trace amount of the isolated hormone was detected in the ether extract of (I), though it was repeated several times. Quantitative estimation of estrone in each extract was done by using a calibration curve of estrone.

Table 4

Statistical comparison of extracted total lipid content from grains (I) and (II)

Source	SS	DF	MS	F
Treatments	0.327	1	0.327	0.2665
Blocks	2.374	2	1.187	0.9683
Error	2.452	2	1.226	
Total	5.152	5		

Table 5

Statistical comparison of hormone concentration of grains (I) and (II)

Source	SS	DF	MS	F
Treatments	1543.369	1	1543.369	3.0775
Blocks	568.763	2	284.381	0.5671
Error	1003.013	2	501.506	
Total	3115.144	5		

Also, qualitative examination of the presence of estrone in each extract, was carried out by TLC and the spot obtained in each chromatogram was found to be identical with an authentic sample of pure estrone.

Therefore it seems that estrone is found in the two examined species of the Gramineae of which grains of *Phalaris canariensis* contain more estrone hormone than *Echinochloa crusgalli* grains.

Statistical analysis of the data obtained, by applying the two ways analysis of variance (ANOVA), shows that the difference is non-significant between mean values of the extracted total lipid content from (I) and (II) (Table 4).

On applying the same statistical analysis to the obtained hormone concentration data we found that the difference is non-significant between samples (Table 5).

3. Conclusions

Total lipids obtained with different solvents from (I) and (II) were 6.65% and 4.46%, respectively. Results of statistical analysis do not show significant differences between the extracted total lipids from grains (I) and (II).

The total concentration of extracted estrone from the lipid fractions of grain (I) was 131.55 mg per 100 g, while of grain (II) was 100.3 mg per 100 g. According to the statistical analysis there is no significant difference between the hormone content of samples.

Literature

AMIN, EL S. & BASSIOUNY, A. (1979): Estrone in *Olea europaea* kernel. *Phytochemistry*, *18*, 344.

AMIN, EL S., AWAD, O., ABDEL SAMAD, M. & ISKANDER, M. (1969): Isolation of estrone from moghat roots and from pollen grains of Egyptian date palm. *Phytochemistry*, *8*, 295.

FEBREL, J. & CARHALLIDE, A. (1965): Composition of canary grass seed. *An. Bromat.*, *17*, 345–360.

FISYUNOV, A. (1970): Effect of herbicides on the germinating capacity of weed seeds. *Khim. Sel'sk. Khoz.*, *8*, 928–930.

LIN, J. T. & HEFTMANN, E. (1981): High performance liquid chromatography of naturally occurring estrogens. *J. Chromat.*, *212*, 239.

MALIC, M. & WILLIAMS, W. (1966): Composition of the seed oil of *Phalaris canariensis*. *J. Sci. Fd Agric.*, *17*, 174–175.

Acta Alimentaria, Vol. 17 (2), pp. 147–157 (1988)

MEAT-BORNE PARASITIC INFECTIONS IN POLAND AND POSSIBLE WAYS OF PREVENTION[a]

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The most important parasitic infections in Poland transmitted by beef and pork are trichinellosis, toxoplasmosis and *Taenia saginata* taeniasis. In the early seventies the percentage of trichinellosis in pigs and the number of human trichinellosis cases in Poland decreased markedly. However, in recent years an increase in human trichinellosis was observed. The infection rate of toxoplasmosis in humans varied between 0.8% and 51.5%. The general pattern of *Taenia saginata* transmission in Poland is endemic, characterized by the existence of a small number of human carriers and a moderate prevalence of cysticercosis in cattle, mostly of low intensity.

Although in many situations it is possible to exclude intensively infected meat owing to proper diagnostics, the eradication of human parasitic infections in most situations is not realistic. Mass treatment, public health education and methods, hitherto existing for destroying infective stages of parasites by cooking and freezing, have not always been very successful. Following registration and controlled slaughter in Poland most of the foci of pig trichinellosis were liquidated. However, the possibility of persistence of potential foci in pigs, as well as in rats, cats and dogs, and the increasing role of wild boar meat have to be taken into account as a source of human infection. Owing to the unique character of the infection with *Taenia saginata* the total eradication of this infection seems very possible; any break in the link between man — as the sole definite host — and the intermediate host should result in the total elimination of the parasite. However, there are some limitations in meat inspection because of the difficulty in the detection of lightly parasitized carcasses. Also, only a fraction of the slaughtered animals passes through official meat inspection. In consequence of the wide distribution of *Toxoplasma gondii* in a wide range of hosts and of various routes of infection, the activities to control or prevent the disease may be negligible; official meat inspection is not concerned with the diagnosis of this infection.

In this situation the irradiation of food as a method of disinfecting it is receiving a considerable attention. As a result of experimental studies there arose the possibility of the application of low-dose irradiation for the control of meat-borne parasites. However, improvement in sanitary and hygienic measures in rearing farms and in diagnostics of parasitic infections in humans and animals should complement health education and lead to eradication of natural foci of parasitic infections or at least to the isolation of domestic animals from these foci.

Keywords: meat-borne parasitic infections, epidemiology, prevention

The list of food-borne parasitic infections is broad and comprises over twenty species of parasitic protozoa and helminths which form a threat to

[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
Kluwer Academic Publishers, Dordrecht

man. The prevalence of these infections and the importance of the problem depends very much on social, economic and cultural factors, such as: meat inspection and compulsory notification of parasitic infections, level of sanitation, animal husbandry patterns, and human behaviour and food habits.

Worldwide distribution of some food-borne parasitic infections plays a decisive role as regards human health and the economy of many people. Highly prevalent in most countries is *Toxoplasma gondii* infection. Trichinella infection is found in most countries of the northern hemisphere, and in parts of South America, Africa and Asia. Both these parasites have numerous natural foci in vast areas of all the continents, and are very often found in food producing domestic animals.

On the other hand, the high endemic character of some parasitic food-borne infections, even restricted to some areas, affects the economic status of these countries; e.g. in Thailand 7 million people are infected with *Opishorchis viverini* in consequence of raw freshwater fish consumption (BHAMARAPRAVATI et al., 1978). Although *Taenia saginata* infection in man is distributed globally, yet the importance of this infection is different in certain countries or regions and the prevalence varies according to the sanitation level, human food habits, meat inspection and compulsory notification of taeniasis cases.

Poland belongs to the countries in which food-borne parasitic infections are of moderate prevalence. Therefore the assessment of some meat-borne parasitic infections in this country corresponds to the situation in most countries, despite of the differences in factors affecting the transmission of the parasite. The most important parasitic infections in Poland transmitted by beef and pork are trichinellosis, toxoplasmosis and *Taenia saginata* taeniasis.

1. The trichinellosis problem in Poland

In the life cycle of *Trichinella spiralis* the same animal acts as definitive and intermediate host in which the adult parasite harbours temporarily in the small intestine and the encysted larvae remain alive for a long period in striated muscle. For completing the life cycle the parasitized flesh with encysted larvae has to be ingested by another host. Over 120 species of wild and domestic animals were found to be infected with Trichinella (WHO, 1979). The pig is the main source of infection in humans. Recent data confirm the differences among strains of *Trichinella spiralis* (MURRELL et al., 1985) and the existence of intraspecific variants: *Trichinella spiralis spiralis*, *T. s. nativa* and *T. s. nelsoni*, and a new species: *Trichinella pseudospiralis* (PAWŁOWSKI, 1981). The proposed new speciation is reflected in differences in the invasiveness of these variants to various hosts which maintain the parasite in two types of cycles: the sylvatic (natural) and the synanthropic (SMITH, 1985). Despite the

low infectivity of parasite isolates from the sylvatic cycle (arctic isolates) for pig and rat (SMITH, 1985), the danger of transmission of sylvatic isolates to pigs is sufficient to encourage prohibition in any control strategy (MURRELL et al., 1985; PENKOVA et al., 1985).

In Poland intensive epidemiological studies were carried out by several medical and veterinary teams. Among others large scale investigations were peformed by a team conducted by Gerwel and Pawłowski since 1963 for 11 years in cooperation with the Center for Disease Control in Atlanta, GA. The data which have been summarized by GERWEL and PAWŁOWSKI (1975) concerned the epidemiological relations between the natural and synanthropic foci of trichinellosis and created the basis for rational control and prevention of trichinellosis in Poland.

The frequency of trichinellosis in pigs in Poland in the early sixties was 0.011% to 0.016%, and the number of human cases in the same period (1960–1963) was 3954. There were numerous severe human cases with 14 deaths which occurred mainly in small family foci. There were also big epidemics reported; between 1953 and 1972 nine epidemics were registered, however, the clinical course of the infections was mild. The infection in rural inhabitants was mainly due to uncontrolled pig slaughter whereas in towns it was mostly the consequence of incompetent work of the veterinary service. The eradication of trichinellosis in Poznań province was attempted by the organization of a proper collaboration between the veterinary and medical services and the improvement in diagnostics of human and porcine trichinellosis. In the early seventies the percentage of infected pigs in this region decreased markedly to 0.001–0.004% and the number of human trichinellosis decreased also, with a tendency to focal distribution; in the period of 1970–1973 the number of registered cases was 1384. Most of the cases were of low intensity and thus hospitalization was not necessary.

However, in recent years an increase in human trichinellosis was observed (RAMISZ, 1985). It seems that the increased number of "urban" type epidemics is responsible in part for that situation (PAWŁOWSKI, 1981). On the other hand, an increasing role of wild boar meat as a source of human trichinellosis is observed; in Poland in about 35% of all clinical cases the source of infection was wild boar meat (CHMARZYŃSKI, 1983). Although man has no influence on the spreading of trichinellosis in wild animals, yet he may play a role in transmission of the parasite from wildlife to synanthropic foci; e.g. carcasses of shot foxes are very common source of trichinellosis in pigs. Garbage feeding is currently believed to play an important role as the source of the infection, and fur animals from the rearing farms are one of the common sources.

2. Epidemiology of toxoplasmosis

The live cycle of *Toxoplasma gondii*, poorly understood until recently, differs from the life cycles of other Coccidia. These differences are the consequence of wide and varied transmission between numerous host species. In cat, as definitive host, the parasite undergoes sexual development in the small intestine, but some parasites reproduce asexually in various parts of the host body. The intermediate stages of development occur in the tissues (mainly striated muscle, retina and brain) of a wide range of animals and man after swallowing the oocysts passed in the faces of cat. The parasites proliferate asexually in the intermediate host cells forming tachyzoites which are free in the blood and abdominal serous exudate after host cell rupture and enter new host cells to initiate further asexual cycle. When the host begins to produce antibodies, the parasites form intracellular cysts with numerous bradyzoites.

The intermediate hosts may aquire infection — besides by ingestion of oocysts from the feces of cats — with the vegetative forms (tachyzoites and bradyzoites) in the flesh of another intermediate host. In addition to the oral route of infection toxoplasmosis may occur as congenital infection when the proliferous phase with free parasites in the circulatory system occurs in pregnancy.

Toxoplasma gondii is widely distributed in many countries and epidemics have occurred both in man and animal (BEYER & SHEVKUNOVA, 1986; DUBEY, 1986). In most countries the infection is highly prevalent in pigs; e.g. in West Berlin and Japan the percentage of infected pigs was 97! (An excellent review is given by DUBEY, 1986.) In the USSR *Toxoplasma gondii* infections were also more prevalent in pigs (up to 40%) than in other food-producing animals (cattle 25.4%, sheep 30.1%, goats 20.0%, rabbits 25.4%) (BEYER & SHEVKUNOVA, 1986). The examination for *Toxoplasma gondii* of domestic animals at a slaughterhouse in Czechoslovakia showed that pigs but not cattle may be important in the transmission of the parasite. In Poland the prevalence of the infection in pigs was 46% (RAMISZ & ZEMBUROWA, 1978) or 90% (TARCZYŃSKI, 1986) and in sheep 50% (TARCZYŃSKI, 1986).

The variable prevalence of the infection is most probably related to the region (survival of oocysts in the environment), the management systems of rearing the animals, the density of cyst population, and also to the serologic survey technique.

Toxoplasma gondii in edible tissue of living pigs can survive for at least 171 days (DUBEY et al., 1984) and in pork at +4 °C up to 3 weeks (WHO, 1979). The parasites persist longer in the brain, heart and tongue than in other tissues, therefore these tissues may be most suitable for parasitologic surveys (DUBEY et al., 1984).

In human toxoplasmosis the major route of infection is the consumption of raw or undercooked meat or meat products (pork, mutton, beef or game). It is worth noting that the contamination of hands and utensils with infective stages of parasite in preparation of the meat-dish may be a possible source of infection.

There are numerous reports on human toxoplasmosis. Although the percentage varies, toxoplasmosis may be considered as a highly prevalent meat-borne parasitic infection in human. KRICK and REMINGTON (1978) found that the prevalence of chronic asymptomatic toxoplasmosis in the USA is 50%. In a random survey of 1% of the total population of Guadeloupe (French West Indies) 60% of 3238 persons were positive for *Toxoplasma gondii* antibodies (BARBIER et al., 1983). CHATAR and co-workers (1985) found that as many as 95.4% women engaged in the slaughter of rabbits were infected with Toxoplasma and consider that rabbits represent a particular risk for human. In France (region of Strasbourg) males were seropositive in 45.8% in 1970, and in 44.01% in 1980 (HIMY-DAHAN et al., 1983).

In Poland the infection rate of toxoplasmosis in human varied from 0.8% (STARZYK, 1972) to 51.5% (KOZAR, 1953). IFAT titer to Toxoplasma investigated in 9524 people in the north-west region of Poland in 1985 was positive in 47.07% (RYDZEWSKI, 1986). The titres of 1 : 1024 accepted as indicative of clinical toxoplasmosis was found in 26.63% and the highest prevalence was among the workers of slaughterhouse and of the veterinary service.

The most tragic form of toxoplasmosis in human is congenital infection particularly severe when it occurs in the first semester of pregnancy. MAYER and co-workers (1983) found that 46.03% of 18 170 pregnant women from Steiermark District (Austria) had positive titres in IFAT. The newborns in Lower Saxony (GFR) were positive for *Toxoplasma gondii* in a ratio of 45.6%; the authors (SANDER & NIEHAUS, 1983) suggested that among 10 000 pregnancies 70 to 80 mothers will undergo primary infection, and that 30 to 40 cases of congenital infection will occur. In Nagasaki City (Japan) the positive rate of the IHAT and the dye test in pregnant women was 6.4% (SUZUKI et al., 1983). The authors suspected that every year about 0.2% of pregnant women became newly infected. LAPIERRE and co-workers (1983) examined over 15 thousand pregnant women in France and estimated the risk of aquired infection during pregnancy at 1.2% and of the transmission of the parasite to the foetus at 1%. Using the IFAT MARTY and co-workers (1985) found in West Cameroon that 48.5% of pregnant women were positive to toxoplasmosis. The authors believed that about 6% of women become infected during pregnancy.

3. Prevalence and transmission of human taeniasis in Poland

Man as the sole definitive host of *Taenia saginata* and *Taenia solium* is infected by the ingestion of raw or undercooked meat parasitized with cysticerci, the larval forms. The larval form of *Taenia saginata* (cysticercus bovis) is found in cattle *(Bos taurus, Bos buffelus, Bos indicus, Bos gruniens)*, and in some regions, in reindeer *(Rangifer tarandus)*. The main host for the larvae of *Taenia solium* (cysticercus cellulosae) is domestic pig.

In Poland, like in many other countries in Europe, *Taenia solium* has disappeared, and only sporadic cases are observed (WHO, 1979). This is due to more efficient meat inspection, changes in pig husbandry, and to the increased standard of living and hygiene. The frequency of porcine cysticercosis in 1971 in slaughterhouses of Poland was only 0.001–0.002% (TARCZYŃSKI, 1986). Man may also be infected with the larval stage of *Taenia solium* (cysticercosis), and because the infection may lead to serious neuro-ocular and cerebral cysticercosis, even sporadic cases of *Taenia solium* taeniasis are a very important source of infection for human.

The general pattern of *Taenia saginata* transmission in Poland is endemic, characterized by the existence of a small number of human carriers and a moderate prevalence of cysticercosis in cattle, mostly of low intensity. The results of studies on epidemiology of *Taenia saginata* taeniasis and cysticercosis in Poznań province (Poland), performed through 1972–1977, have been summarized by PAWŁOWSKI (1980). The epidemiological situation in Poznań can be characterized as follows:

— The number of *Taenia saginata* cysticerci ingested by inhabitants of Poznań in officially examined raw beef was evaluated to be 3300 per year. This value was estimated by examining annual raw meat consumption, the number of undiagnosed cysticercosis in cattle, and the mean number of cysticerci in carcasses, in which the routine inspection did not reveal the infection.

— The annual number of *Taenia saginata* taeniasis among Poznań inhabitants was estimated at 350. This estimated value is obtained on the basis of reported and unreported but diagnosed or treated cases of taeniasis.

— The uncontrolled and clandestine staughtering plays insignificant role in the transmission of *Taenia saginata* taeniasis from cattle to man. For only 1.6% of human *Taenia saginata* carriers consume beef not examined by the meat inspector service.

— It has been found that the infection rate with *Taenia saginata* in man is related mainly to the frequency of eating raw beef, most of all steak tartare and raw ground-meat sausage (LISOWSKA, 1979). Most of the carriers (90%) are admitted to eat raw meat; 44% of them eat the meat exclusively at home, and these are mostly married women and children, 22% — single men and women — eat exclusively out, and 24% — mostly married men —

eat both at home and out (PAWŁOWSKI, 1970, 1982). The risk of being infected is determined also by profession (14 times greater in workers of the meat industry and restaurants) (PAWŁOWSKI, 1970).

— The prevalence of *Taenia saginata* taeniasis registered among the rural inhabitants of Poznań province is very low (0.0042%). This is the consequence of the habit of consuming mostly thoroughly cooked meat (LISOWSKA, 1979).

— Using "trace-back" methods in Poznań province there was found a concentration of localities with bovine cysticercosis around urban conglomerations, recreation areas, regions with developed agricultural industry, and along rivers, main roads and railway tracts (PAWŁOWSKI, 1980). The data from slaughterhouses showed that *Taenia saginata* became common in Poland after 1950. In Poznań the frequency of bovine cysticercosis rose from 0.5% in 1955 to 2.3% in 1962 (PAWŁOWSKI & SCHULTZ, 1972). In the period of 1972 to 1985 the frequency increased in the whole country from 0.67% to 1.5% (TARCZYNSKI, 1986).

4. Prevention and control of meat-borne parasitic infections

The eradication of trichinellosis in man could be realized by eliminating the source of infection of pigs and destruction of the larvae in pork. The examination of pigs in a slaughterhouse in Poznań performed after organized prophylaxis and favourable changes introduced in the sanitary and economic conditions in the rural districts, showed marked decrease of the infected animals. Following registration and controlled slaughter most of the foci of pig trichinellosis were liquidated (GERWEL & PAWŁOWSKI, 1975). However, the possibility of persistence of potential foci in pigs, as well as in rats, cats and dogs has to be taken into account. Most probably *Trichinella spiralis* could easily spread both from persistent single focus of trichinellosis and from pigs imported to other hitherto Trichinella-free areas (PAWŁOWSKI, 1981). The prohibition of raw garbage feeding, which should contribute to reducing pig trichinellosis, is also not completely successful (WHO, 1979).

According to PAWŁOWSKI (1981) "man cannot intervene in the transmission of Trichinella in nature, and we can only learn how to isolate natural *Trichinella spiralis* foci from synanthropic ones and how to prevent people from eating raw meat from infected wild animals".

Owing to the unique character of the infection with *Taenia saginata* and *Taenia solium*, whose life cycles are completed by man as the sole definitive host, the total eradication of these infections seems very possible; any break in the link between man and the intermediate host should result in the total elimination of the parasite. To control the infection, besides the treatment of infected persons, health education, sewage treatment, meat inspection have to

be introduced. However, there are some limitations in meat inspection in the detection of bovine cysticercosis because of the difficulty in detecting lightly parasitized carcasses.

On the other hand, in some countries only a fraction of the slaughtered animals passes through official meat inspection. Among others, in Poland the compulsory notification of human *Taenia saginata* infections is not always complied with; the survey conducted in Poznań showed that five years after the introduction of compulsory notification, 20% of *Taenia saginata* infections were not formally registered (PAWŁOWSKI & SCHULTZ, 1972; PAWŁOWSKI, 1984). To reduce the risk of infection there is a proposal in Poland to introduce limitation of steak tartare supply and to prepare it from meat of highest quality and inspected twice for cysticercosis (LISOWSKA, 1979). Safe meat production will be connected with an increase in cost.

One of the important activities in preventing and control of human taeniasis is health education. The observations conducted in Poznań showed that nearly half of the *Taenia saginata* carriers did not know how the infection in man is realized.

We may presume that the preconditions for eradication of *Taenia saginata* and *Taenia solium*, as well as for other meat-borne parasitic infections, are rarely met with in any country, therefore a realistic objective in most situations will be control rather than eradication.

In consequence of the wide distribution of *Toxoplasma gondii* in a wide range of hosts and of various routes of infection, the activities in controlling or preventing of the disease may be negligible. Austria is the first country in the world where toxoplasmosis control in pregnancy is obligatory (FLAMM & ASPÖCK, 1981). As a result of the surveillance performed since 1975 in all observed cases of pregnancy (about 52 thousands) no child with congenital toxoplasmosis was found. One of the control measures was the prophylaxis – "no contact with cats, no consumption of raw meat in pregnancy" – however, chemotherapy was probably also a very effective activity. Nevertheless, the authors suspected that in about half of the cases the therapy was groundless, and that is why toxoplasmosis prophylaxis is so expensive.

5. Possible role of irradiation in control of meat-borne parasitic infections

In the control of trichinellosis, as well as other meat-borne parasitic infections, there are few steps to be taken (PAWŁOWSKI, 1981). The first step is the exclusion of infected food. Although in many situations it is possible to exclude intensively infected meat owing to proper diagnostics, the eradication of human parasitic infections in most situations is not realistic. On the other hand, the detection procedure used in individual examination of slaughter

animals, in contrast to mass treatment, is impracticable. Unfortunately, many cases of trichinellosis have as the source the meat from individual uncontrolled and clandestine slaughtering. In the case of toxoplasmosis the official meat inspection is not concerned with the diagnosis of this very frequent infection of slaughter animals.

We have to admit that control measures, including mass treatment, public health education and hitherto existing methods for destroying the infective stages of parasites (cooking and freezing), have not always been very successful. Even an effective treatment does not really mean the possibility of successful control or eradication of the infection, even — on the contrary — may encourage consumers to eat parasitized raw meat which would bring increased number of infected people (BHAIBULAYA, 1985). Contamination of meat with parasitic protozoa and helminths is a serious problem of human diseases and of economic losses. The medical costs and productivity losses caused by trichinellosis, toxoplasmosis and *Taenia saginata* taeniasis in the USA were estimated roughly at 270 million dollars annually (ROBERTS, 1985).

Irradiation of food as a method of disinfection is receiving a considerable attention. As the result of experimental studies conducted on different species of parasites, the possible application of low-dose irradiation for control of meat-borne parasites emerged (VERSTER et al., 1977; BHAIBULAYA, 1985; BRAKE et al., 1985; DUBEY et al., 1986). These low doses prevented the reproduction and maturation of the infective parasite stages. Citing the review of FARKAS (1987), doses of 0.15–0.6 kGy suppressed the invasiveness of *Trichinella* larvae, *Toxoplasma* trophozoties, *Taenia saginata* and *Taenia solium* cysticerci, and of *Opisthorchis viverini* metacercariae. This radiation treatment promise significant reduction in the risk of human infections with the above-mentioned parasites.

It seems that irradiation will be an effective and safe technique in the control of meat-borne parasitic disease. It should be complementary to health education, improvement in sanitary and hygienic measures in rearing farms and in diagnostics of parasitic infections in humans and animals, and to the eradication of natural foci of parasitic infections or at least to the isolation of domestic animals from these foci. However, this opinion on the safety of the irradiation procedure has to be accepted by the consumer. Unfortunately, most people associate "radiation" with danger and with the noxious effects of nuclear energy.

Literature

BARBIER, D., ANCELL, E. T. & MARTIN-BOUYER, G. (1983): Seroepidemiological survey of toxoplasmosis in LaGuadelupe, French West Indies. *Am. J. trop. Med. Hyg.*, *32*, 935–942.

BEYER, T. V. & SHEVKUNOVA, E. A. (1986): A review of toxoplasmosis of animals in the USSR. *Vet. Parasitol.*, *19*, 225–243.

BHAIBULAYA, M. (1985): Effect of gamma ray on the metacercariae of liver fluke *(Opisthorchis viverrini)* infective stages of parasite caused by consumption of raw or semiprocessed fish. *Fd Irradiat. Newsl., 9*, 8.

BHAMARAPRAVATI, N., THAMMAVIT, W. & VAJRASTHIRA, S. (1978): Liver changes in hamsters infected with a liver fluke of man, *Opisthorchis viverrini*. *Am. J. trop. Med. Hyg., 27*, 787–794.

BRAKE, R. J., MURELL, K. D., RAY, E. E., THOMAS, J. D., MUGGENBERG, B. A. & SIVINSKY, J. S. (1985): Destruction of *Trichinella spiralis* by low-dose irradiation of infected pork. *J. Fd Saf., 7*, 127–143.

CHATAR, G., GOLKOVA, A. & MAVLINA, M. (1985): Toksoplazmoz kak vazhnyi zoonoz. *Med. Parazit. Parazit. Bol.*, 51–55.

CHMARZYŃSKI, W. (1983): Włosnica dzików w Polsce. (Trichinellosis of boar in Poland.) *Med. Weter., 39*, 428–431.

DUBEY, J. P. (1986): A review of toxoplasmosis in pigs. *Vet. Parasitol., 19*, 181–223.

DUBEY, J. P., BRAKE, R. J., MURRELL, K. D. & FAYER, R. (1986): Effect of irradiation on the viability of *Toxoplasma gondii* cysts in tissue of mice and pigs. *Am. J. vet. Res., 47*, 518–522.

DUBEY, J. P., MURRELL, K. D. & FAYER, R. (1984): Persistence of encysted *Toxoplasma gondii* in tissues of pigs fed oocysts. *Am. J. vet. Res., 45*, 1941–1943.

FARKAS, J. (1987): Decontamination, including parasite control, of dried, chilled and frozen food by irradiation. (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.) *Acta Alimentaria, 16*, 343–382.

FLAMM, H. & ASPÖCK, H. (1981): Die Toxoplasmose-Überwachung der Schwangerschaft in Österreich — Ergebnisse und Probleme. *Pädiatrie, 20*, 27–34.

GERWEL, C. & PAWŁOWSKI, Z. (1975): Studies on the epidemiology and biology of trichinellosis in Poland (1964–1974). *Wiad. Parazytol., 21*, 513–540.

HIMY-DAHAN, R., HEINRICH, A., FERRY, R., KIEN, T. & KREMER, M. (1983): La toxoplasmose humaine et animale dans la région strasbourgeoise en 1980. Modifications observées depuis 1970. *Méd. mal. infect., 13*, 457–459.

KOZAR, Z. (1953): Wartość i znaczenie próby śródskórnej dla rozpoznawania toksoplazmozy. (The importance and value of connective tissue tests for the detection of toxoplasmosis.) *Acta Parasitol. Pol., 1*, 159–229.

KRICK, J. A. & REMINGTON, J. S. (1978): Toxoplasmosis in the adults. *N. Engl. J. Med., 298*, 550–553.

LAPIERRE, J., TOURTE-SCHAEFFER, C., HEYER, F., NESSMANN, C., TIMBART, M. F. & BEAUJOUAN, L. (1983): Toxoplasmose congénitale. Réflexions à propos de la surveillance sérologique de quinze mille femmes enceintes. *Sem. Hôp., 59*, 2741–2745.

LISOWSKA, M. (1979): *Analiza epidemiologiczna tasiemczycy* Taenia saginata *na terenie miasta Poznania*. (Epidemiological analysis of the incidence of *Taenia saginata* infections in Poznan.) Thesis, Academy of Medicine, Poznań.

MARTY, P., REYNEY, J. & LE FICHOUX, Y. (1985): Contribution à l'étude de la toxoplasmose chez les femmes enceintes au Cameroun. *Bull. Soc. Path. exot., 78*, 623–628.

MAYER, H. O., STÜNZER, D. & ROSANELLI, K. (1983): Wertigkeit eines Toxoplasmose-Screening. *Zentbl. Gynäk., 105*, 1097–1100.

MURRELL, K. D., LIEBY, D. A., DUFFY, C. & SCHAD, G. A. (1985): Susceptibility of domestic swine to wild animal isolates of *Trichinella spiralis*. — in: KIM, C. W. *Trichinellosis*. The State University of New York Press, Albany, New York, pp. 301–305.

PAWŁOWSKI, Z. S. (1970): On control of taeniarhynchosis in urban population. *J. Parasitol., 56*, Sec. II. 261–262.

PAWŁOWSKI, Z. (1980): Epidemiologia tasiemczycy i wągrzycy. (Epidemiology of *Taenia saginata* and cysticercus bovis.) *Wiad. Parazytol., 26*, 539–552.

PAWŁOWSKI, Z. S. (1981): Control of trichinellosis. — in: KIM, C., RUITENBERG, E. J. & TEPPEMA, J. S. *Trichinellosis*. Reedbooks, England, pp. 7–20.

PAWŁOWSKI, Z. S. (1982): Epidemiology and prevention of *Taenia saginata*. — in: FLISSER, A., WILLMS, K., LACTETTE, J. P., LARRALDE, C., RIDAVRA, C. & BELTRAN, F. *Cysticercosis. Present state of knowledge and perspectives*. Academic Press, New York, pp. 69–85.

PAWŁOWSKI, Z. S. (1984): Cestodiasis. — in: WARREN, K. & MAHMOUD, A. A. F. *Tropical and geographical medicine*. McGraw Hill Book Company, New York, pp. 471–486.

PAWŁOWSKI, Z. S. & SCHULTZ, M. G. (1972): Taeniasis and cysticercosis *(T. saginata)*. *Adv. Parasitol.*, *10*, 269–343.

PENKOVA, R. A., BESONOV, A. S. & BELUSOV, M. N. (1985): The role of *Trichinella spiralis* isolates from wild animals in the epizootiology of pig trichinellosis. — in: KIM, C. W. *Trichinellosis*. The State University of New York Press, New York, pp. 295–300.

RAMISZ, A. (1985): Current problems of epidemiology and epizootiology of trichinellosis. — in: KIM, C. W. *Trichinellosis*. The State University of New York Press, New York, pp. 190–200.

RAMISZ, A. & ZEMBUROWA, K. (1978): Serological survey of Toxoplasma antibodies in animal livestock. *Communic. IV. Int. Congr. Parasitol.*, Warszawa, *E*, p. 85.

RYDZEWSKI, A. (1986): Personal communication.

ROBERTS, T. (1985): Microbial pathogens in raw pork, chicken and beef: Benefit estimates for control using irradiation. — ref.: FARKAS, J. (1987): Decontamination, including parasite control of dried, chilled and frozen food by irradiation. (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.) *Acta Alimentaria*, *16*, 343–382.

SANDER, J. & NIEHAUS, C. (1983): Häufigkeit der Toxoplasmose-Erstinfektion bei Schwangeren. *Dt. med. Wschr.*, *108*, 455–457.

SMITH, H. J. (1985): Epidemiology of *Trichinella spiralis nativa* in swine. — in: KIM, C. W. *Trichinellosis*. The State University of New York Press, Albany, New York. pp. 291–294.

STARZYK, J. (1972): Badania nad epidemiologia toksoplazmozy w Polsce. (Study of toxoplasmosis epidemiology in Poland.) *Wiad. Parazytol.*, *18*, 217–223.

SUZUKI, H., TSUCHIHASHI, K., MIYAZAKI, T., NAKASHIMA, H. & MATSUMOTO, K. (1983): Serological diagnosis and epidemiological study of toxoplasmosis in Nagasaki City. *Trop. Med.*, *25*, 83–89.

TARCZYŃSKI, S. (1986): Personal communication.

VERSTER, A., DU PLESSIS, T. A. & VAN DEN HEEVER, W. (1977): The eradication of tapeworms in pork and beef carcasses by irradiation. *Radiat. phys. Chem.*, *9*, 769.

WHO (1979): *Parasitic zoonoses*. Report of a WHO Expert Committee. Technical Report. No. 637, pp. 75–79.

Acta Alimentaria, Vol. 17 (2), pp. 159–167 (1988)

MODIFIED METHOD FOR DIAMINOPIMELIC ACID DETERMINATION IN SAMPLES OF BIOLOGICAL ORIGIN

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(Received: 23 February 1987; accepted: 17 July 1987)

A modified ion exchange column chromatographic method was developed for the determination of diaminopimelic acid (DAP) in samples of biological origin. The essence of the new method is the performic acid oxidation of the sample prior to HCl-hydrolysis. During the process methionine is transformed into methionine-sulfonium and migrates from its original place between valine and isoleucine to that between aspartic acid and threonine making room thereby for DAP between valine and iso-leucine. By oxidation with performic acid and the buffer systems applied the determination of minute quantities of DAP present (1–2 nmol) became possible. Using the newly developed analytical method DAP was determined in the rumen liquor (0.010 g DAP per 100 g sample and 0.67 g DAP per 100 g protein), in the centrifuged rumen liquor residue (0.32; 0.67).

Keywords: diaminopimelic acid determination, ion exchange column chromatography, performic acid oxidation, diaminopimelic acid in rumen content

In ruminants a part of the feed protein breaks down in the rumen and from the ammonia thus developing microorganisms inhabiting the rumen build up their own protein and thus a part of the feed protein is converted into bacterial protein. This transformation may be useful if from feed protein of low biological value and of NPN materials bacterial protein of higher biological value is formed. However, in most of the cases the breaking down of the feed proteins of high biological value in the rumen is disadvantageous. In the future it will be more and more important to get to know the proportion of protein breaking down in the rumen and the percentage protein reaching the duodenum originating from the feed or bacteria, respectively.

In recent years several methods were developed to determine the part of microbiological origin of the nitrogen containing substances getting from the rumen into the abomasum or into the ileum. Attempts were made to use nucleic acid, vitamin B_{12} or sulfur 35 isotope to follow up the nitrogen containing substances originating from microbes. Lately, it became possible to conclude the presence of protozoa nitrogen by measuring 2-aminoethylphosphonic acid or the presence of bacterial nitrogen by measuring 2-6-diaminopimelic acid. This is possible because 2-aminoethylphosphonic acid is mainly found in protozoa, while 2-6-diaminopimelic acid only in the mucopeptides of the cell wall in bacteria. In spite of the fact that the amount of diaminopimelic

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acid (DAP) in the cell wall is highly species depending, the proportion of DAP in relation to the total bacterial protein is, under steady feeding conditions constant, thus in comparative analyses DAP is suitable for the estimation of the part of bacterial protein in the total protein content.

Different methods were tried out for DAP determination in the rumen liquor or the intestine content. HUTTON and co-workers (1971) determined DAP on the amino acid analyzer, utilizing the characteristic of DAP that in contrast to other amino acids, but like proline, forms yellow color with the acidic solution of ninhydrin. The maximum light absorption was observed at 420 nm.

CZERKAWSKI (1974) developed a method for the determination of 2-aminoetylphosphonic acid and of DAP. To determine the latter the protein was hydrolyzed with acid, the hydrolysate was purified by running it through a bone charcoal column, DAP was separated from proline on an ion exchange column and determined with acidic ninhydrin.

PONGOR and BAINTNER (1980) developed a simple and rapid ion exchange thin-layer-chromatographic method to determine DAP. However, it did not spread in general use.

EDOLS (1985) determined DAP from the rumen liquor hydrolysate with automatic amino acid analyzer utilizing two columns. By optimizing the buffer composition he made DAP appear between methionine and iso-leucine in the chromatogram, well separated from the two amino acids in a clear-cut peak, easily evaluated.

Of the methods mentioned above that of EDOLS (1985) was tried out first, because our laboratory is provided with two amino acid analyzers and, besides, the authors were interested in all the amino acids present in the rumen liquor and in the ileum content.

If the determination was carried out in accordance with specifications separation and evaluation was satisfactory as long as the quantity of DAP and the total amino acid concentration was in the same order of magnitude or the concentration of the two amino acids, methionine and iso-leucine, next to DAP, did not exceed 8 to 10 times that of DAP. In this case, namely, DAP appeared as a shoulder peak of methionine or iso-leucine in the chromatogram causing evaluation to become uncertain or even impossible. Because of the above it was necessary to modify the method and develop a new one for the determination of DAP in the rumen liquor or the intestine content. In this paper the modified method is described.

1. Materials and methods

1.1. Principle of the method

By changing the pH of the buffers, the sodium ion concentration and the temperature of the ion exchange column it is possible to shift, DAP between certain limits, in the chromatogram. The problem arising when the amino acids next to DAP being present in 8–10 times higher amount than DAP depress the DAP peak or cause it to appear as a shoulder peak occurs also when DAP is removed to in between methionine and valine. Therefore, the sample to be analysed was oxidized with performic acid (HIRS, 1956) during which cystine is transformed into cysteic acid and methionine into methionine sulfonium. In the chromatogram cysteic acid appears directly behind the front and in front of aspartic acid and the methionine sulfonium between aspartic acid and threonine, thereby liberating the space between valine and iso-leucine. By changing the composition of the buffers it was achieved to make DAP appear in the place of methionine or a little before it in the chromatogram. By the above changes it was achieved that DAP appeared between valine and iso-leucine just in the middle and since being far enough from both valine and iso-leucine even a high concentration of these two amino acids does not disturb DAP determination.

1.2. Materials tested

To develop the method fish meal of 67% protein content with a high percentage of methionine and iso-leucine was selected as a model material, DL-2,6-diaminopimelic acid standard was purchased from the firm Fluka AG, Buchs, SG. When the development of the method was accomplished rumen liquor samples were taken from Holstein Friz cows. DAP determination was carried out in aliquots taken from the rumen liquor dried by lyophilization. Prior to the amino acid analysis the raw protein, true protein, digestible protein content of the samples was determined on the Kjel-Foss (Foss-Electric, Danmark) rapid nitrogen analyzer, using 6.25 as conversion factor.

1.3. Hydrolysis and processing the hydrolysate

Air-dried samples containing about 10 mg protein were weighed in medical ampoules washed previously with chromic sulfate. These were then oxidized with performic acid prepared according to HIRS (1956) and directly after cooled to −55 °C. Using a laboratory lyophilizing apparatus (Labor MIM, Hungary, Type OE-950) the ampoules were evaporated to dry. The

residues were hydrolized according to CSAPÓ and co-workers (1986) with 6 mol HCl at 110 °C for 24 h. The hydrolysate was processed as described in the above paper (CSAPÓ et al., 1986).

1.4. Analysis

The amino acid composition of the samples was established with LKB-4101 automatic amino acid analyzer (LKB Biochrom Ltd., UK) using Merck amino acid standards for calibration. The size of the ion exchange column and the composition of the buffers was as follows:

Column: 500×6 mm;

Resin: CHROMEX UA-8;

Flow rate of buffer: 80 $cm^3\ h^{-1}$;

Flow rate of ninhydrin: 40 $cm^3\ h^{-1}$;

Temperature of the column: 50 °C for 60 min, then 70 °C for another 60 min;

Buffer A: pH = 3.18; Na molarity 0.2; 25 min;

Buffer B: pH = 4.35; Na molarity 0.2; 60 min;

Buffer C: pH = 6.35; Na molarity 1.2; 55 min;

NaOH: 0.4 mol; 15 min;

Equilibration: buffer A; 60 min.

Chromatograms were evaluated by comparing them to the region below the peak of the calibration standard. Results of parallel tests were calculated with a HT PTK-1050 type pocket computer (Híradástechnikai Szövetkezet, Hungary).

2. Results

2.1. Model experiments to determine DAP

Using the buffer composition as suggested by EDOLS (1985) DAP appears in the chromatogram between methionine and iso-leucine. Separation and evaluation is considered optimal as long as the concentration of DAP and the other amino acids is nearly identical. However, when DAP has to be determined not by the standard but from the rumen liquor, the situation is altogether completely different. Because of the presence of methionine and iso-leucine in large amounts sometimes DAP cannot even be determined. Taking into account the above prior to hydrolysis with HCl the samples were oxidized with performic acid, prepared according to HIRS (1956), thereby transforming cystine into cysteic acid and methionine into methionine sulfonium. This transformation freed the place between valine and iso-leucine for DAP and

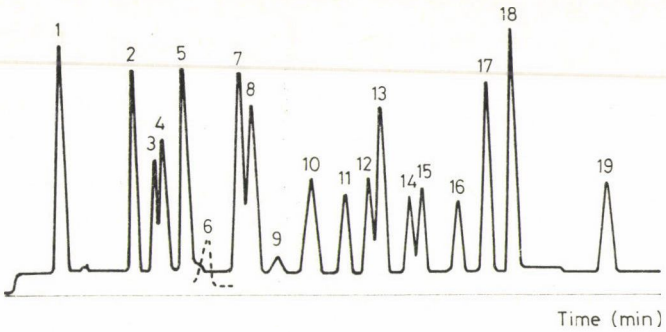

Fig. 1. Chromatogram of the amino acid profile of fish meal. 1: cysteic acid standard; 2: aspartic acid; 3: threonine; 4: serine; 5: glutamic acid; 6: proline; 7: glycine; 8: alanine; 9: cystine; 10: valine; 11: methionine; 12: iso-leucine; 13: leucine; 14: tyrosine; 15: phenylalanine; 16: histidine; 17: lysine; 18: ammonia; 19: arginine

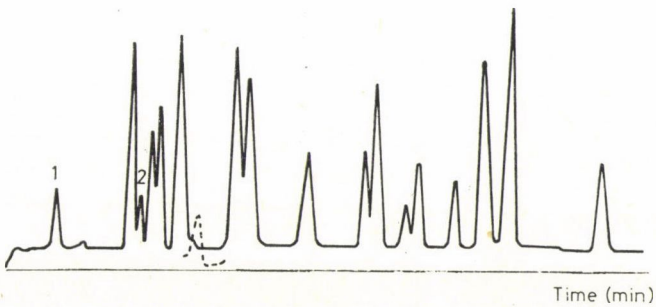

Fig. 2. Chromatogram of the amino acid profile of fish meal oxidized with performic acid. 1: cysteic acid; 2: methionine sulfonium

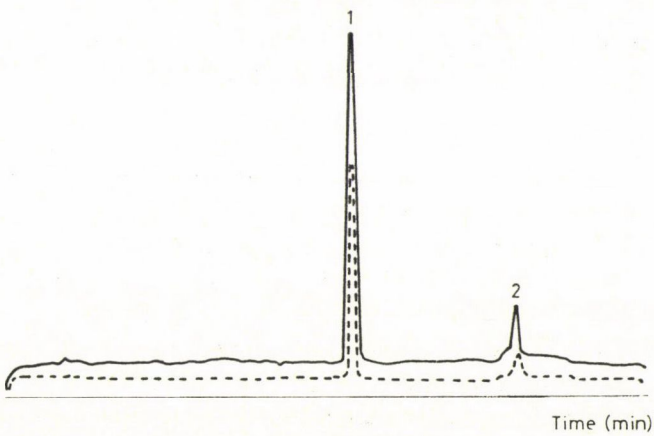

Fig. 3. Chromatogram of the amino acid profile of diaminopimelic acid. 1: diaminopimelic acid; 2: ammonia

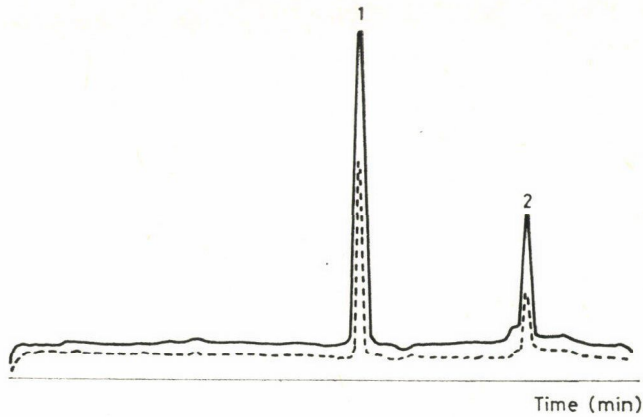

Fig. 4. Chromatogram of the amino acid profile of diaminopimelic acid oxidized with performic acid. 1: diaminopimelic acid; 2: ammonia

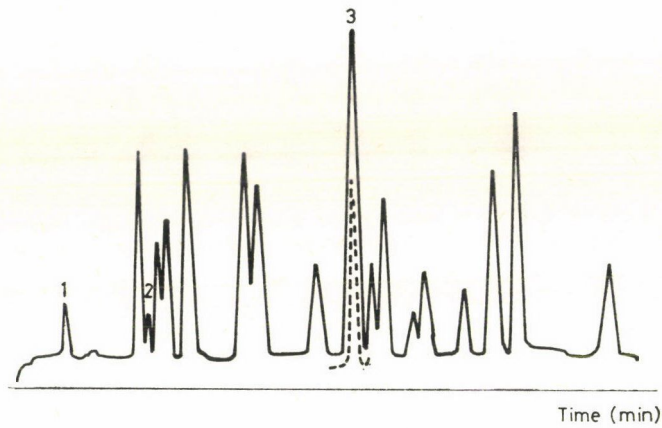

Fig. 5. Chromatogram of the amino acid profile of fish meal with added diaminopimelic acid after oxidation with performic acid. 1: cysteic acid; 2: methionine sulfonium; 3: diaminopimelic acid

by the change of the buffer composition even very small amounts of DAP can be evaluated in the chromatogram beside other amino acids.

The observations mentioned above are well illustrated by the amino acid chromatograms attached. Figure 1 shows the amino acid profile of fish meal, Fig. 2 that of fish meal oxidized with performic acid, Fig. 3 that of DAP, Fig. 4 that of DAP oxidized with performic acid while Fig. 5 that of fish meal with added DAP after oxidation with performic acid. As it can be well seen in the chromatograms DAP is practically not affected by performic acid oxidation (although in the chromatogram of the oxidized sample the amount of ammonia increased) and of the amino acids only the breaking down of tyrosine needs to be reckoned with.

2.2. Determination of DAP in rumen liquor samples prepared in different ways

After development of the analytical method with model materials it was used to determine the DAP composition of the rumen liquor, the sediment of the liquor obtained by centrifuging. Figure 6 shows the composition of the rumen liquor, Fig. 7 that of the sediment of centrifuged rumen liquor. The results calculated from the data in the chromatograms are given in Table 1.

As seen in the Table the centrifuged residue of the rumen liquor contains about 3-times the amount of DAP in the rumen liquor.

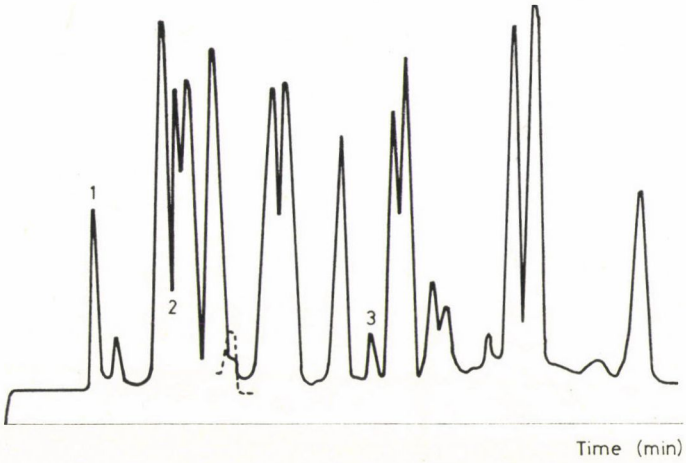

Fig. 6. Chromatogram of the composition of the rumen liquor. 1: cysteic acid; 2: methionine sulfonium; 3: diaminopimelic acid

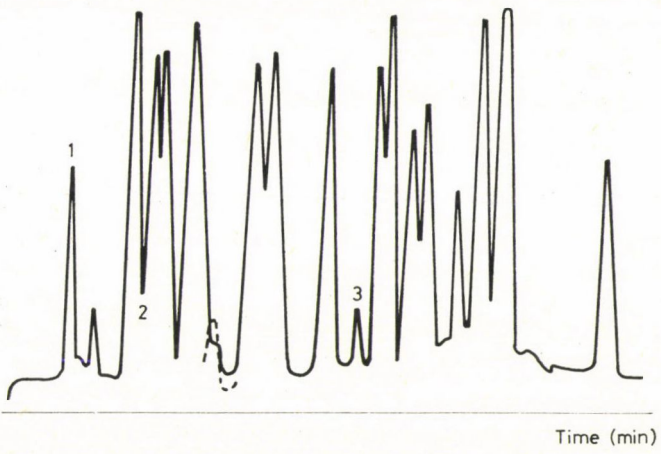

Fig. 7. Chromatogram of the composition of the sediment of centrifuged rumen liquor. 1: cysteic acid; 2: methionine sulfonium; 3: diaminopimelic acid

Table 1

Diaminopimelic acid content of different fractions

Fraction	Diaminopimelic acid content		Raw protein (g per 100 g sample)
	(g DAP per 100 g sample)	(g DAP per 100 g protein)	
Rumen liquor	0.010	0.67	1.50
Centrifuged residue I of rumen liquor	0.034	0.61	5.58
Centrifuged residue II of rumen liquor	0.029	0.73	4.00

3. Conclusions

Of the four literatures cited only that of CZERKAWSKI (1974) contains concrete data on the DAP content of the original rumen liquor. The result found by him (28–30 μg per g) is almost three times lower than that of the present study (0.010 g DAP per 100 g sample). Since the other papers do not contain exact data the data obtained in this study cannot be compared with them.

The chromatograms as well as the results calculated from them show that this method with an accuracy stipulated by amino acid analysis ($\pm 3\%$) and with the sensitivity of amino acid analysis (1–4 nmol) is suitable for the determination of DAP in samples of biological origin. Data in Table 2 prove good reproducibility since the variation coefficient as calculated from 5 parallel measurements is below 2.

Apart from the analytical findings this study permits of other conclusions, too. According to these measurements DAP can be determined directly

Table 2

Parallel measurements of the DAP content of rumen liquor

Serial number of measurement	Diaminopimelic acid content		Raw protein (g per 100 g sample)
	(g DAP per 100 g sample)	(g DAP per 100 g protein)	
1	0.0102	0.68	1.50
2	0.0104	0.69	1.50
3	0.0100	0.67	1.50
4	0.0099	0.66	1.50
5	0.0101	0.67	1.50
Average	0.01012	0.674	
Standard deviation	0.000192	0.0114	
Variation coefficient	1.90	1.69	

from the rumen liquor without any preliminary manipulations. It is not worthwhile to centrifuge the rumen liquor because although increasing the amount of DAP simultaneously the amount of other amino acids is also increased thereby the detectability of DAP is reduced thus it is of no advantage whatsoever from the analytical point of view. However, it should be stressed that DAP can be determined directly from the rumen liquor with the appropriate accuracy.

Since there is not much difference in the DAP content and other amino acid composition of the rumen and the small intestine this method is suitable for the measurement of the amount of DAP getting into the small intestine of ruminants. From the DAP content, on the other hand, the quantity of bacterial protein formed in the rumen can be established with great accuracy and this is of great practical importance to the animal breeder.

Literature

CZERKAWSKI, W. J. (1974): Methods for determining 2-6-diaminopimelic acid and 2-aminoethylphosphonic acid in gut contents. *J. Sci. Fd Agric., 25*, 45–55.

CSAPÓ, J., TÓTH-PÓSFAI, I. & CSAPÓ-KISS, ZS. (1986): Optimization of hydrolysis at determination of amino acid content in food and feed products. *Acta Alimentaria, 15*, 3–21.

EDOLS, R. W. (1985): Simple method for the determination of diaminopimelic acid in rumen liquor hydrolysates. *J. Chromat., 329*, 199–201.

HIRS, C. H. W. (1956): The oxidation of ribonuclease with performic acid. *J. biol. Chem., 219*, 611–621.

HUTTON, K., BAILEY, F. J. & ANNISON, E. F. (1971): Measurement of the bacterial nitrogen entering the duodenum of the ruminant using diaminopimelic acid as a marker. *Br. J. Nutr., 25*, 165–173.

PONGOR, S. & BAINTNER, K. (1980): Quantitative evaluation of ion exchange thin-layer chromatograms by video-densitometry. — Part IV. Determination of diaminopimelic acid. *Acta Biochim. Biophys. Acad. Sci. Hung., 15*, 1–4.

Acta Alimentaria, Vol. 17 (2), pp. 169–175 (1988)

STUDIES OF SOME PHYSICAL AND CHEMICAL CHARACTERISTICS OF AJEEBA WHEAT CULTIVAR AND SOME OF ITS MUTANTS

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(Received: 25 March 1987; accepted: 25 June 1987)

The rheological and baking quality of Ajeeba wheat cultivar and seven of its mutants were examined. All mutants were significantly lower in ash and protein content than that of the control. Elemental content were higher in K, Ca, Mg, Fe, Mn, Na, Zn and Cu for most tested mutants.

Farinograph data showed a long stability time for B13, B117 and B7 mutants indicating that their flours could be used successfully for bread making. These mutants gave higher values for mixing tolerance, falling number and amylograph peak except the falling number value for B7.

The mutants B13, B117 and B7 also showed significantly higher loaf volume than the control with lighter color.

Keywords: Ajeeba wheat cultivar, mutants, rheological and baking quality, chemical characteristics

Our recent breeding efforts have produced wheat mutants from the local common wheat *(Triticum aestivum* L.) var. Ajeeba with improved agronomic characteristics (ALKALISI et al., 1984). Maintenance of nutrient levels and rheological and baking characteristics in newly developed crop varieties with improved agronomic characteristics is one of the factors which contributes to increased agricultural production (SENTI & RIZEK, 1975).

Several studies have related mineral composition in wheat to nutritional value and such factors as variety, location and climate (TOEPFER et al., 1972; ZOOK et al., 1970). LORENZ and LOEWE (1977), who correlated protein with mineral components in commercial wheat blends, found significant correlation between protein and Ca, Fe, K and Cu and between ash and Ca, Mg, Na, K, Mn and Cu.

Flours from various varieties of wheat display great diversity in their dough and baking properties. Therefore the rheological and dough development properties of flour are essential to judge suitability of any wheat variety for bread making and its capability to produce a high quality product (SHELLENBERGER, 1981).

This study reports the milling and baking qualities of Ajeeba wheat cultivar and seven of its promising mutants.

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

1. Materials and methods

1.1. Samples

Wheat cultivar Ajeeba and seven of its mutants were obtained from our plant breeding section. Samples were milled using a Buhler laboratory mill. All flour data are expressed on a 14% moisture basis. All chemicals used were reagent grade. Flour samples were stored in polyethylene bags in a refrigerator at 5 °C for further analysis.

1.2. Flour analysis

For mineral analysis by atomic absorption spectroscopy, 2 g flour samples were ashed at 600 °C temperature then dissolved in 20% HCl. Absorption measurements were made on clear digests by Shimadzu Model AA-670 atomic absorption spectrophotometer. Optimum instrument parameters were adjusted according to manufacturer's instructions. An air–acetylene flame was used for all elements examined. All samples were analyzed in duplicate.

Protein ($N \times 5.7$) was determined by the micro-Kjeldhal method. Ash content and falling numbers were determined by AACC (1983) methods (No. 08-01 and 56-81 B, respectively).

1.3. Physical dough testing

The farinograph was used to determine the physical dough properties of the flours. Farinograms were determined according to AACC (1983) method No. 54-21 using a constant flour weight of 300 g on a 14% moisture basis.

1.4. Amylograph data

Maximum viscosity of the various flour samples was determined with the Brabender Amylograph according to standard AACC (1983) procedure method No. 22-10.

1.5. Wet gluten

A Glutomatic 2200 (Doty Labs, Inc., Kansas City, Mo.) was used to determine wet gluten. Determinations were made in duplicate, using 10.0 g flour and were expressed on a 14% moisture basis.

1.6. Determination of reducing sugars

Reducing sugars were determined by the AACC (1983) method No. 80-60 and were expressed as mg maltose per 10 g flour.

1.7. Baking studies

The flour samples were baked in triplicate using a conventional straight-dough procedure containing 1.5% yeast, 1.5% sugar, 1.5% salt, 2.0% shortening and water as determined by the farinograph. After a 1 h 25 min fermentation period, the doughs were sheeted, hand-molded and proofed for 50 min at 30 °C temperature and 75–85% relative humidity. Baking was carried out for 17–20 min at 230 °C oven temperature. The bread was allowed to cool and the volume was measured by rapeseed displacement. Color values were determined with the Kent-Jones and Martin flour color grader.

Table 1

Ash, protein and elemental contents (on dry basis) for the flours of control Ajeeba wheat cultivar and its mutants[a]

Samples	Ash[b] (%)	Protein[c] (%)	Elemental contents (mg per 100 g)		
			K	Ca	Mg
Control	1.134 *d*	15.451 *f*	121.79 *a*	26.40 *a*	44.53 *a*
B1	0.767 *bc*	13.711 *b*	180.61 *b*	33.40 *b*	61.98 *cd*
B13	0.607 *a*	13.720 *b*	230.16 *c*	38.51 *b*	68.86 *d*
B100	0.752 *bc*	14.035 *d*	190.59 *b*	35.15 *b*	75.08 *e*
B117	0.609 *a*	13.681 *b*	197.90 *b*	33.67 *b*	63.86 *cd*
B7	0.753 *bc*	13.881 *c*	171.12 *b*	33.22 *b*	57.01 *bc*
A70	0.688 *ab*	11.845 *a*	172.65 *b*	33.35 *b*	54.13 *bc*
A 6-1	0.797 *c*	15.012 *e*	126.91 *a*	27.20 *a*	48.80 *ab*

Samples	Elemental contents (mg per 100 g)				
	Fe[d]	Mn[d]	Na	Zn	Cu
Control	2.079	1.205	1.830 *b*	0.959 *ab*	0.332 *b*
B1	2.580	1.490	1.379 *a*	1.502 *cd*	0.333 *b*
B13	4.471	1.720	2.600 *b*	1.766 *de*	0.452 *c*
B100	4.238	1.760	2.500 *b*	1.942 *e*	0.487 *cd*
B117	2.835	1.320	3.700 *c*	1.772 *de*	0.721 *e*
B7	3.207	1.130	3.348 *c*	1.199 *b*	0.164 *a*
A70	4.115	1.490	1.249 *a*	1.238 *bc*	0.574 *d*
A 6-1	2.604	1.325	1.669 *a*	0.778 *a*	0.428 *c*

[a] Values within a column followed by different letters are significantly different at the 0.05 probability level, using least significant differences

[b] Mean value of duplicate determinations

[c] $N \times 5.7$

[d] The mean values do not differ significantly

2. Results and discussion

Ash, protein and mineral contents are summarized in Table 1. All mutants were significantly lower in ash and protein quantities than that of the control. Our results also indicated that protein content of the wheat com-

Table 2

Farinograph, falling number and amylograph peak viscosity data for the flours of control Ajeeba wheat cultivar and its mutants[a]

Sample	Water absorption[b] (%)	Arrival time[c] (min)	Mixing time (min)	Stability (min)	Mixing tolerance index[c] (Bu)[d]	Falling number[b,c] (sec)	Amylograph peak height[b,c] (Bu)[d]
Control	74.4	3.8	5.3	5.5	40	532 *ab*	1160 *c*
B1	73.0	3.3	5.0	6.3	40	534 *b*	1200 *c*
B13	65.3	2.5	6.5	20.0	10	607 *f*	1275 *d*
B100	75.8	2.1	3.3	4.3	60	637 *g*	780 *a*
B117	67.8	1.9	5.5	10.0	45	543 *c*	1500 *e*
B7	70.6	2.2	4.7	10.0	50	530 *a*	1285 *d*
A70	69.0	2.3	3.5	3.9	75	548 *d*	1330 *e*
A 6-1	75.2	2.5	3.7	3.8	58	566 *e*	1045 *b*

[a] Values within a column followed by different letters are significantly different at the 0.05 probability level, using least significant differences
[b] Results expressed on a 14% moisture basis
[c] Mean value of duplicate determinations
[d] Brabender units

Table 3

Quality measurements of control Ajeeba wheat cultivar and its mutants[a]

Samples	Flour yield (%)	1000 kernel weight[b] (g)	Loaf volume[c] (cm^3)	Color volume[c,d]	Wet gluten[b,e] (%)	Maltose value[b,e,f]
Control	79.4	29.2 *b*	740 *b*	8.2 *e*	42.5 *g*	41 *a*
B1	80.9	32.2 *bc*	731 *a*	4.1 *b*	34.8 *c*	51 *c*
B13	74.3	29.8 *ab*	789 *e*	2.4 *b*	36.7 *d*	56 *d*
B100	79.7	29.5 *b*	724 *a*	5.7 *d*	34.8 *c*	57 *d*
B117	76.7	32.0 *bc*	761 *d*	2.9 *a*	33.1 *a*	51 *c*
B7	78.8	33.6 *b*	771 *d*	6.0 *d*	41.2 *f*	46 *b*
A70	82.3	29.5 *a*	746 *c*	4.2 *b*	34.3 *b*	47 *b*
A 6-1	84.2	34.0 *c*	736 *a*	5.3 *c*	40.8 *e*	51 *c*

[a] Values within a column followed by different letters are significantly different at the 0.05 level using least significant differences
[b] Results expressed on a 14% moisture base
[c] Mean value of triplicate determinations
[d] Color value was determined with the Kent-Jones and Martin flour color grader
[e] Mean value of duplicate determinations
[f] In mg of maltose per 10 g of flour

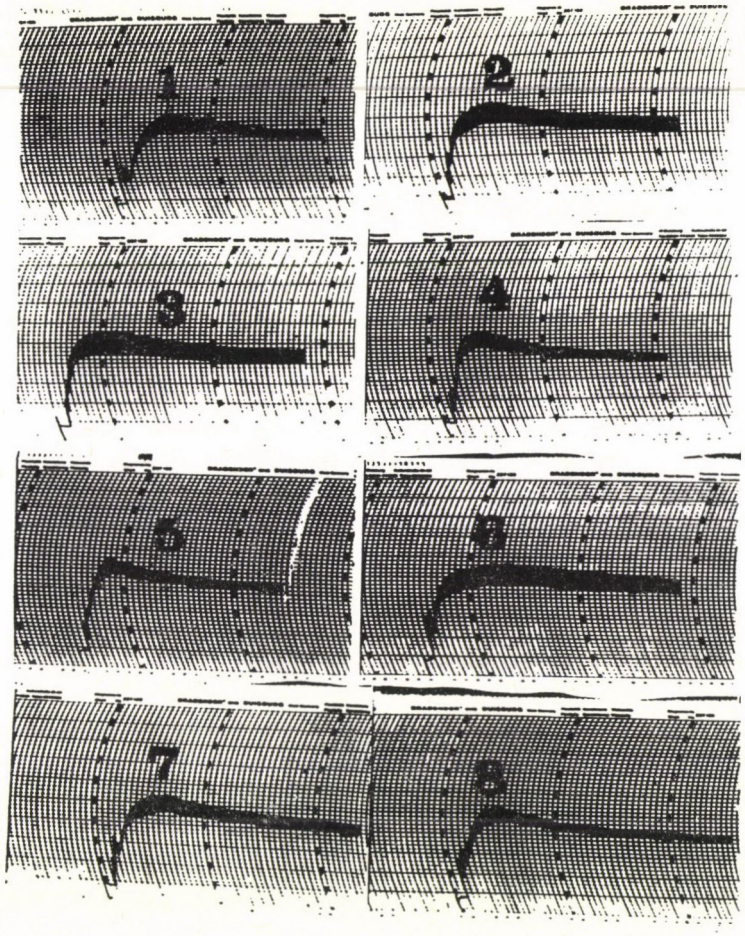

Fig. 1. Farinograph curves of control Ajeeba wheat cultivar and its mutants. 1: control; 2: B7; 3: B117; 4: A70; 5: B1; 6: B13; 7: B100 and 8: A 6-1

posites was not related to ash content. This was in agreement with DIKEMAN and co-workers (1982) and contrary to the findings of MILLER and JOHNSON (1954). On the other hand, elemental contents were higher for K, Ca, Mg, Fe, Mn, Na, Zn and Cu for most tested mutants compared with the control, indicating varietal differences between the cultivars and as was reported by RASMUSSON and co-workers (1971).

Table 2 shows the farinograph data obtained for the control and the mutant flour samples. Farinograph absorption was slightly higher for B100 and A 6-1 and lower for the other mutants. In general arrival time decreased for all mutants while mixing time was variable. The farinograph data also showed a long stability time for B13, B117 and B7. These results indicated

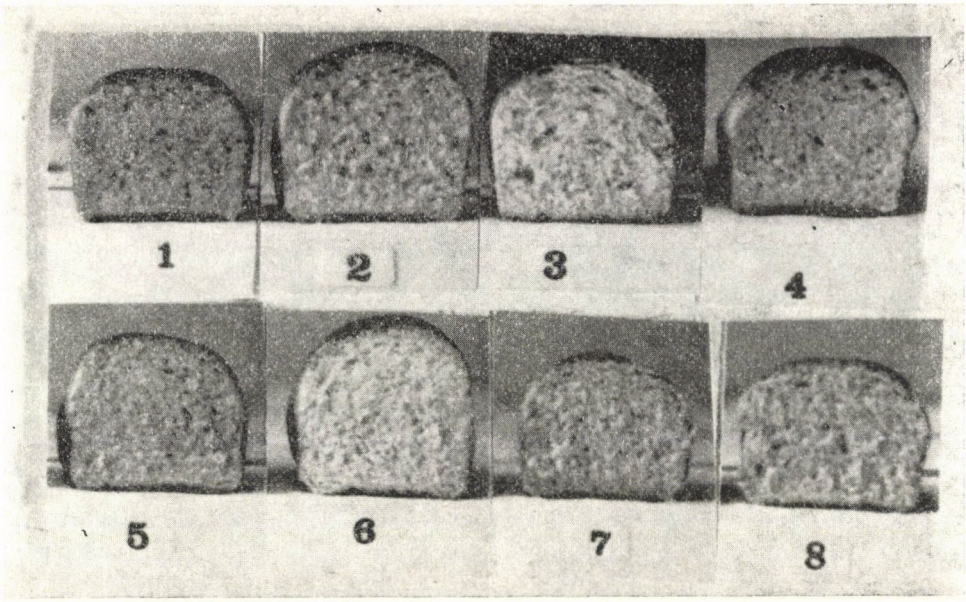

Fig. 2. Internal loaf characteristics of the straight-dough breads. 1: control; 2: B7 3: B117; 4: A70; 5: B1; 6: B13; 7: B100 and 8: A 6-1

that their flours could be used successfully for breadmaking and that they conform well to the classification criteria of wheats for breadmaking (POMERANZ, 1978). The results also compared well with rheological data presented on hard red spring wheat flour (VOLPE & ZABIK, 1981). Mixing tolerance index was also higher for most mutants. The farinograph curves of control and mutants' flours are shown in Fig. 1. The mutants B13, B117 and B7 also gave higher values for falling number and amylograph peak height except the falling number value for B7 indicating a superior breadmaking quality.

Baking quality data for the control flours and those derived from the mutants are shown in Table 3. The mutants B13, B117 and B7 showed significantly higher loaf volume than their control. Figure 2 shows the internal loaf characteristics of the breads made from the dough of the control and the mutants. Crumb color was darker in the control bread than all the breads made from the flours of the mutants. Wet gluten is higher in the control flour than all the mutants. It is generally recognized that gluten strength plays a major role in determining loaf volume potential and bread dough mixing properties (TIPPLES & KILBORN, 1974). Both quality and quantity of the gluten play a major role in the baking performance of wheat flour. It is most likely that gluten quality of the mutants B13, B117 and B7 is higher than that of the control. At the same time maltose value of the control is significantly lower than the values found for all the mutants.

Literature

AACC (1983): *Approved methods of the AACC.* (Method 08-01, approved April 1961; method 56-81 B, approved November 1972; method 54-21, approved April 1961; method 22-10, approved March 1960; method 80-60, approved April 1961.) American Association of Cereal Chemists, St. Paul, Mn.

ALKALISI, F. M. H., AL-JANNABI, K. & JIAWOOK, N. (1984): Induced mutations in a local *Triticum aestivum* L. var. Ajeeba by physical and chemical mutangens. — in: *Proc. 3rd Arab Scientific Conf. of Bio. Sci.* Amman, Jordan, p. 100.

DIKEMAN, E., POMERANZ, Y. & LAI, F. S. (1982): Minerals and protein contents in hard red Winter wheat. *Cereal Chem., 59,* 139–142.

LORENZ, K. & LOEWE, R. (1977): Mineral composition of U.S. and Canadian wheats and wheat blends. *J. agric. Fd Chem., 25,* 806–809.

MILLER, B. S. & JOHNSON, J. A. (1954): A review of methods for determining the quality of wheat and flour breadmaking. *Kans. agric. expl. Stn. tech. Bull.* No. 7.

POMERANZ, Y. (Ed.) (1978): *Wheat chemistry and technology.* American Association of Cereal Chemists, St. Paul, Mn.

RASMUSSON, D. C., HESTER, A. J., FICK, G. N. & BYRNE, I. (1971): Breeding for mineral content in wheat and barley. *Crop Sci., 11,* 623–626.

SENTI, F. R. & RIZEK, R. L. (1975): Nutrient levels in horticultural crops. *HortScience, 10,* 5.

SHELLENBERGER, J. A. (1981): The baking industry and wheat quality evaluation. *Bakers' Dig.,* (June), 22–23.

TIPPLES, K. H. & KILBORN, R. H. (1974): Baking strength index and the relation of protein content to loaf volume. *Can. J. Pl. Sci., 54,* 231–234.

TOEPFER, E. W., POLANSKY, M. M., EHEART, J. F., SLOVER, H. T., MORRIS, E. R., HEPBURN, F. N. & QUACKENBUSH, F. M. (1972): Nutrient composition of selected wheats and wheat products. — Part II. Summary. *Cereal Chem., 49,* 173–186.

VOLPE, T. A. & ZABIK, M. E. (1981): Single-cell protein substitution in bread system: Rheological properties and product quality. *Cereal Chem., 58,* 441–443.

ZOOK, E. G., GREEN, F. E. & MORRIS, E. R. (1970): Nutrient composition of selected wheats and wheat products. — Part 6. Distribution of Mn, Cu, Ni, Zn, Mg, Pb, Sn, Cd, Cr and Se as determined by atomic absorption spectroscopy and colorimetry. *Cereal Chem., 47,* 720–731.

Acta Alimentaria, Vol. 17 (2), pp. 177–182 (1988)

THE DEFINITION OF TWO CATALAN VITICULTURAL REGIONS BY CLASSIFICATION METHODS

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(Received: 24 April 1987; accepted: 13 July 1987)

Several pattern recognition techniques: Statistical Linear Discriminant Analysis (SLDA), K-Nearest Neighbours (KNN), Least-Squares Linear Regression Analysis, Linear Learning Machine (LLM), Soft Independent Modelling of Class Analogy (SIMCA) and Discriminant Analysis Based on Non-Parametric Density Estimation (ALLOC) have been used to classify twenty eight red wine samples into two geographically contiguous zones. The oenological parameters: ethanol content, reducing sugars, conductivity and the sum of malic and lactic acids together with the metal ions magnesium and zinc, have been found to be the most important variables to differentiate the two areas according to three variable selection procedures. In spite of the similarity in varietal, edafologic and cultural factors, the two zones can be conclusively distinguished, therefore climatic and orographic conditions have enough influence to confer to the wines studied their own character. All assayed classification methods have given prediction rates higher than 88% while the non-parametric methods ALLOC, KNN and Least-Squares Linear Regression have provided the maximum percentage of correct prediction (97.0%).

Keywords: wine, oenological parameters, classification methods, metal ions, pattern recognition

In previous papers (TAPIAS et al., 1986, 1987), the suitability of pattern recognition methods to distinguish wines according to their geographic origin, has been shown. These papers confirm that wines from very different origins or elaborated in adjacent areas but with patent varietal differences, are easily recognized. More difficulties arise whenever climatic and orographic conditions have to be the responsible for the major differences existing between adjacent contiguous wine producing areas since a close similarity in varietal, edafologic and cultural factors does exist.

After having visualized, by means of cluster analysis methods, the objective presence of different wines (LARRECHI & RIUS, 1987), the aim of the present paper consists of demonstrating the actual existence of two separated viticultural regions, within only one geographical province, by developing a classification rule which will be able to correctly classify the wine samples produced in this D.O. into one of the defined areas. On the basis of the known viticultural region membership of a set of wine samples, the most relevant variables to characterize each zone are selected. Several feature selection methods are employed and the variables chosen are used to evaluate the performance of different supervised methods of analysis (SHARAF et al., 1986).

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1. Materials and methods

1.1. Source of data

Twenty eight red wines (objects), all from 1983 vintage were collected in two production areas; eleven samples from Camp de Tarragona region (class 1) and seventeen samples from Comarca de Falset region (class 2). All wines were guaranteed by the Spanish D.O. legislation. Although a total number of twenty five physicochemical parameters (variables) were determined (LARRECHI & RIUS, 1987), only those related to the geographical origin of the samples were taken into account; eleven of them named as oenological parameters: density (DEN), pH, ash content (A), alkalinity of the ashes (AA), conductivity (COND), ethanol content (ETOH), reducing sugars (SUG), dry extract (EXT), titratable acidity (T), tartaric acid (HTa) and the sum of malic and lactic acids (HMaLa), all of them determined at the Viticultural, Oenological and Fructicultural station (INCAVI) of Reus, following the procedures recommended by the O.I.V. (1965), except for malic acid which was determined enzimatically (RIBEREAU-GAYON et al., 1976). Seven metal ion concentrations were also considered; calcium, magnesium, strontium, zinc and manganese measured by atomic absorption spectrometry and potassium and lithium determined by flame emission spectrometry by employing an Instrumentation Laboratory AA/AE 551 spectrophotometer.

1.2. Pattern recognition methods

The supervised methods of analysis: Statistical Linear Discriminant Analysis (SLDA) from the SPSSx package (ANON., 1981b). K-Nearest Neighbours (KNN), Linear Learning Machine (LLM), Soft Independent Modelling of Class Analogy (SIMCA) and Least-Squares Linear Regression analysis from the well-known pattern recognition package ARTHUR (ANON., 1981a) and the use of Discriminant Analysis based on Non-Parametric Density Estimation (ALLOC) from ALLOC80 package (HERMANS et al., 1982) permitted, after some preprocessing and the most convenient selection of variables, the assignement of the wine samples into one of the previously established groups. All calculations were carried out on an IBM 3083 computer at the Informatics Centre of the University of Barcelona.

2. Results and discussion

Before computing a classification rule which would classify the objects into one of the previously defined classes, some preprocessing were considered necessary: (i) as parametric methods of analysis have been used, the normal

distribution of the variables in each class has been tested by applying subroutine CONDESCRIPTIVE from SPSSx. Only one too high value of kurtosis for variable Zn (17.00) was detected in the Comarca de Falset class. A careful revision of the data showed the presence of an atypical value for this variable in object 2097. The substitution of this figure by a missing value gave rise to a new kurtosis value (0.52) which fell into the usual range found (1.40).

The second preprocessing step (ii) consisted of autoscaling the variables (KOWALSKI & BENDER, 1972) in order to minimize the effect of the different magnitudes employed (iii). Several reasons have already been given to reduce the number of austoscaled variables before deriving the classification functions (VAN DER VOET et al., 1984). Following them, three feature selection procedures were applied. Subprogram DISCRIMINANT from SPSSx contains five feature reduction procedures based on different criterions (ANON., 1981b) and all of them gave Mg, EtOH, SUG, COND and HMaLa as the best set of variables with which a 95.5% of correct classification was obtained when SLDA was applied, i.e. only one out of 11 wines from class 1 was assigned incorrectly while all 17 wines from class 2 were correctly classified. A similar result was obtained when applying SELECT procedure from ARTHUR; both variance weight and Fisher weight gave the same optimum set: EtOH, COND, Zn, HMaLa and SUG when taking the KNN rule as the method to evaluate their performance. The maximum classification rates obtained were 1 NN = 92.9%, 3 NN = 89.3% and 4 NN = 85.7%. Finally, a selection was undertaken by using ALLOC80 program and following COOMANS and co-workers (1981) methodology. The best set in the latter case was confirmed by seven variables; Mg, Mn, Zn, EtOH, SUG, TA and DEN, which gave rise to the same maximum classification score (97.0%) obtained with other procedures.

The classification ability, resulting from the assignation of the same objects which had been used to derive the classification functions are reported in Table 1. It is known that these results give an optimistic evaluation of the actual classification power of the derived functions (MASSART et al., 1978) but they allow the selection of the best set of variables for each classification method. It has already been claimed (SJOSTROM & KOWALSKI, 1979) that the variables selected by DISCRIMINANT are the best set to classify objects according to SLDA. With the present data, also the heuristic selection procedure of ALLOC80 provided the group of variables which best form with ALLOC classification method and SELECT gave the most suitable set according to the KNN classification rule as can be observed in Table 1. It can also be noticed that the best classification results with LLM, SIMCA or Least-Squares Linear Regression Analysis are achieved with the variables selected by DISCRIMINANT.

The best combination of variables and classification methods were used to obtain the actual classification power or prediction ability (MASSART et al.,

Table 1

Classification results

Feature selection procedures	Classification methods						
	SLDA	ALLOC	KNN		LLM	Least squares	SIMCA
			1 NN	3 NN			
DISCRIMINANT	95.5	95.5	92.5	95.5	100	95.5	88.2
SELECT		74.3	92.5	95.5	100	95.5	52.9
Heuristic ALLOC 80		97.0	97.0	97.0	100	89.6	52.9

1978) which was determined in all cases except for ALLOC by applying the leave-one-out procedure (VARMUZA, 1980). All results are displayed in Table 2. SLDA is the easiest visualizable method since the two classification functions, one for each class, can be assimilated to orthogonal axes describing a two dimensional space where the wines, considered as points, can be represented as in Fig. 1. It can be observed that no straight line exists which divides the plane in two regions each of them including all wines from a single class, therefore always one out of 28 wines is missclassified. These results agree with those obtained by the LLM method in which no hyperplane was capable to fully separate the two considered classes. The SIMCA method used, implemented in five subroutines of the ARTHUR package, has given the lowest classification results. In spite of employing the best number of principal components according to the crossvalidation, subprogram SICSVA, it is recognized that much better results can be achieved with the improved version of the SIMCA package (ANON, 1983).

Table 2

Correctly predicted wines

Classification method		Camp de Tarragona (n = 11)	Comarca de Falset (n = 17)
SLDA[a]		10	17
ALLOC[b]		11	16
KNN[c]	1 NN	11	16
	3 NN	11	16
	5 NN	10	17
Least-Squares[a]		11	16
LLM[a]		10	15
SIMCA[a]		11	13

[a] DISCRIMINANT feature selection; [b] Heuristic ALLOC feature selection; [c] SELECT feature selection

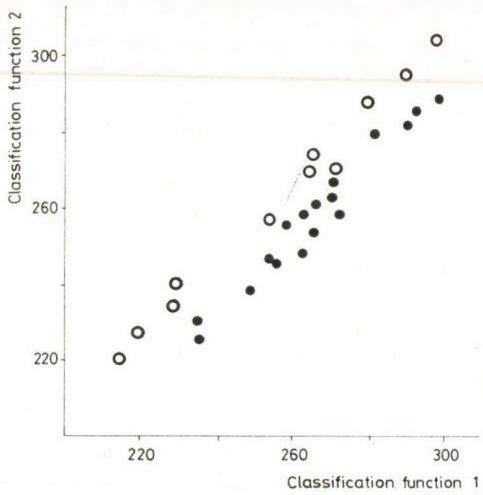

Fig. 1. Distribution of wine samples in the bidimensional space defined by the classification functions derived by the SLDA method. ●: Comarca de Falset wines; ○: Camp de Tarragona wines

To conclude, it can be stated that a clear differentiation of the two viticultural regions studied has been confirmed. Oenological variables as ethanol content, reducing sugars, conductivity, the sum of malic and lactic acids and metal ions as zinc or magnesium play an important role in the discrimination between the two areas. In spite of the similarities due to the geographic neighbourhood, all assayed methods gave conclusive results of the zone differentiation, however, none of them were able to attain a 100% of prediction ability. Many of them erroneously classify only one wine, being this object a different one in each method, assessing in this way the absence of outliers, i.e. wines with peculiar characteristics which diffeentiate them from all other wines in their class, observed in the cluster analysis study. Although relatively small groups of samples have been computed, SLDA, a parametric method has provided the same results as the non-parametric methods ALLOC, KNN or Least-Squares Linear Regression. Nevertheless the three later ones seem more appropriate in the present study due to their independence on statistic parameters of the classes. More definitive conclusions will be drawn by taking into account different vintages, which would contemplate the variability introduced by the climatic conditions along several years. These studies are in progress in our laboratory.

*

The authors wish to thank Estació de Viticultura, Enologia i Fruticultura (INCAVI) de Reus for the oenological analyses of the samples and the CAICYT for financial support given (Project No. 2838/83).

Literature

ANON. (1981a): ARTHUR 81 User's manual. Package distributed by Infometrix Inc. PO Box 25808, Seattle, WA 98125, USA, pp. 21–112.

ANON. (1981b): SPSSx User's manual. Package distributed by SPSS Inc. Suite 3300, 444 North Michigan Avenue, Chicago IL60611, USA, pp. 623–647.

ANON. (1983): AIMCA 3B manual. Package distributed by Sepanova AB Ostrandsv 14, S-122 43 Enskede, Sweden, pp. 101–525.

COOMANS, D., DERDE, M., MASSART, D. L. & BROECKAERT, I. (1981): Potential methods in pattern recognition. — Part 3. Feature selection with ALLOC. *Analytica chim. Acta, 133,* 241–250.

HERMANS, J., HABBEMA, J. D., KASANMOENTALIB, T. K. & RAATGEVER, J. W. (1982): *ALLOC 80 discriminant analysis program.* Department of Medical Statistics, University of Leiden. The Netherlands, pp. II. 1–II. 33.

KOWALSKI, B. R. & BENDER, G. F. (1972): Pattern recognition. A powerful approach to interpreting chemical data. *J. Am. chem. Soc., 94,* 5632–5639.

LARRECHI, M. S. & RIUS, F. X. (1987): Multivariate data analysis applied to the definition of two Catalan viticultural regions. *Z. Lebensmittelunters. u. Forsch. 185,* 181–184.

MASSART, D. L., DIJKSTRA, A. & KAUFMAN, L. (1978): *Evaluation and optimization of laboratory methods and analytical procedures.* Elsevier, Amsterdam, pp. 409–428.

O.I.V. (1965): *Recueil des methodes internationales d'analyses des vins.* Office International de la Vigne et du Vin. Paris, pp. 31–275.

RIBEREAU-GAYON, J., PEYNAUD, E., SUDRAUD, P. & RIBEREAU-GAYON, P. (1976): *Traité d'oenologie, sciences et techniques du vin.* Bordas, Paris, Vol. 1, pp. 179–181.

SHARAF, M. A., ILLMAN, D. L. & KOWALSKI, B. R. (1986): *Chemometrics.* John Wiley & Sons, New York, pp. 228–242.

SJOSTROM, M. & KOWALSKI, B. R. (1979): A comparison of five pattern recognition methods based in the classification results from six real data bases. *Analytica chim. Acta, 112,* 11–22.

TAPIAS, R. M., LARRECHI, M. S., GUASCH, J., RUBIO, J. & RIUS, F. X. (1986): Enological parameters and pattern recognition methods in the geographic differentiation of Spanish red wines. *Am. J. Enol. Vitic., 37,* 195–201.

TAPIAS, R. M., CALLAO, P., LARRECHI, M. S., GUASCH, J. & RIUS, F. X. (1987): Application de l'analyse multidimensionelle des donnes a la reconnaissance des vins rouges de la Rioja. *Connaissance Vigne Vin, 21,* 43–57.

VAN DER VOET, H., DOORNBOS, D. A., MEEMS, M. & VAN DE HAAR, G. (1984): The use of pattern recognition techniques in chemical differentiation between Bordeaux and Bourgogne wines. *Analytica chim. Acta, 159,* 159–171.

VARMUZA, K. (1980): *Pattern recognition in chemistry.* Springer Verlag, Berlin, pp. 8–10.

Acta Alimentaria, Vol. 17 (2), pp. 183–192 (1988)

EFFECT OF LOW RESIDUAL O_2 AND CO_2 ON THE PRESERVATION OF CO_2- AND N_2-PACKED SPONGE-CAKE

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(Received: 24 April 1987; accepted: 13 July 1987)

Preservation of sponge-cake in CO_2/N_2 atmospheres was studied, along with the choice of an appropriate packaging material. The effect of storage duration, temperature, residual oxygen on mold growth, was investigated. Much more than the ratio of CO_2 in the athmosphere, a low concentration of residual oxygen was found preponderant in growth inhibition. The effect of CO_2 on water activity (a_w) of preserved sponge-cake was studied. It was found that an important decrease in a_w of thin slices of cake may be observed while it is very slowly decreased in thick samples. The decrease of a_w could be an important component of CO_2 action in CO_2 preserved baked goods.

Keywords: sponge-cake, CO_2 preservation, water activity (a_w) mold growth

The use of carbonic anhydride in order to increase shelf-life of packaged foodstuffs is well documented. For high water activity foodstuffs like meat, the combination of the actions of low temperatures and CO_2 proves to be the most efficient way to preserve sensorial quality while increasing shelf-life and freshness for several weeks. When the gas is produced naturally as in vegetable respiration or the ripening of certain kinds of cheeses, the ratio of CO_2 in the atmosphere surrounding the product may be controlled in order to control the preservation conditions.

However, although controlled atmospheres and CO_2 packaging technologies are mastered, the mechanism of action of carbonic anhydride on the product, or on the micro-organisms, is not always elucidated. It happens that a special phenomenon such as the "package snugging" or "pack collapse" leads the user to pose questions about the adsorption of CO_2 by one or another phase of the product or on the permeability of the packaging material. Among the components of food products, water is preponderant and essential to the development of micro-organisms. An interpretation of the mechanism of action of CO_2 during the storage of CO_2 packaged foodstuffs could be based on the structural and physico-chemical properties of simple aqueous solutions (salt, sugar, proteins, etc.) in the presence of CO_2.

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The chemistry of CO_2 in water, under its relationship to carbonate and bicarbonate ions, is well understood by chemists, probably because of the problems posed by water treatment and the importance of such ions in geology. Food industries have utilized CO_2 as a preservative for more than one century (KOLBE, 1882), but no systematic study has been devoted to the food–CO_2 interactions. These are essentially due to the structural characteristics of CO_2. Indeed, in this linear molecule the order of the C=O bond is approximately 2.5 instead of 2 (PIMENTEL & SPRATLEY, 1971) and two charged structures $^{-}O{\equiv}C\text{-}O^{+}$ and $^{+}O\text{-}C{\equiv}O^{-}$ exist which may interact with food constituents.

Dissolved CO_2 in water is partially hydrated. The degree of hydration is ill defined (ROQUES, 1964). It is noted CO_2, nH_2O. The nature of hydration is of the clathrate-type (JEFFREY, 1969), induced by the hydrophobic character of CO_2. When the solubility of CO_2 in water is considered, a number of reactions take place which differ in their kinetics and their influences on water structure. First, the hydration of CO_2 occurs without any direct bond between water and carbon dioxide. The water molecules are repelled by the solute and this induces an increase in hydrogen bond strength of the solvent. The entropy of the solution is decreased (KING, 1953). However after a few seconds, ionization reactions take place. These reactions are not instantaneous, which may be demonstrated easily by addition of saturated aqueous CO_2 on the one hand and dilute acetic acid on the other to solutions of dilute NaOH containing phenolphthalein indicator. The acetic acid neutralization is instantaneous whereas with the CO_2 neutralization it takes several seconds for the color to fade. The effect of ions on the dissolution of CO_2 in water depends on their hydration (BURMAKINA et al., 1982). The salting out effect does not follow Setchenov's equation when the concentration is varied. Carbon dioxide was also found (MITSUDA et al., 1975) to be adsorbed by proteins and amino-acids. The rate of adsorption depends on moisture content. A large proportion (90%) of gas adsorbed by the proteins is desorbed in low CO_2 concentration medium, while the adsorption by amino-acids (free bases) and histamine is not reversible.

Some aspects of CO_2 adsorption by proteins are similar to the adsorption and attack of molecular oxygen of certain molecular groups to give free radicals. This takes place when the level of moisture is less than a monolayer. In the case of CO_2, adsorption is favoured by the presence of hydrophobic cavities in the structure of proteins. It was also shown (PAUCHARD et al., 1980) that CO_2 has a higher affinity for fats than for water. Among fats, unsaturated fatty acids dissolve more CO_2 than saturated acids. It seems that the hydrophobic character of CO_2 together with its lipophilic affinity are at the origin of this high solubility of CO_2 in fats. Interactions of CO_2 with water, proteins and fats in simple model systems may help in the elucidation of its role in the inhibition of microorganisms during the storage of CO_2-packed sponge-cake. In all cases of CO_2 packing of foods, the efficiency is connected with a low

ratio of O_2. It is the case for meat which should be maintained at a temperature of -1 °C to $+2$ °C and an atmosphere composed of 15% CO_2, the rest being N_2 or rare gases and less than 0.1% O_2 (WINTER, 1978). For bread slices preserved under CO_2 the ratio of O_2 may vary during the time in the same package but it has to be maintained below 3% (BRUEMMER et al., 1980). Our objective is to study the preservation of the cake in a gastight package in the presence of different ratios of CO_2, a minimized residual O_2 at different temperatures.

1. Materials and methods

The industrial sponge cake investigated was prepared by S.B.P. Dijon from a mixture composed of sucrose, wheat flour, baking powder and E 471 and E 472 emulsifiers. A quantity of whole egg equal to the quantity of the mixture was added. After mixing the ingredients, the cake was cooked for 20 minutes at 180 °C temperature.

Packaging films were supplied by Rhône-Poulenc-Films. Two opaque films (Clarylene^R F and Clarylene^R HB) and a transparent one (Terthene^R DX 24) were used. Terthene is a complex of polymers composed of PET or coated PET and polyolefins. Clarylene HB is a high barrier complex with reinforced impermeability composed of metallized PET (PET M HB) and polyolefins. Clarylene F is a combination of metallized PET and polyolefins.

The parameters controlled during the sponge-cake preservation study were: water activity (a_w), residual oxygen in the atmosphere surrounding the sample and the fungal flora growing at the surface of the product. The a_w was measured with Novasina electric hygrometer at 20 °C. The fungal flora of sponge-cake was left to develop on cake samples at an a_w close to 1.0. After 10 days, the samples were covered with molds, which were isolated on potato agar at pH 5 and identified according to classical methods (MOREAU, 1980). Analysis of residual oxygen was performed with a polarographic probe (Clark electrode) attached to ARELCO, TAI 340 FBS analyzer. Analysis of the solubility of CO_2 in saturated aqueous solutions at different temperatures was achieved by a titrimetric method after precipitation as barium carbonate (JACOBS, 1960).

2. Results and discussion

2.1. Permeability of films

The permeability characteristics of the packaging films are reported in Fig. 1. Terthene is transparent over the whole range of wavelengths while Clarylene F and Clarylene HB are not transparent.

2.2. Microbiological results

The principal fungal species identified on the surface of the sponge cake were: *Aspergillus niger*, *Aspergillus chevalierii*, *Penicillium expansum* and *Penicillium implicatum*. This cake was also very often contaminated by yeasts especially *Saccharomyces cerevisiae*. The four mold species were grown on potato agar in plates packaged in each of the three films (Terthene, Clarylene F and Clarylene HB). No difference was observed in presence (Terthene) or absence (Clarylenes) of light.

2.2.1. Effect of CO_2 on yeast growth. Plates were placed in Terthene films to allow direct observation of microbiological results through the transparent package. Filling the pouches with CO_2–N_2 mixtures and sweeping away the air took at least 10 min per pouch to try to insure that no oxygen was left. As expected, yeast growth was not affected by the absence of air and the rate of growth was almost the same in air and in CO_2–N_2 atmospheres. Yeasts are, of course, mostly aerobic-anaerobic facultative microorganisms and can develop

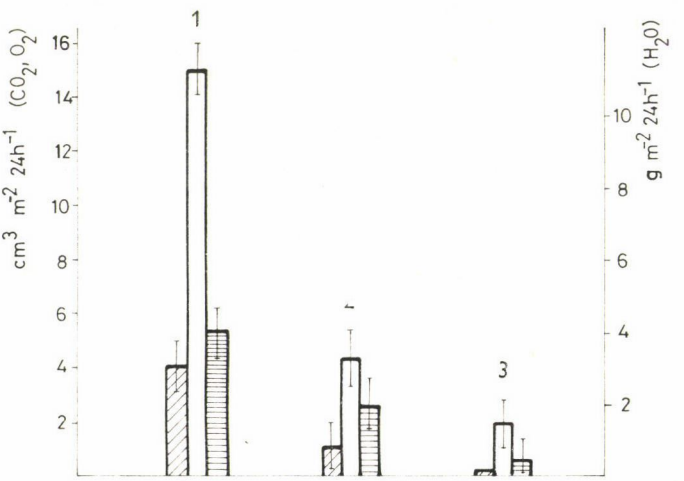

Fig. 1. Permeability of Terthene (1), Clarylene F (2) and Clarylene HB (3) to O_2 (▨), CO_2 (□) and water vapour (▤) at 21 °C temperature

in air with an oxidative (respiration) metabolism and in CO_2 with a fermentative metabolism.

2.2.2. Mold growth in CO_2/N_2 modified atmospheres. Aspergillus niger growth was observed at 20 °C in 3 different atmospheres: air, 15% CO_2–85% N_2, 85% CO_2–15% N_2. The results are given in Table 1.

Table 1

Mold (Aspergillus niger) *growth in covered dilution plates with different atmospheres*

	Air	15% CO_2[a]	85% CO_2[a]
1 week	++	±	0
3 weeks	+++	±	0

±: a white spot of mycelium on the plate; ++ and +++: spore colonies of increasing size; 0: total inhibition
[a] containing about 4% residual O_2

Analysis of residual oxygen showed that the level was, in fact, relatively high (4%). This was probably due to the cover of the dilution plate. Indeed, even with a prolonged sweeping of CO_2–N_2 mixture, residual air was not removed between the cover and the plate. Another experiment was therefore carried out under the same conditions as previously, the dilution plates containing agar being placed in the package without their covers. This resulted in a residual amount of oxygen concentration of approximately 1%.

Observation of the growth of *Aspergillus niger* at 20 °C showed that, under these conditions, after 3 weeks, total inhibition was obtained with 15% as well as with 85% CO_2. Normal growth was observed in air. This indicates that the principal factor in the inhibition of mold growth was the low oxygen level (about 1%) in the atmosphere. However, a high rate of CO_2 (85%) seems to have its own inhibition effect (Table 1). Interpretation of the effect of high amounts of CO_2 on the inhibition of growth is difficult. It is possible that a synergistic effect due to high CO_2 and low O_2 concentrations in the atmosphere contributes to the inhibition (ENFORS & MOLIN, 1980) of mold growth. It is known that a small amount of O_2 is sufficient to initiate vegetative growth. Lack of oxygen and a high amount of CO_2 prevents spore development, so that only mycelium is observed (Table 1).

2.2.3. Mold growth in N_2 atmosphere. Aspergillus niger was grown at 20 °C on dilution plates without cover in gastight Terthene pouches filled with air, 10% CO_2–90% N_2 and 100% N_2, respectively. Results of mold growth after 3 weeks showed that growth was normal in air and inhibited in the 10% CO_2 and 100% N_2 atmospheres. Residual oxygen amounts were 1 to 1.5%.

Table 2

A. niger *growth after 3 weeks at 4 °C and different atmospheres*

Atmosphere	Temperature (°C)	Residual O_2 (%)	Time of incubation (week)	Observed growth
Air	20	—	1	++
			3	+++
15% CO_2–85% N_2	20	4	1	±
			3	±
85% CO_2–15% N_2	20	4	1	0
			3	0
10% CO_2–90% N_2	4	1	3	0
15% CO_2–85% N_2	4	1	3	0
85% CO_2–15% N_2	4	1	3	0
100% N_2	4	1	3	0

For legend see Table 1

These results show that the lack of oxygen has a preponderant role in the inhibition of mold growth. Indeed, inhibition was also obtained with 100% N_2 in the presence of only 1% O_2. However, nitrogen is not efficient in the presence of bacteria, whereas CO_2 has an inhibiting effect (OORAIKUL, 1982; HUFFMAN, 1974).

2.2.4. Effect of low temperature. The preceding experiments on the growth of *Aspergillus niger* in air, and different amounts of CO_2 in CO_2–N_2 mixtures

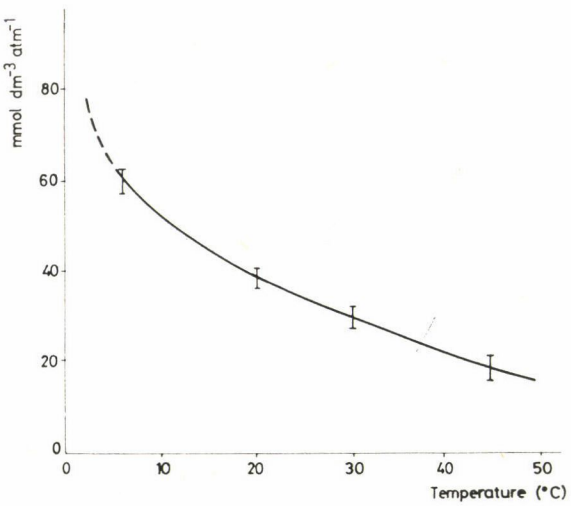

Fig. 2. Variation of the solubility of CO_2 in water as a function of temperature

were repeated at 4 °C. The dilution plates were covered in a first experiment and gave the results listed in Table 2.

With the cover on the plate, the remaining oxygen in the atmosphere analysed after 3 weeks was 4%. The effect of lowering the temperature was to slow down growth. The effect of low temperature with a high amount of CO_2 leads to total inhibition of *A. niger* growth even in the presence of 4% oxygen. This is probably due to the use of two preservation agents: cold and CO_2. On the other hand, solubility of CO_2 gas in the medium increases as temperature is decreased (Fig. 2). Its effect when dissolved in the aqueous medium is higher probably because of pH lowering (WANG & BROWN, 1983). The effects of confined atmospheres and low temperatures were investigated on *A. niger* and *P. expansum* strains. Cultures were made in non covered dilution-plates placed in gastight Terthene pouches filled with air and propagated at different CO_2/N_2 ratios. Results are given in Table 2 for the growth after 3 weeks at 4 °C.

These results clearly show that the efficiency of modified atmospheres (other than air) is improved at low temperature. However, a low O_2 rate (1.5%) is required to ensure a total absence of mold growth.

2.3. Preservation results

2.3.1. Package permeability to O_2. Sponge-cake slices were packed in pouches each made of the three films (Terthene, Clarylene F and Clarylene HB) filled with air, 15% CO_2–85% N_2 and 50% CO_2–50% N_2 mixtures, respectively. After 10 weeks of storage at laboratory temperature ($\simeq$20 °C), analysis of oxygen in the different pouches was made. It was found that only Clarylene HB was completely impermeable to oxygen. The amount of O_2 was 1 to 1.5% and did not change during the 10 weeks. Clarylene F is slightly more permeable to oxygen. At the end of the 10 weeks O_2 increased to 4%. Terthene, the most permeable film to oxygen had 6% residual O_2 at the end of the storage period. Cake preservation was continued with the more efficient film Clarylene HB, while it was more convenient to use the transparent Terthene film for the microbiological growth study.

2.3.2. Effect of CO_2 on water activity of sponge-cake. Two sets of experiments were undertaken. In the first, very thin lamellas of cake (5 mm thick) were conditioned in Clarylene pouches with different atmospheres: air, 15% CO_2–85% N_2, 50% CO_2–50% N_2 and 85% CO_2–15% N_2.

The second set of experiments dealt with 30 mm thick slices of sponge-cake conditioned in the same gas mixtures. The water activity of the cakes before conditioning was equal, at 0.91. It was measured after several weeks of storage at 20 °C. Results are given in Table 3 for 5 mm thick lamellas and Table 4 for sponge-cake slices.

Table 3

Changes in water activity of sponge-cake lamellas stored at 20 °C in different time and atmospheres

Atmosphere	Storage time		
	2 weeks	3 weeks	8 weeks
Air	0.89 ± 0.01 (3)	0.87 ± 0.01 (3)	0.81 ± 0.01 (3)
15% CO_2—85% N_2	0.86 ± 0.01 (4)	0.84 ± 0.01 (4)	0.76 ± 0.015 (4)
50% CO_2—50% N_2	0.86 ± 0.01 (4)	0.78 ± 0.015 (4)	0.79 ± 0.015 (4)
85% CO_2—15% N_2	0.82 ± 0.01 (4)	0.75 ± 0.015 (4)	0.73 ± 0.015 (4)

The numbers of measurements are in brackets

Table 4

Changes in water activity of sponge-cake slices stored at 20 °C in different time and atmospheres

Atmosphere	Storage time		
	2 weeks	3 weeks	8 weeks
Air	0.89 ± 0.01 (4)	0.88 ± 0.01 (4)	0.88 ± 0.01 (4)
15% CO_2—85% N_2	0.88 ± 0.01 (4)	0.88 ± 0.01 (4)	0.87 ± 0.01 (4)
50% CO_2—50% N_2	0.88 ± 0.01 (4)	0.87 ± 0.01 (4)	0.87 ± 0.01 (4)
85% CO_2—15% N_2	0.89 ± 0.01 (4)	0.88 ± 0.01 (4)	0.87 ± 0.01 (4)

The numbers of measurements are in brackets

It may be observed that the a_w decreased during storage of 5 mm thick samples of sponge-cake. Decrease in a_w seemed to be more important as the ratio of CO_2 was increased. The samples in air were covered with mold after the second week of storage. No mold was observed in the other pouches with different ratios of CO_2, the residual amount of oxygen being 1.5 to 2%. All sponge-cake lamellas with a_w less than 0.80 were crisp and lost the softness generally desired in such a baked good. The objective of preventing mold growth was achieved but the intrinsic properties of the sponge-cake were modified. The experiment on thin lamellas permitted rapid observation of water vapour diffusion from the sample to the surrounding atmosphere. The general use of modified atmospheres concerns thick slices of product or very often the whole baked goods. In order to minimize the effect of the size of the sample on a_w equilibration and work in conditions close to industrial practice, the same experiment was conducted with 5 cm thick slices occupying approximately 80% of the 15×15 cm pouches. Changes of a_w during storage is given in Table 4.

The a_w decrease is clearly much less when the size of sample and the ratio of its volume to gas volume is increased. In such conditions water vapour

diffusion is slower. Moreover, the relatively long ($\simeq$10 minutes) sweeping of dry CO_2 gas mixtures acts as a drying process that is particularly efficient in the case of thin lamella. In this experiment, as in the previous one, only samples stored in the air atmosphere were moldy. In the other pouches the rate of residual O_2 was 1.5%. The aspect of the thick slices and their softness did not change except for the slices stored for 8 weeks which showed white areas in the crumb probably due to starch retrogradation, known as the staling of baked goods.

3. Conclusions

The investigated product was an industrial sponge-cake commonly used as a raw material for different preparations. Its water activity was relatively high ($a_w = 0.91$) as no humectants were added to its formula. Improving the shelf-life of such a product could be useful in choosing the preservation conditions for other baked goods with lower water activity. Among the packaging films investigated the less impermeable to water vapour, Clarylene HB (see Fig. 1) was found to give the better results. Although microbiological counts were made in transparent Terthene film, without opening the pouch, in sponge-cake preservation experiments Clarylene HB was used. The microbiological results showed that a low rate of oxygen (about 1%) is essential to inhibit mold growth. This condition is more important than the presence or absence of CO_2 in the atmosphere. The second conclusion to be noticed is the inhibiting effect of lowering the temperature of storage. Although mold growth may be inhibited in a 100% nitrogen atmosphere, it should be recalled that CO_2 has a specific inhibitory effect on bacteria (OORAIKUL, 1982).

The mechanism of action of CO_2–N_2 atmospheres on the stability of sponge-cake during a 2 months storage may find an explanation in the modification of water activity. The decrease of a_w (Table 3) in case of the storage of thin (5 mm) slices of cake was very important (from 0.91 to 0.73) and is probably fundamental to the increase in shelf-life of the product. Although thin slices of cake did not mold, the taste characteristics of fresh samples were not preserved. The slices were dehydrated due to a long time of CO_2–N_2 sweeping and because the volume of gas in the pouch was important relatively to that of the cake. Part of the moisture of the sponge-cake diffused to equilibrate with the surrounding volume of gas. As a_w decrease is due to the diffusion of water out of the cake, it obeys Fick's law and depends on the surface and thickness of the sample. That is why the decrease in a_w of thick samples is much less important. Such an a_w (0.88–0.87) at the end of storage is compatible with sensory properties of sponge-cake like softness. The only observed defect is staling which could be canceled out by adding humectants to the formula of the product. Preservation of intermediate moisture foods of the

sponge-cake type could be achieved by combining the effects of low temperature (4 °C), modified atmospheres (50% CO_2–50% N_2, less than 1.5% O_2) and depressing the a_w with humectant additives.

*

The authors thank the Society "S.I.A.C." for supplying the gas and for financial support to P.G.

Literature

BRUEMMER, J. M., STEPHAN, H. & MORGENSTERN, H. (1980): Possibilities of use of CO_2. *Verpack. Rdsch., 31*, 21–27.

BURMAKINA, G. V., EFANOV, L. N. & SHNET, M. A. (1982): Solubility of CO_2 in aqueous solutions of electrolytes and of sucrose. *Russ. J. phys. Chem., 56*, 705–707.

ENFORS, S. O. & MOLIN, G. (1980): Effect of high concentrations of CO_2 on growth rate of *Pseudomonas fragi, Bacillus cereus, Streptococcus Cremoris. J. appl. Bact., 48*, 409–416.

HUFFMAN, D. L. (1974): Effect of gas atmospheres on microbial quality of pork. *J. Fd Sci., 39*, 723–725.

JACOBS, M. B. (1960): *The chemical analysis of air pollutants*. Interscience Pub., New York, NY, pp. 44–45.

JEFFREY, G. A. (1969): Water structure in organic hydrates. *Acct. chem. Res., 2*, 344–352.

KING, E. L. (1953): Entropy changes accompanying association reactions in solution. *J. chem. Educ., 30*, 71–77.

KOLBE, H. (1882): Antiseptische Eigenschaften der Kohlensaure. *J. prakt. Chem., 26*, 140.

MITSUDA, H., KAWAI, F., YAMAMOTO, A. & NAKAJIMA, H. (1975): Carbon dioxide–protein interaction in a gas-solid phase. *J. nutr. Sci. Vitaminol., 21*, 151–162.

MOREAU, C. (1980): Les moisissures. — in: BOURGEOIS, C. M. & LEVEAU, J. Y. (Eds) *Les techniques d'analyse et de controle dans les industries agricoles et alimentaires*. Lavoisier, Paris. Tome 3, pp. 146–173.

OORAIKUL, B. (1982): Gas packaging for a bakery product. *Can. Inst. Fd Sci. Technol. J., 15*, 313–315.

PAUCHARD, J. P., FLUEKIGER, E., BOSSET, J. O. & BLANC, B. (1980): CO_2-Löslichkeit-Konzentration bei Entstechung der Löcher und -Verteilung im Emmentalerkäse. *Schweiz. Milchwirtsch. Forsch., 9*, 69–74.

PIMENTEL, G. C. & SPRATLEY, R. D. (1971): *Understanding chemistry*. Holden Day, San Francisco, CA.

ROQUES, H. (1964): Thesis, University of Toulouse.

WANG, M. Y. & BROWN, W. D. (1983): Effects of elevated carbon dioxide atmosphere on storage of freshwater crayfish. *J. Fd Sci., 48*, 158–162.

WINTER, F. F. (1978): CO_2-Anwendung in Theorie und Praxis. *Fleischerei, 29*, 17–20.

Acta Alimentaria, Vol. 17 (2), pp. 193–199 (1988)

TEA AROMATIZATION WITH BETA-CYCLODEXTRIN COMPLEXED FLAVOURS

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(Received: 4 June 1987; accepted: 23 July 1987)

Beta-cyclodextrin (β-CD) complexed bergamot, jasmine, peppermint and cinnamon oils were added to Ceylon black tea of standard quality. On contacting with hot water the complexed volatile oils get released immediately. Comparative stability and sensory tests showed, that the use of β-cyclodextrin entrapped flavouring materials for tea aromatizations resulted in products of remarkably improved stability, storability and sensory value.

Keywords: cyclodextrin, flavour, stabilization, molecular encapsulation

Increasing number of new tea products offering improved sensory quality and broader selection show, that there is a constant demand for new tea sorts giving new sensory experiences for tea consumers. In the early 70's many novel tea preparations have been introduced to the market with rather new and even unusual tastes. For instance dried jasmine flower, orange and lemon peels, cinnamon, clove as well as their extracts were added to black tea leaves, to provide extra aroma.

The popular filter teabags were also aromatized with formulated (adsorbed, encapsulated, etc.) flavours, like lemon, bergamot, strawberry, raspberry, apple, smoke, cherry, rum and others.

Adding extra aroma to tea products of any kind (leaves, teabags, instant tea powders, etc.) proved to be a convenient way to improve the sensory value of rather poor tea sorts, which would be otherwise difficult to sell.

The majority of flavour compositions employed for tea aromatization, are however, volatile, multicomponent systems, which exhibit rather high sensitivity towards oxidation and destruction by light (REYMOND, 1977; YAMANISHI, 1975; WICKREMASINGHE et al., 1973). Therefore aromatized tea products need to be packed hermetically with exclusion of air and light by employing tightly sealed containers, boxes, etc. After launching the β-cyclodextrin complexed spice extracts (garlic, dill, onion, caraway, etc.) to the market in Hungary, it seemed to be quite logical to aromatize also tea, packed into filter bags with β-cyclodextrin complexed essential oils of bergamot, jasmine, cinnamon, peppermint and lemon. All the above essential oils form inclusion complexes with β-cyclodextrin (SZEJTLI et al., 1980a). The molecular

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encapsulation of these flavours might lead to the introduction of aromatized tea products containing solid, chemically stable flavours, enabling longer preservation (SZEJTLI et al., 1980b).

1. Materials and methods

1.1. Materials

The β-cyclodextrin (14% moisture content) is the product of Chinoin Pharmaceutical Chemical Works Ltd. Budapest (Hungary). The essential oils were purchased from Givaudan (bergamot oil), Payan Bertrand (jasmine oil) and Dragoco Co. (lemon oil, peppermint oil). All other chemicals and reagents used were of analytical purity.

1.2. Methods

1.2.1. Preparation of crystalline β-cyclodextrin inclusion complexes. The complexes were prepared in two different ways:

1.2.1.1. — by cocrystallization; 0.1 mol 14% moisture containing β-CD (129.4 g) was dissolved in 1 dm^3 of ethanol–water 1 : 2 mixture at 58–60 °C with vigorous stirring. Fifteen g of the essential oils (equivalent to 0.1 mol, referring to the average mol weight of constituents of the actual oil) was dissolved in 120–150 cm^3 of 96% ethanol and dropwise added to the cyclodextrin solution. The mixture was slowly cooled to room temperature under stirring (approx. 5 h) and kept at +3 – +5 °C overnight. The crystalline precipitate was filtered off on a glass filter and dried in air to constant weight.

1.2.1.2. — by co-grinding; β-cyclodextrin and the flavouring oils in 10 : 1 weight ratio were ground in a mortar in the presence of a small amount of water (10 g cyclodextrin + 1 cm^3 water) until the mixture became powdery, solid material. The formulations were then dried to constant weight in air at 22 °C.

1.2.2. Determination of essential oil content of the solid formulations. The flavour content of cyclodextrin complexes was determined by comparative gas-chromatography (HARANGI & NÁNÁSI, 1984). Gas-chromatographic parameters used for the analyses were as follows:

Column: glass capillary column coated with OV-101; length: 24.5 m; internal diameter: 0.2 mm,

Carrier gas: nitrogen, at a flow rate of 19.5 $cm^3\ min^{-1}$,

Injector: 200 °C; FID detector: 300 °C.

The quantitative determination of model flavour substances (e.g. cinnamaldehyde, geraniol, citral) was carried out by UV-spectroscopy. The UV-spectra were registered on a Specord UV-VIS photometer in 50% (v/v) ethanolic solutions. The characteristic UV absorption maxima at which determinations

were carried out were 287 nm for cinnamaldehyde, 238 nm for geraniol, and 240 nm for citral.

1.2.3. Granulation of crystalline flavour β-cyclodextrin complexes with dextran. Two hundred and fifty g of the flavour-complexes were mixed with 50 g of dextran (Mwt: 43000) — as binder — 25 cm^3 of glycerol and 25 cm^3 of water at 22 °C. The binder of the granules has to be rapidly soluble in hot water, without resulting in any cloudiness. For experimental purposes dextran seemed to be acceptable, but of course for industrial purpose there are various conventional granulating binders and methods. The mixture was spread in a thin layer and dried at room temperature. The dried plates were than crushed and sieved on a screen of 1.0 mm aperture. The volume of 50 g of granulated substance was 120 cm^3. The flavour complex content of these granulates was 74–77% by weight. Mixing tea leaf powders to the CD complex before granulating the salient white colour of the granules can be covered.

1.2.4. Heat stability studies. The heat stability of free and complexed flavours was characterized by thermoanalytical investigations, using a Du Pont 990 Thermal Analyzer System (TG, DTG, TEA analyses).

In 916 TEA (Thermal Evolution Analysis) apparatus test samples were heated in nitrogen atmosphere at a heating rate of 8 °C min^{-1}, and the evolved organic volatiles were detected by means of flame ionization detector.

1.2.5. Storability studies. Absorbed and complexed flavour samples were stored in open test tubes at 80 °C temperature for three days. Samples were taken every day and the actual flavour content of the formulations was determined by gas-chromatography and UV-spectrophotometry.

1.2.6. Test of clumping tendency of samples prepared. Crystalline β-cyclodextrin complexes and corresponding lactose flavour adsorbates with identical flavour content were stored for 4 and 8 hours at 22 °C in humid air (cca 90% R.H.) and then sieved through a screen of 400 μm aperture. The weight percentages of the passed and retained sample fraction were used for the characterization of clumping tendencies of formulations due to hygroscopicity. (Instead of the structurally related glucose, lactose was used for preparation of flavour absorbates, because glucose itself is hygroscopic.)

2. Results and discussion

2.1. Characterization of β-cyclodextrin flavour complexes in solid state

2.1.1. Flavour contents entrapped in β-cyclodextrin. The gas-chromatographic and UV-spectrophotometric results of flavour contents of inclusion complexes are listed in Table 1. All data on the flavour content of the formulations are presented as mean values of 5 parallel measurements.

Table 1

Flavour content of the β-cyclodextrin complexes

Complex	Flavour content (w/w %)	Note
Lemon oil–β-CD	9.7 ± 0.3	(determined by GC)
Bergamot oil–β-CD	9.0 ± 0.5	(determined by GC)
Jasmin oil–β-CD	10.6 ± 0.1	(determined by GC)
Peppermint oil–β-CD	9.3 ± 0.3	(determined by GC)
Cinnamon oil–β-CD	9.9 ± 0.2	(determined by GC)
Citral–β-CD	9.2 ± 0.2	(determined by UV photometry)
Geraniol–β-CD	10.4 ± 0.3	(determined by UV photometry)
Cinnamaldehyde–β-CD	10.6 ± 0.1	(determined by UV photometry)

Gas-chromatographic and previous sensory investigations proved, that no differences in composition and character between the original and the molecularly encapsulated flavours were found, due to the loss or deterioration of volatile components upon the complexing processes. (Fig. 1 demonstrates the above-mentioned on the example of bergamot-oil and its β-CD complex.)

These flavour β-cyclodextrin inclusion complexes are white, microcrystalline, free flowing, not-hygroscopic formulations.

2.1.2. Assay of the clumping tendency of complexed and adsorbed flavours. None of the β-cyclodextrin complexed essential oils and flavour substances were found to exhibit considerable hygroscopicity up to about 90% R.H.

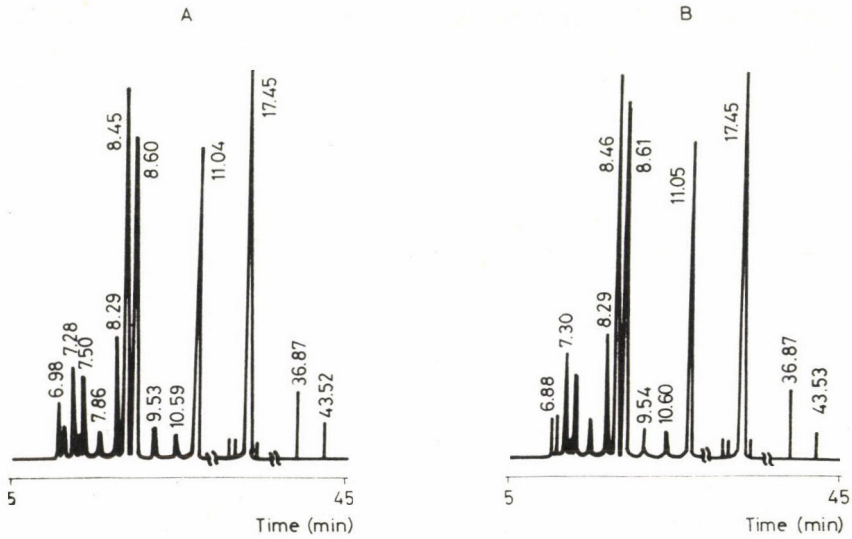

Fig. 1. Comparative gas chromatograms of original bergamot oil and the bergamot oil re-extracted from β-cyclodextrin complex. A: original oil; B: complexed oil

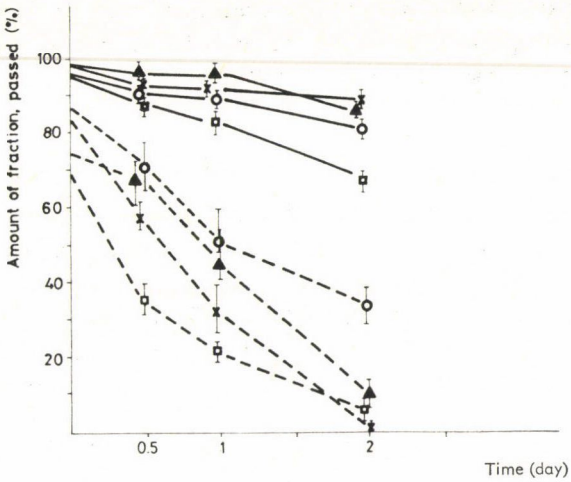

Fig. 2. Clumping tendency of flavour-β-cyclodextrin complexes and corresponding lactose-flavour adsorbates upon storage in humid air (90% R.H.) for two days at 22 °C (Screen test). ×: Jasmine oil; ▲: bergamot oil; ○: lemon oil; □: peppermint oil; ——: complexes; – – – –: adsorbates on lactose

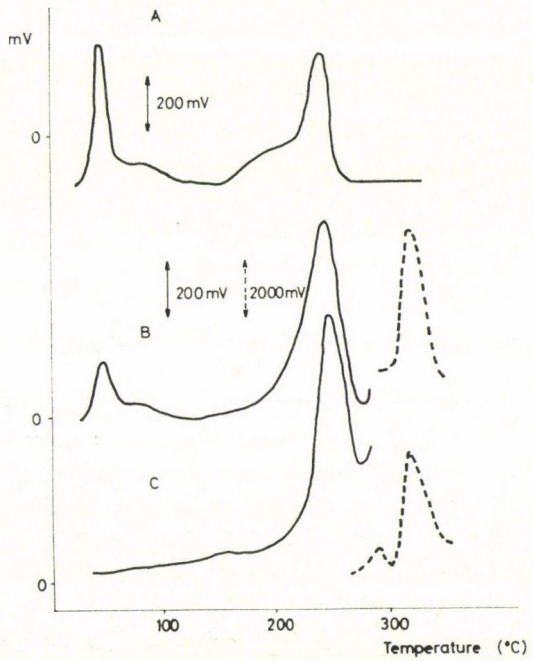

Fig. 3. TEA (Thermal Evolution Analysis) curves of free lemon oil (A); lemon oil + β-cyclodextrin mixture (B) and β-cyclodextrin lemon oil inclusion complex (C)

Lactose-flavour adsorbates (which were prepared in the same way as co-ground β-cyclodextrin complexes) however, showed to be rather hygroscopic, even after a few hours, storage in humid air (Fig. 2).

2.2. Stability studies

2.2.1. Thermoanalytical studies. (Presented on the example of lemon oil.) The TEA thermoanalytical investigations show that the evaporation of the highly volatile constituents of free lemon oil takes place between 100–130 °C, that of the less volatile ones at 170–270 °C (Fig. 3).

The TEA curves of lemon oil β-cyclodextrin mechanical mixture represent a similar thermal release of the lemon oil volatiles. Significantly retarded escape of volatiles can be detected, however, from the β-cyclodextrin lemon oil complex. The evaporation of lemon oil started from the complex only above 180 °C and continuously proceeded till the thermodegradation of the carbohydrate matrix. Similar thermoanalytical data were obtained with all other flavour formulations discussed in this paper.

2.2.2. Storage at elevated temperature. The behaviour of cyclodextrin complexed and adsorbed flavours upon long term storage was approached by treating the samples (containing identical amounts of flavours) at 80 °C for three days.

This heat stress showed, that the flavour–lactose adsorbates suffered a severe loss of volatiles, while flavours properly entrapped into β-cyclodextrin exhibited remarkable persistency under this treatment.

The results of the storage test are summarized in Table 2.

Comparative thin-layer chromatography proved that in β-cyclodextrin complexes even the most volatile and heat sensitive constituents were retained after heat treatment for three days and the TLC patterns of untreated and treated complexed oils were almost identical.

2.3. Application of the granulated form of cyclodextrin flavour complexes

Upon filling and packing teabags and teaboxes with tealeaves aromatized with powdery β-cyclodextrin complexed flavours, a significant size-sorting takes place resulting in tea products of rather heterogenous flavour distribution.

Therefore to ensure the possible highest degree of homogeneity of the complexed flavours in the mixture of tea leaves and complexes the microcrystalline solid complexes were formulated to granules of similar specific gravity with that of the tea leaves.

The use of common granulating materials such as different types of PVP (polyvinylpirrolidone), carboxymethyl-cellulose, carboxymethyl starch, various dextrin, etc. for granulation of flavour complexes resulted in severe

Table 2

Effect of storage time at 80 °C on the flavour content of lactose adsorbates and β-cyclodextrin complexes

Samples		Flavour content (%)[a]			
		0 day	1 day	2 day	3 day
Bergamot oil	complex	9.3 ± 0.6	9.0 ± 0.2	10.1 ± 0.4	8.2 ± 0.5
	adsorbate	9.5 ± 0.2	5.7 ± 0.2	2.7 ± 0.7	1.3 ± 0.4
Lemon oil	complex	9.7 ± 0.1	9.1 ± 0.3	9.3 ± 0.2	8.8 ± 0.4
	adsorbate	9.5 ± 0.2	2.4 ± 0.4	2.8 ± 0.3	1.8 ± 0.6
Peppermint oil	complex	9.0 ± 0.3	9.2 ± 0.3	9.2 ± 0.4	8.0 ± 0.3
	adsorbate	9.0 ± 0.4	6.0 ± 0.3	4.4 ± 0.2	2.8 ± 0.5
Citral	complex	9.3 ± 0.1	9.5 ± 0.1	9.0 ± 0.2	9.0 ± 0.1
	adsorbate	9.5 ± 0.2	4.4[b] ± 0.8	4.0[b] ± 0.8	0.0[b]
Geraniol	complex	10.5 ± 0.1	10.7 ± 0.3	10.0 ± 0.2	8.8 ± 0.3
	adsorbate	10.0 ± 0.2	6.6 ± 0.5	2.8[b] ± 0.9	3.0[b] ± 1.0
Cinnamaldehyde	complex	10.7 ± 0.2	10.4 ± 0.3	8.8 ± 0.1	8.9 ± 0.4
	adsorbate	10.5 ± 0.3	7.0 ± 0.5	2.2 ± 0.5	0.0[b]

[a] All data represent the mean value and the standard deviation of 5 parallel determinations

[b] The UV-spectra of these treated samples seemed to be distorted, so that the results given here should not be considered as informative ones

turbidity of tea infusions due to the precipitation of tannic substances by binders at higher temperatures.

Dextran, or pyrodextrin (yellow dextrin) and saccharose were, however, appropriate binders for granulation.

One of the most important requirement to such flavour granules is: to provide rapid dissolution and release of the flavour entrapped, without precipitation in hot water in the presence of tea-leaf extracts. The above dextran based complex containing granules were found to meet all these requirements. It is to be noted that the granulated cyclodextrin complexed flavours can not be employed, however, for the aromatization of instant tea powders used for iced tea beverages, because of the very slow and slight release of the entrapped flavours at lower temperatures.

Literature

HARANGI, J. & NÁNÁSI, P. (1984): Measurement of the essential oil in inclusion complexes with cyclodextrin by means of capillary gas chromatography. *Analytica Chim. Acta, 156*, 103–109.

REYMOND, D. (1977): Flavor chemistry — coffee, cocoa and tea. *Chemtech. J.*, (Nov.), 664–670.

SZEJTLI, J., SZEJTLI, M. & SZENTE, L. (1980a): *Tea aromatizing compositions and tea aromatized therewith.* Hung. Pat. 358/80.

SZEJTLI, J., SZENTE, L. & BÁNKY-ELŐD, E. (1980b): Molecular encapsulation of volatile, easily oxydizable labile flavour substances by cyclodextrins. *Acta chim. hung., 101*, 27–46.

YAMANISHI, T. (1975): Tea aroma. *Nippon Nogei Kagaku Kaishi, 49*, (9), R1–R9.

WICKREMASINGHE, R. L., WICK, E. L. & YAMANISHI, T. (1973): Gas-chromatographic mass-spectrometric analyses of "flavory" and "non-flavory" Ceylon black tea aroma concentrates prepared by two different methods. *J. Chromat., 79*, 75–80.

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BOOK REVIEW

Changing food technology

M. KROGER & R. SHAPIRO (Eds)

Technomic Publishing Company, Inc., Lancaster, PA, 1987; 170 pages

The book contains fourteen original selected papers from the Fourth "Eastern" Food Science & Technology Conference, held October 6—9, 1985, in Lancaster, PA, U.S.A. The declared theme of this conference was "Innovation and Communication: Partners for Progress in the Food Industry."

This volume of proceedings is divided into three subject areas. The first part contains four papers about the problems of automation of food processes, under the title Process Automation for Quality and Cost Control. The second part involves six papers about the results and trends of new food product research and development with special regard to the aspects of nutrition. The group of these proceedings is entitled Laboratory Productivity and New Food Products. The third area is entitled Future Processing Technologies and Packaging Trends. The four selected papers introduce the innovations of crossflow membrane technology, of food preservation by irradiation, of instantizing food ingredients and of food packaging as an integrated technology in food processing.

Concurrent with the conference was a food science graduate student poster session. Descriptions of all twelve posters are in the Appendix.

The book is useful for technologists, engineers and graduate students interested in the recent results of research and development in the field of food industry.

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Acta Alimentaria, Vol. 17 (2), pp. 203 (1988)

ANNOUNCEMENT

7th INTERNATIONAL MEETING ON RADIATION PROCESSING

A BIENNAL CONFERENCE DEDICATED TO THE DISSEMINATION AND ADVANCEMENT OF THE TECHNOLOGY OF INDUSTRIAL RADIATION PROCESSING

April 23rd to 28th 1989

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The programme includes a mixture of invited lectures, contributed papers, poster sessions, panels, open discussions and tutorials.

The main body of the programme will consist of contributed papers.

The topics will include:

1. Radiation sterilisation
2. Waste treatment
3. Food preservation
4. Polymer modification
5. Materials for application in radiation environments
6. Semiconductors
7. Industrial irradiation facilities
8. Dosimetry

Important issues will be addressed in panel discussions. Provisional titles are:

— Public perception of radiation processes

— Why use radiation in industrial processes? Why not?

Abstracts of papers (300 words or less) should be forwarded to Mr E. Franken, the conference secretary, before 1st June, 1988.

Official language: English.

Correspondence address: Mr E. Franken, 7th International Meeting on Radiation Processing, P.O. Box 4240, 6710 EE Ede. The Netherlands.

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Pigment extraction from alfalfa protein condensates
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ACTA ALIMENTARIA

VOLUME 17 No. 2 — 1988

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ACTA ALIMENTARIA

An International Journal of Food Science

Editor

J. HOLLÓ

Volume 17 **September 1988** **Number 3**

Akadémiai Kiadó
Budapest

Kluwer Academic Publishers
Dordrecht/Boston/London

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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Kluwer Academic Publishers
P.O. Box 17, 3300 AA Dordrecht, Holland
and 101 Philip Drive, Norwel, MA 02061 U. S. A

Publication programme, 1988: Volume 17 (4 issues).
Subscription prices per volume: Dfl. 276,—/$ 130.00 including postage.

Acta Alimentaria is published 4 times per annum: March, June, September and December

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.

ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

VOLUME 17

1988

AKADÉMIAI KIADÓ
BUDAPEST

KLUWER ACADEMIC PUBLISHERS
DORDRECHT/BOSTON/LONDON

PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda Vállalat, Budapest

Acta Alimentaria, Vol. 17 (3), pp. 205–210 (1988)

STUDIES INTO THE CHANGES IN SULPHUR AMINO ACIDS OF BEEF EXPOSED TO DIFFERENT TIME AND TEMPERATURE CONDITIONS

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(Received: 20 November 1986; revision received: 25 August 1987; accepted: 8 September 1987)

The present paper deals with the influence of time and temperature combinations during sterilization of beef in its own juice within the range of 75–135 °C and 5–95 min on the losses of sulphur amino acids studied as the loss of the total, protein and non-protein —SH groups.

Experimentally obtained losses varied from 0.75% to 29.01%, 12.30% to 44.84% and from 0.32% to 28.71% for total, non-protein and protein —SH groups, respectively.

Regression equations were obtained for the losses using the two-factor plan of experiment.

Keywords: beef, sterilization, combination treatment, sulphur amino acids, plan of experiment

Heating of protein containing foods brings about changes in nutrition value, taste, odour, appearance and texture. Sulphur amino acids, i.e. cysteine cystine and methionine are mainly participating in these changes. Not only does heat cause changes in the secondary and tertiary structure of proteins e.g. denaturation, but at higher temperatures chemical changes also occur caused by reactions of their functional groups and by breaking of covalent bonds. According to the research of SAMEJIMA and co-workers (1969) denaturation of proteins takes place up to 70 °C. Conclusions drawn by RANDAL and BRATZLER (1970) and also BOWERS (1972) show that the total sulfhydryl group content treated with temperatures up to 70 °C is constant by using denaturant. Quantitative increase of the total sulfhydryl group content up to this temperature is caused by opening the actomyozin molecule when masked groups are made accessible to the reagent by denaturation — already reported by JACOBSON and HENDERSON (1973).

Using temperatures higher than 70 °C revealed the decrease of sulfhydryl group content. Using temperatures above 85 °C changes of the SH groups do not take place as a consequence of their oxidation, but as referred

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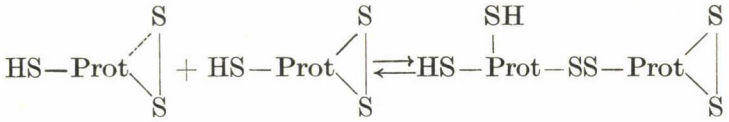
to by MALYUTIN (1970), protein aggregation is a consequence of the changes of the SH—SS system



This reaction does not necessitate the change of SH—SS groups. The decrease of these groups above these temperatures as observed could be caused by reduced reactivity of the sulfhydryl groups in comparison to their original reactivity. This is proposed in the literature only as a hypothesis, it is not yet confirmed. The loss of the total sulfhydryl groups becomes significant only at temperatures above 85 °C, while beside the original the disulfite bonds and hydrogen sulfite is also released. During heating at these higher temperatures for a long time, as in the case of sterilization of meat cans, the strongly nucleofil sulfhydryl group is oxidized to disulfite SS group. Mainly the influences of neighbouring functional groups as hydrogen bonds, as well as the coulomb forces and also the pH value of the surrounding medium are responsible for the big differences in reaction activity of sulfhydryl groups. USUNOV and ZOLOVA (1976) suggest that the SH group content in meat is influenced also by factors such as the species, age, sex and feeding of animal and its general condition before slaughtering. The content of SH groups also differs in dependence on the kind of used muscle.

1. Materials and methods

1.1. Sample preparation

Model samples of beef meat were prepared as the beef in its own juice. Cans were filled up to 425 g weight. These were prepared according to the conditions determined by two-factor plan of experiment (Table 1). The factor changes ranged within the temperature limits of 75–135 °C and sterilization time varied from 5 to 95 min.

1.2. Methods

The content of SH groups was determined by spectrophotometry at 412 nm using Ellman's reagent (ELLMAN, 1959). We used urea for denaturation (C_{urea} = 8.5 mol l^{-1}).

The non-protein SH groups were determined after their dissolution in trichloracetic acid and they were separated from protein SH groups.

The content of protein SH groups was determined as a difference between total and non-protein SH groups.

Table 1

The conditions and results of experimental measurements

Sample number	Temperature (°C)	code	Time (min)	code	Total SH	Losses (%) non-protein SH	protein SH
1	126	+1	82	+1	23.88	44.84	23.49
2	84	−1	82	+1	5.09	36.58	4.40
3	126	+1	18	−1	7.70	29.47	7.22
4	84	−1	18	−1	0.75	20.42	0.32
5	135	+1.414	50	0	29.01	42.63	28.71
6	75	−1.414	50	0	3.03	12.37	2.82
7	105	0	95	+1.414	25.28	32.26	25.13
8	105	0	5	−1.414	2.05	17.16	1.71
9	105	0	50	0	19.53	25.79	19.39
10	105	0	50	0	20.03	23.16	19.96
11	105	0	50	0	19.63	26.05	19.49
12	105	0	50	0	20.46	25.26	20.40
13	105	0	50	0	19.40	25.26	19.27

2. Results

The following losses were discovered within the temperature range of 75 to 135 °C and the sterilization period of 5 to 95 min as applied in the experiments: in total sulfhydryl groups from 0.75 to 29.01%, in non-protein sulfhydryl groups from 12.37 to 44.84% and in protein sulfhydryl groups from 0.32% to 28.71%. The relative deviation between parallel determinations was 0.8%.

The regression equations (1,2,3) for the changes of total SH groups

$$y_{CSH} = 97.44 - 1.675\,T - 0.187\,t + 0.007\,T^2 + 0.004\,t^2 - 0.004\,T\,t \qquad (1)$$

for those of non-protein SH groups

$$y_{NSH} = -41.621 + 0.847\,T - 0.215\,t - 0.006\,T^2 - 0.0021\,t^2 + 0.001\,T\,t \qquad (2)$$

for the changes of protein SH groups

$$y_{BSH} = 100.640 - 1.734\,T - 0.187\,t + 0.007\,T^2 + 0.004\,t^2 - 0.004\,T\,t \qquad (3)$$

were obtained by processing the experimental data using the least square methode.

The diagrams shown in Figs. 1, 2 and 3 represent graphically the changes in the core temperature (T, °C) as a function of time (t, min) in the temperature and time range as applied in the experiments. They were plotted on the basis of the regression equations obtained.

3. Discussion

The contour diagram (Fig. 1) allows to conclude that the lowest losses are in the temperature range of 75–135 °C and the time range of 5–95 min where the losses of the total SH groups are increasing with the increase of temperature and time. That means that the loss of total SH groups increases with the increase of temperature and prolongation of sterilization time. The lowest losses (contour 0.75) are in the temperature range of 75–135 °C and range of time of 5–95 min, where the longest sterilization time corresponds to the lowest temperature. Yet from the microbiological aspect these time–temperature combinations are not sufficient.

A contours perpendicular to the time axis within the time range of 5–34 min expresses constant loss of total sulfhydryl groups at constant time and increase of sterilization temperature above 105 °C.

In cooked meat the loss in TSH (total SH groups) was found to be 11%. Following these losses on contour diagrams we can say, that these low losses are achieved by using not satisfactory time–temperature combinations. On stewing the meat losses in TSH reach 22%.

According to the contour diagram, which represents constant loss of TSH in dependence on processing temperature, a higher temperature treatment for shorter time is proposed.

Following the losses of non-protein SH groups by contour diagram (Fig. 2) we find that under the influence of sterilization conditions the losses increase from 17–42% depending on the time–temperature combination used.

In comparison with the losses in total SH groups it is evident that in the course of heat sterilization preferentially the non-protein SH groups decrease, was assumed by KUNSMAN and RILLEY (1975).

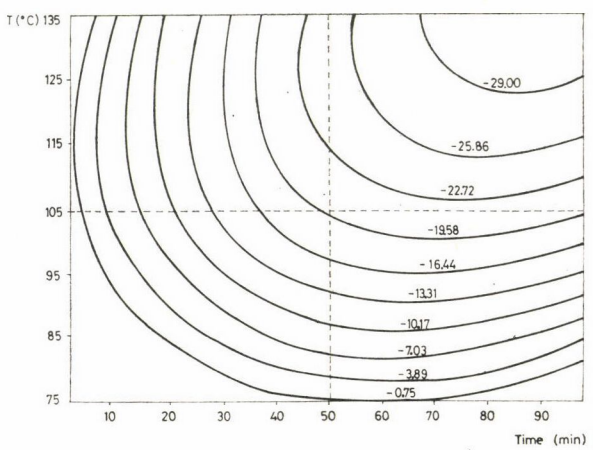


Fig. 1. Contour diagram of the losses (%) in total sulfhydryl group content

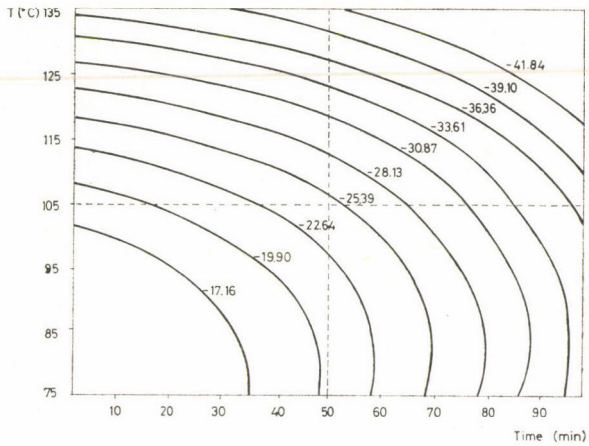


Fig. 2. Contour diagram of the losses (%) in non-protein sulfhydryl group content

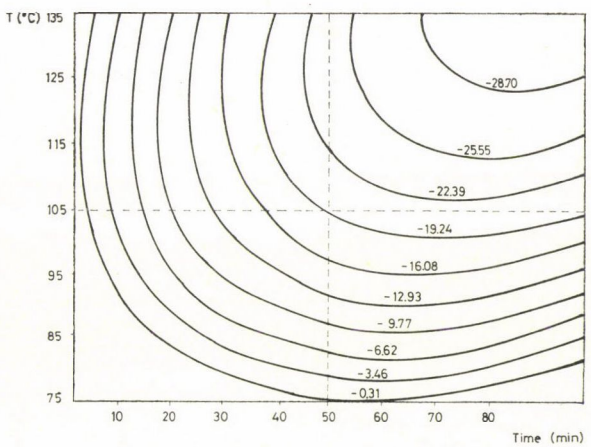


Fig. 3. Contour diagram of the losses (%) in protein sulfhydryl group content

All changes in SH radicals caused by heating relate to protein sulfhydryl groups, because 97% of the SH content in tissues is bound to proteins. The progress of contours in the diagram is similar to the losses of total SH groups (Figs. 1 and 3). With rising temperature and sterilization time the loss of protein SH groups increases and the dependences are similar to those for total SH groups.

4. Conclusions

KRYLOVA and KUZNECOVA (1964) found the loss in SH groups in the range from 7% to 29% when beef is heated to 70–80 °C. Amperometric titration was used for analysis. HOFFMAN (1964) heating beef to 70 °C found only negligible losses. In nitrogen atmosphere using 120 °C he found a loss of 25%, this being 5% higher than we found in the contour diagram. Applying 90 °C the losses amount to 15% while according to our contour diagram the assumed loss is 10%. The author used amperometric titration at pH 7.4.

It can be concluded that the mentioned equations sufficiently describe the changes in SH groups in the material used not only on the base of mathematical statistical evaluation of the adequacy of equations (SORMAN et al., 1987) but also on the base of previously published results.

Literature

BOWERS, J. A. (1972): Eating quality, sulfhydryl content and TBA value of turkey breast muscle. *J. agr. Fd Chem., 20*, 706–709.

ELLMAN, G. L. (1959): Tissue sulfhydryl groups. *Archs Biochem. Biophys., 82*, 70–77.

HOFFMAN, K. (1964): Untersuchung des Einflusses der termischen Behandlung von Fleisch auf die funktionellen Gruppen der strukturellen Muskelproteine. PhD Thesis, Justus Liebig Univ., Giessen, Germany.

JACOBSON, A. L. & HENDERSON, J. (1973): Temperature sensitivity of myozin and actomyozin. *Can. J. Biochim., 51*, 71–74.

KRYLOVA, N. N. & KUZNECOVA, V. V. (1964): Changes of sulfhydryl groups in meat as influenced by a kind of treatment. *Eur. Congr. Meat Res. Inst.*, 10th Rockilde Denmark, Rep. 6.12.

KUNSMAN, J. E. & RILLEY, M. L. (1975): A comparison of hydrogensulfide evolution from cooked lamb and other meats. *J. Fd Sci., 40*, 506–508.

MALYUTIN, A. F. (1970): Changes in the content of sulfhydryl groups in meat under superhigh frequency heating. *Obsch. Pit., 29*, -ref.: *Chem. Abst.*, (1971), *75*, 18805 L.

RANDAL, C. J. & BRATZLER, L. J. (1970): Changes in various protein properties of pork muscle during the smoking process. *J. Fd Sci., 35*, 248–253.

SAMEJIMA, K., MASHIMOTO, Y., YASUI, T. & FUKUZAWA, T. (1969): Heat gelling properties of myozin, actin, actomyozin and myozinsubunits in saline system. *J. Fd Sci., 34*, 242–246.

ŠORMAN, L., DUDÁŠOVÁ, S., KRÁLIK, M. & DUDÁŠ, J. (1987): Metódy plánovania experimentu v potravinárskom vyskume. (Methods of experiments planning in food research.) *Potravinářske vědy, 5*, (23), (in press).

USUNOV, G & ZOLOVA, L. (1976): Untersuchungen der SH Gruppen und der Milchsäure in der PSE-Muskulatur von Schwein. *Fleischwirtschaft, 56*, 253–255.

Acta Alimentaria, Vol. 17 (3), pp. 211–218 (1988)

INFLUENCE OF DIETARY OILS IN EXPERIMENTAL LIPID METABOLISM DISTURBANCE

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Sexually mature Wistar male rats (OÉTI) were fed isoenergetically with synthetic lipogenic diet containing 20% of sunflower oil (P/S = 5.2) i.e. the same quantity of a mixture containing 33% sunflower oil and 67% MCT C_8C_{10} (Medium Chain Triglycerides)-oil (P/S = 0.3) for 6 weeks and certain indices of the serum and liver as well as the distribution of fatty acids in the liver, were studied.

— It has been found that as a response to the lipogenic diet the lipid indices of the serum and the liver increased considerably compared to the control animals consuming a normal diet, for both kinds of fats. On consumption of sunflower oil the level of serum lipids decreased significantly in comparison with feeding on fat mixture, but

— at the same time, the total lipid, triglyceride and total cholesterol content of the liver was significantly higher than in case of the fat mixture;

— P/S ratio of the liver fat was nearly as high (4.2) as the P/S ratio of the diet (5.2) in case of sunflower oil consumption, however, on consumption of the oil mixture of lower P/S ratio (0.3) the P/S ratio of liver fat was also lower (1.36).

— Compared to the control, the proportion of total unsaturated fatty acids increased in the liver in consequence of development of disturbance in fat metabolism. In case of sunflower oil consumption the quantity of the polyunsaturated fatty acids and on consumption of the fat mixture rich in MCT the proportion of the monounsaturated fatty acids increased.

— Compared to the control values, after feeding the lipogenic diet the proportion of $C_{20:4}$ (arachidic acid) decreased significantly but as a response to the fat mixture in a much lower degree than in the case of sunflower oil.

Keywords: mixture of MCT (C_8C_{10}) and sunflower oil, lipogenic diet, serum- and liver lipids, fatty acid composition of the liver

In our earlier studies, the effect of the quantity and quality of dietary fats used most frequently in Hungary on certain lipid indices was investigated feeding a normal diet to the rats in other aspects adequate (J. N. ZSINKA et al., 1986, 1987a, b). Thereafter we studied the influence of pathologic lipogenic diet on some indices of fat metabolism. Upon feeding a lipogenic diet (developed by us) it has been found that an excessive fat intake — in combination with other diet mistakes — increased significantly the lipid content of the liver and there

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were differences in lipid indices depending upon the quality of fat, too. We found, that in case of excessive fat consumption the fats of medium chain triglycerides (MCT) ($C_8C_{10}C_{12}$) and the fat mixture rich in MCT caused a lower lipid accumulation in the liver than sunflower oil alone, under normal and pathological conditions, and that in normal diet the fat mixture containing $C_8$and C_{10} fatty acids only — given in a proportion of 20% — seemed to be more favourable than a fat mixture of otherwise similar composition but containing $C_8C_{10}C_{12}$. In the present experiment we wanted to study how in lipogenic diet the sunflower-oil and its mixture with MCT-oil (C_8C_{10}) of very different P/S and C-chain lengths influences the lipid indices of the serum and liver.

1. Materials and methods

With the lipogenic diet (containing 1% of cholesterol among others according to J. N. ZSINKA et al., 1988) 20 sexually mature male rats (219±12 g per groups) were fed for 6 weeks isoenergetically. Control rats were fed with normal powdered diet with 5% fat content. The composition of the diets is given in Table 1

The C_8C_{10} fatty acid content of the oil (named "DELIOS-S", rich in MCT) was 99.5 % C_{12} fatty acid content was less than 0.5%. (Gas chromatographic determination of the fatty acids was carried out in the Vegetable Oil and Detergent Research Institute.) The mixture of oil rich in MCT and of sunflower oil corresponded to the ratio used in our former experiments (J. N. ZSINKA, 1988).

Table 1

Composition of experimental diets

	Groups		
	I	II	III
Feed	Normal rat chow	Sunflower oil	Mixture of 67% DELIOS-S[a] + 33% sunflower oil
Fat content (%)	5	20	20
Total satured fatty acids (S) (%)	33.5	15.4	72.1
Polyunsaturated fatty acid (P) (%)	35	70.9	21.4
P/S	1.08	5.2	0.3
MCT (%)	—	—	67.7
Energy content			
J per 100 g feed	1680	1737	1726
Cal per 100 g feed	400	415	412

[a] Oil rich in MCT (C_8C_{10})

During the experiments, the feed consumption of rats and changes in their body mass were measured. After feeding for 6 weeks and an 18 h starvation, the blood serum (taken in ether narcosis) and in the liver homogenate (FOLCH et al., 1957), were determined: the total lipid content according to ZÖLLNER and KRISCH (1962), the triglyceride content of the serum by the enzymatic Boehringer test, the triglyceride content of the liver by Reanal extraction test, the free fatty acid level (DOLE & MEINERTZ, 1960), the total serum and the liver cholesterol and serum HDL-cholesterol content by Gödecke test. The relative mass of the liver, kidney, heart and spleen were calculated. From groups of 10 animals each, the fatty acid composition of the liver was determined by gas chromatography. Variance analysis was applied for statistical evaluation of results (SACHS, 1984).

2. Results

Feed and energy consumption of the rats are presented in Table 2. There was no significant difference in energy consumption among the groups.

Table 3 represents the gain in body mass. It can be seen that with similar energy intake there was a significantly greater body mass gain as a response to lipogenic diet compared to normal. As an effect of the fat mix-

Table 2

Feed and energy consumption of experimental groups

	Groups		
	I	II	III
Feed consumption (g per rat per day)	18.9	16.3	16.7
Energy intake			
J per rat per day	301	288	295
Cal per rat per day	71.8	68.5	70.3

Table 3

Changes in body mass
(g per 6 weeks)

		Groups		
		I	II	III
Change	$\bar{x}$	131	176[a]	155[a]
	$\pm s$	28	25	26

[a] significant difference
$\bar{x}$: mean value; $\pm s$: standard deviation

ture rich in MCT, the body mass was by about 12% lower than in the case of sunflower oil consumption. A difference in relative organ mass could be observed only in the liver as shown in Table 4.

Greater relative liver masses were observed with lipogenic diet compared to the control, but consumption of fat mixture rich in MCT resulted in a lower average in relative liver mass than feeding with sunflower oil.

The development of serum lipids is summarized in Table 5.

Table 4

Change in relative liver mass
(g per 100 g body mass)

		Groups		
		I	II	III
Change	$\bar{x}$	3.3	4.7[a]	4.25[ab]
	$\pm s$	0.26	0.34	0.36

Significant differences: [a] I—II, III $P < 0.05$
[b] II—III $P < 0.05$

Table 5

Serum lipid content

		Groups		
		I	II	III
Total lipid (g per l)	$\bar{x}$	3.8	4.65[a]	5.7[a,b]
	$\pm s$	0.5	1.5	1
Triglyceride (mmol per l)	$\bar{x}$	0.73	0.51[a]	0.48[a]
	$\pm s$	0.23	0.13	0.15
Total cholesterol (mmol per l)	$\bar{x}$	1.7	2.7[a]	4.67[a,b]
	$\pm s$	0.4	0.69	1.3
HDL_{chol} (mmol per l)	$\bar{x}$	1.16	1.30	0.93
	$\pm s$	0.28	0.31	0.47
Free fatty acid (mmol per l)	$\bar{x}$	0.45	0.59	0.55
	$\pm s$	0.09	0.3	0.25
Total cholesterol/HDL_{chol}		1.46	1.95	5.02[a,b]

Significant differences: [a] I—II, III $P < 0.05$
[b] II—III $P < 0.05$

On consumption of the lipogenic diet, the total lipids and the total cholesterol content increased significantly compared to the control values, while the triglyceride content decreased in both cases.

As a response to the fat mixture, significantly higher total lipid and total cholesterol contents were found than in case of the consumption of sunflower oil. The HDL-cholesterol content did not change significantly.

The same was reflected in the calculated index as well, being more favourable as a response to sunflower oil.

The composition of the lipid content of the liver is presented in Table 6.

Table 6

Liver lipid content

		Groups		
		I	II	III
Total lipid (mg per g)	$\bar{x}$	73.7	398[a]	292[a,b]
	$\pm s$	11.4	112	68
Triglyceride (mg per g)	$\bar{x}$	14.2	86[a]	68.4[a,b]
	$\pm s$	5	15	17
Total cholesterol (mg per g)	$\bar{x}$	6.3	116[a]	93.5[a,b]
	$\pm s$	1.2	37	17
Free fatty acid (mmol per g)	$\bar{x}$	14.1	10[a]	11.4[a]
	$\pm s$	6	3.9	4

Significant differences: [a] I—II, III $P < 0.01$; [b] II—III $P < 0.05$

Table 7

Fatty acid composition of liver fat

Fatty acid (%)	Groups		
	I	II	III
Total saturated fatty acid content (S)	41.4	13.2[a]	23[a]
C_8C_{10}-fatty acid	—	—	0.86
Monounsaturated fatty acid	28.3	30.9	45.6[a,b]
Polyunsaturated fatty acid (P)	30.1	55.6[a]	31.4[b]
Total unsaturated fatty acid	58.4	86.5[a]	77[a]
$C_{20:4}$ (arachidic acid)	14.7	4.3[a]	7.5[a,b]
P/S	0.72	4.2	1.36

Significant differences: [a] I—II, III $P < 0.05$; [b] II—III $P < 0.05$

The visually well visible liver fatness was found to be correlated with a very high total lipid and total cholesterol content in the experimental groups and the amount of triglyceride increased particularly. The fat mixture rich in MCT showed in all liver indices significantly lower values compared to sunflower oil — except for the free fatty acid content.

The fatty acid composition of the liver fat is given in Table 7.

In comparison to the control values, the amount of total saturated fatty acids decreased and the total unsaturated fatty acids increased. A greater difference was observed in the liver due to sunflower oil than to the fat mixture and this is shown by the P/S ratio, as well. As an effect of sunflower oil, the arachidic acid content decreased more significantly compared to the control than in case of fat mixture consumption. The diet with fat mixture resulted a higher monounsaturated fatty acid percentage, with sunflower oil a higher polyunsaturated ratio than the normal diet.

3. Conclusions

As a response to the "lipogenic" diet the changes were similar to those found in our former experiments: the lipid indices of the serum and the liver increased significantly after consumption of both kinds of fats. According to our former results this was attributed to the lipogenic change of other dietary factors associated with the high fat consumption (J. N. ZSINKA, 1988). It is known, that as an effect of a high fat and cholesterol intake the accumulation of tissue cholesterol is increasing and the LDL cholesterol content of the serum becomes higher as well (TEARS, 1978), inhibiting the activity of LDL receptors of the liver enabling normally the elimination of LDL cholesterol content (BROWN, 1971) and because of that the cholesterol contents of the serum and the liver increase. A very important role is played by the genetical control of cholesterol transport, synthesis and of the elimination (GOLDSTEIN et al., 1983).

We suggested that another factor can also be of interest: the high ratio of sunflower oil in the diet means the introduction of much unsaturated fatty acid and the unsaturated bonds are very reactive, they can cause lipid accumulation in the liver being initiator of lipid peroxidation (MUTO et al., 1983, FEHÉR, 1983; FEHÉR & VERECZKEY, 1983).

Some of the authors are of the opinion that in the development of pathogenic lipid indices the structure of the triglycerides can be as important as the degree of unsaturation of the fatty acids (KRITCHEVSKY et al., 1982; KRITCHEVSKY, 1984).

In our experiments there was a difference in the fatty acid content of the livers. While on consumption of sunflower oil of P/S = 5.2 the P/S ratio of the liver fat approached a similarly high value (P/S = 4.2), upon consumption

of the fat mixture (P/S = 0.3) the P/S ratio of the liver fat (1.36) was near to the normal value. After the consumption of sunflower oil, the percentage of polyunsaturated fatty acids in the liver was lower than that of the consumed sunflower oil, while the fat mixture caused a higher polyunsaturated fatty acid level in the liver than that of the diet.

Compared to the control, an increase of the total unsaturated fatty acid content in the liver accompanies the disturbances in fat metabolism. With the consumption of sunflower oil the polyunsaturated fatty acid ratio and with fat mixture rich in MCT the monounsaturated fatty acid proportion was increased as a characteristic parameter of the normal liver (CANUTO et al., 1986). The reduction in arachidic acid was more intensive in case of sunflower oil consumption. With the increase of the lipid indices of the liver, the prostaglandin precursor unsaturated fatty acid content — mainly arachidic acid — indicates also the development of lipid metabolism troubles which phenomenon was compensated to a certain extent by the consumption of the C_8C_{10} MCT oil + sunflower oil mixture. Meanwhile, as its effect in some serum values unfavourably stronger growth was compared to sunflower oil consumption.

Interaction between cholesterol intake and P/S ratio was also studied e.g. by WALKER (1984) who found that the P/S ratio of the diet changed the serum cholesterol level when cholesterol was given with a diet rich in unsaturated fatty acids. MUHLWILL and WALKER (1984) suggested that with a diet containing 1% cholesterol, the increasing P/S ratio of the diet lowered the HDL- and LDL-cholesterol content in the blood serum.

In our results, the higher quantity of unsaturated fatty acids did not change the serum HDL level but the total cholesterol level increased with the consumption of lipogenic diet in the case of both fats, but mainly as a response to the mixture. KRISCH-ETHERTON and co-workers (1984) reported that after feeding with fat containing polyunsaturated fatty acid, the serum HDL_{chol} content remained unchanged with a low serum triglyceride level but the HDL_{chol} depot of the liver increased. According to their data, saturated fats did not influence the cholesterol level of the liver, but the lipoproteins of the liver contained more linoleic acid as a response to polyunsaturated fat compared to the effect of fats rich in saturated fatty acids.

As an effect of a mixture with C_8C_{10} fatty acids a decreased lipid accumulation of the liver was shown. As we described earlier (J. N. ZSINKA et al., 1987b) there is a metabolic difference between the longer C-chain and C_8C_{10} (or shorter) fatty acids, the latters transforming into lipids at a lower rata than the longer chain fatty acids because of its quick absorption and oxidation (DEMARNE, 1977).

We are of the opinion, that not only the P/S ratio and the amount of fat but other factors of the lipogenic diet — among others the large quantity

of cholesterol and therewith the increase of the proportion of unsaturated bonds and the structure of fats — affected the lipid and cholesterol transport, leading to fatty liver. The development of fat metabolism disturbance was not similarly influenced by the fat mixture: the increase of some lipid indices of the serum was less compensated by it than by sunflower oil but it lowered significantly the lipid indices of fatty liver.

Literature

BROWN, A. B. (1971): Food patterns that lower blood lipids in man. *J. Am. diet. Assoc. 58*, 303–311.

DEMARNE, Y. (1977): Effects of dietary lipids on qualitative aspects of lipogenesis in growing rat and pig. *Biochem. biophys. Acta, 17*, 875–882.

CANUTO, R. A., BIOCCA, M. E., MUZIO, G. & DIANZANI, M. U. (1986): Changes of fatty acid composition of phospholipids during diethylnitrosamine carcinogenesis in rat liver. *Cell Biol. int. Rep., 10*, 472.

DOLE, V. P. & MEINERTZ, H. J. (1960): Microdetermination of long chain fatty acids in plasma and tissues. *J. Biochem.*, 235, 2595–2598.

FEHÉR, J. (1983): The effect of a recently developed hydroquinolin type radical scavenger in cholesterol reduced hyperlipidemia. *Proc. 16th Congress ISP*, Budapest, p. 1069.

FEHÉR, J. & VERECZKEY, A. (1983): *Importance of free radical reactions in medical sciences.* Biotéka, Biogal. Debrecen.

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

GOLDSTEIN, J. L., KITA, T. & BROWN, M. S. (1983): LDL-receptors and cholesterol metalolism. *New Engl. J. Med., 309*, 288–296.

J. N. ZSINKA, Á., SZÉPVÖLGYI, J., FÖLDES, V., PERÉDI, J. & LINDNER-SZOTYORI, K. (1986): Study into the effect of diets containing various amounts of soya fibre in rats. *Acta Alimentaria, 15*, 39–45.

J. N. ZSINKA, Á., FÖLDES, V. & PERÉDI, J. (1987a): Certain serum and liver lipid parameters as affected by edible fats of different quantity and quality. *Acta Alimentaria, 16*, 217–223.

J. N. ZSINKA, Á., FÖLDES, V. & PERÉDI, J. (1987b): Certain serum and liver lipid parameters as affected by different fat mixture. *Acta Alimentaria, 16*, 225–230.

J. N. ZSINKA, Á., FÖLDES, V. & PERÉDI, J. (1988): Indices of lipid metabolism disturbance induced by diet. *Acta Alimentaria, 17*, 43–51.

KRISH-ETHERTON, P. M. CHIN YING HO & FOSMIRE, M. N. (1984): The effect of dietary fat saturation on plasma and hepatic lipoproteins in the rat. *J. Nutr., 114*, 1675–1682.

KRITCHEVSKY, D. (1984): Experimental atherosclerosis in rabbits fed cholesterol free diet. Comparison of peanut and olive oils. *Atherosclerosis, 50*, 253–259.

KRITCHEVSKY, D., TEPPER, S. A., CZARNECZKI, S. K. & KLURFELD, D. M. (1982): Atherogenicity of animal and vegetable protein. *Atherosclerosis, 41*, 429–431.,

MUTO, T., TANAKA, J., MIURA, T. & KIMURA, M. (1983): Autooxidation of cholesterol in the presence of unsaturated fatty acid and iron catalyst. *J. Pharmacobio-Dynamics, 6*, 3–15.

MUHLWILL, J. B. & WALKER, B. A. (1984): Dietary fatty acids cholesterol interaction and serum cholesterol in the hyper-cholesterolemic mouse. *Nutr. Res., 4*, 611–619.

SACHS, L. (1984): *Applied statistics.* A handbook of techniques. Springer Verlag. Berlin.

TEARS, R. (1978): The relationship between changes in the concentration of serum lipoprotein and tissue lipogenesis. *Biochem. Soc. Trans., 6*, 602–604.

WALKER, B. A. (1984): Plasma cholesterol response to dietary saturated and hydrogenated fats in CLR and C_{57} BRcd mice. *Nutr. Res., 4*, 601–610.

ZÖLLNER, N. & KRISCH, K. (1962): Über die quantitative Bestimmung von Lipoiden (Micromethode) mittels der vielen natürliche Lipoiden. *Z. Ges. exp. Med., 135*, 545–562.

Acta Alimentaria, Vol. 17 (3), pp. 219–225 (1988)

COMPARISON OF TWO MATHEMATICAL MODELS FOR THE STRESS OVERSHOOT IN START-SHEAR EXPERIMENTS OF MODEL EMULSIONS

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(Received: 9 April 1987; accepted: 9 October 1987)

The fit of modified Elliott–Green and Bird–Leider models with data obtained for two model emulsions were compared at the shear rate region that approximates to the stimuli associated with the perception of pourability and spreadability of saled dressings. A shortened measurement time was used because of the good correlation of the transient shear stress value, i.e. the maximum stress value obtained over the initial part of the experiment, with textural properties of foods. NaCl or one of two salt substitutes was added to the emulsions to study their influence on the rheological parameter values. It was observed that the modified Elliott–Green model better described the shear stress–time behaviour of the emulsions. Both models failed to describe the shear stress growth accurately, but the modified Elliott–Green model fitted well to the data over the descending-stress period. Except for one case, the transient shear stress value increased remarkably when Morton Lite Salt (50% w/w NaCl and 50% KCl) or Mineral Salt (65% NaCl, 25% KCl and 10% $MgSO_4 \cdot 7H_2O$) replaced NaCl in the emulsion.

Keywords: emulsions, rheological properties, mathematical models, sodium chloride, salt substitutes

A stress overshoot is a typical response in start-shear experiments, i.e. in the measurement of shear stress–time curves at a constant shear rate, conducted on oil-in-water and water-in-oil emulsions (DEMAN, 1969; ELLIOTT & GANZ, 1971). Several mathematical models have been proposed to describe the rheological data representing the stress overshoot phenomenon. The models of VAN DEN TEMPEL (1980), MASON and co-workers (1982) and FIGONI and SHOEMAKER (1983) contain several exponential terms and are thus not very practical when correlations between instrumental and sensory evaluation data are searched for. The models of ELLIOTT and GREEN (1972) and LEIDER and BIRD (1974) contain one and two exponential terms, respectively, but such simple models seldom fit to the rheological data with the same degree of accuracy over the whole measurement time. However, the Bird–Leider model has been used in the calculation of the shear rate values associated with the perception of spreadability and thickness of foods (KOKINI & DICKIE, 1982; DICKIE & KOKINI, 1983).

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The present study is part of a research project that examines the influence of NaCl and salt substitutes on food emulsions. In this part, the agreement of the modified Elliott–Green and Bird–Leider models with the experimental data are compared. The Elliott–Green and Bird–Leider models were chosen for this purpose because the transient shear stress value that is the maximum value of the stress overshoot curve, correlates better with the textural properties of foods than does the steady state value, i.e. the last stress value of the curve (KOKINI & DICKIE, 1982; DICKIE & KOKINI, 1983). This means that a complete start-shear measurement need not be performed to obtain the data for correlations between some rheological parameters and the perceived properties of emulsions. Even a simple model may describe the initial part of the stress overshoot well.

1. Materials and methods

1.1. Modification of the models

ELLIOTT and GREEN (1972) based their description on the modified Bingham body that is built from three elements: a Hooke element, representing an elastic solid, a St. Venant element, representing a plastic flow; and a Newtonian element representing a viscous flow. From the mechanical model,

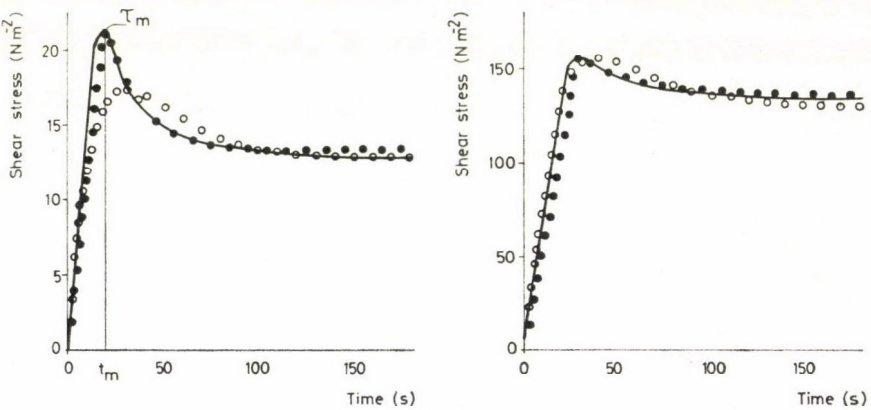

Fig. 1. Typical measured and simulated curves. At the left: emulsion I with Morton Lite Salt, at the right: emulsion II with NaCl. Dotted lines represent the simulated values.

		Emulsion I	Emulsion II
●●●: Elliott–Green model	$s_{\tau t}$ total	1.0	13.3
	$t \leq t_m$	2.1	25.1
	$t > t_m$	0.2	0.7
○○○: Bird–Leider model	$s_{\tau t}$ total	2.1	8.2
	$t \leq t_m$	3.8	13.5
	$t > t_m$	1.2	4.6

elastic effects should govern the behaviour of the material until the transient shear stress value τ_m is reached (Fig. 1). At a constant shear rate, this region is described by the relationship

$$\tau = G\,\dot{\gamma}\,t \qquad t \leq t_m \tag{1}$$

where G is the elastic modulus, $\dot{\gamma}$ is the shear rate and t is the elapsed time. At the point (t_m,τ_m) an instantaneous change from linear shear stress growth to a stress decay occurs and the model becomes

$$\tau - \tau_s = (\tau_m - \tau_s)\, e^{-G(t-t_m)\eta} \qquad t > t_m \tag{2}$$

where τ_s is the experimental steady state stress value at that particular shear rate and η is the coefficient of the Newtonian element. If the measurement is not completed, another parameter τ_e must be used instead of τ_s. The value of this parameter is obtained when fitting the model (2) to the incomplete experimental data.

The Bird–Leider model is expressed by

$$\tau = m\,\dot{\gamma}^n[1 + (b\,\dot{\gamma}\,t - 1)\, e^{-t(an\lambda)}] \tag{3}$$

where a and b are adjustable parameters and λ is the time constant (LEIDER & BIRD, 1974). A typical feature of the Bird–Leider model is that over long periods, it converges to the power-law model

$$\tau = m\,\dot{\gamma}^n \tag{4}$$

where m is the coefficient of consistency and n is the flow behaviour index. Many foods follow this model satisfactorily (HOLDSWORTH, 1971). At a constant shear rate, $m\,\dot{\gamma}^n = \tau_s$ and the model can be rewritten as

$$\tau - \tau_s = (G^*\,\dot{\gamma}\,t - \tau_s)\, e^{-kt} \tag{5}$$

where G^* is the modulus of deformation over small periods of time and k is the decay rate constant. As above, the parameter τ_e replaces τ_s when model (5) is fitted to the incomplete data.

1.2. Preparation of emulsions

A stock emulsion containing 1.0% w/w food grade gelatin, 69% demineralized water and 30% soybean oil was prepared by dissolving the gelatin in hot water (70 °C), adding the oil, and homogenizing at a pressure of 16 MPa in a Rannie Blue Top (Multi-Mic) homogenizer. The pH value of the stock emulsion was then lowered to 4.5 with hydrochloric acid, the emulsion (emul-

sion I) was divided into portions, and 0.50% w/w NaCl or one of two salt substitutes was mixed into the portions using a hand mixer. The mixing time was exactly the same in all cases. The emulsion II that contained 1.1% soy protein isolate, 0.9% acetylated distarch adipate, 68% soybean oil and 30% water was prepared in the same manner as emulsion I, except that cool water was used. The salts were added to the emulsion II to a concentration of 1.00%.

1.3. Ingredients

The individual salts were of analytical grade of Merck (FRG) and the salt substitutes mixed of them were similar to Morton Lite Salt (50% w/w NaCl and 50% KCl) and Mineral Salt (65% NaCl, 25% KCl and 10% $MgSO_4 \cdot 7H_2O$). The gelatin (Italgelatine, Italy) was alkali-processed, the pH value of its water solution was 5.5, and its Bloom value was 250. The protein concentration of the soy isolate (Purina Protein 500E, Ralston Purina Co.,USA) was 92%. The acetylated distarch adipate was Instant Clearjel manufactured by Laing National Ltd, UK. The soybean oil was a commercial product obtained from a local store.

1.4. Rheological measurements

The emulsion portions were kept for two days at + 4 °C and, prior to measurements, for several hours at the measurement temperature of 20.0 °C. Each measurement was taken after the material had rested for twenty minutes in the viscometer cup. The roughened MV I P coaxial cylinder assembly of Haake Rotovisco RV3 viscometer and a Bryans 28 000 t-x-recorder were used. Two replications were made, each using fresh material. The time between the replications was constant so that each sample type was measured once before a new round of measurements. The shear rate values were 2.34 s^{-1} (emulsion I) and 0.936 s^{-1} (emulsion II) that approximate to those associated with the perception of pourability and spreadability of salad dressings (KIOSSEOGLOU & SHERMAN, 1983). The measurement time was 3.0 min from the beginning of the stress decay. Some complete measurements i.e. until the τ_s value were taken from emulsion I.

1.5 Calculations

The value of G or G^* was first calculated from the recorded curve by using the equation $G = \tau_m (\dot{\gamma}\, t_m)^{-1}$ or the equation of line $\tau = G^* \dot{\gamma}\, t$, respectively. The standard error of estimate of τ i.e. $s_{\tau t}$ was then minimized with a computer to produce the parameter values τ_e and $G\, \eta^{-1}$ or τ_e and k either in Eq. (2) or (5). A search method was used in the minimization (BENNINGTON, 1981).

Paired time and shear stress values were collected for the purposes of computation at intervals of two seconds. The same points were used in the case of both models. In addition to the total standard error of the estimate, partial standard errors were computed separately for the rising and the falling part of the curve.

To calculate the standard error of estimate the following formula was used:

$$s_{yx} = \sqrt{\frac{\Sigma(y_i - \hat{y}_i)^2}{n - 2}}$$

where $\Sigma(y_i - \hat{y}_i)^2$ is the sum of the squared differences between measured and estimated values of y and n is the number of (x_i, y_i) points used for the calculation. The significances of the differences between the mean values of parameters were tested statistically by one-way variance analysis and Tukey's test (STEEL & TORRIE, 1960).

2. Results and discussion

ELLIOT and GREEN (1972) assumed that the parameter η of the model they proposed can be calculated from the equation $\tau_s = \tau_{0,s} + \dot{\gamma}\eta$ where $\tau_{0,s}$ is the shear stress that remains after stopping the shearing when the steady state value τ_s is reached. They found, however, that when the calculated value of η was combined with the G value to form the decay rate constant, the model predicted a much shorter time for to reach the τ_s value than those observed. The same observation was made in the present study when the simulated and the experimental curves of the complete measurements were compared: the present empirical form of the Elliott–Green model better fitted to the experimental data than the original one based on the use of the $\tau_{0,s}$ value. When the data obtained during the shortened measurement time was used for the fitting, the agreement between the experimental and simulated shear stress values was even good for both emulsions over the decending-stress period (Fig. 1). However, the model did not describe the shear stress growth successfully (Fig. 1). This means that the parameter G^* of the Bird–Leider model is better suited for characterization of these types of emulsions than the parameter G.

The maximum shear stress values calculated from the modified Bird–Leider model after fitting to the data agreed successfully with the transient shear stress values τ_m only in few cases. In addition, 45–62% higher values were regularly obtained for the corresponding peak times than were observed in the measurements (Fig. 1). This lack of accuracy is consistent with the earlier observations obtained for mayonnaise, butter and margarine (KOKINI & DICKIE, 1981; DICKIE & KOKINI, 1982; MASON et al., 1982). No improve-

ment was thus achieved in this respect as a result of τ_e being a mathematical and not an experimental parameter. Besides, both the total standard error and the partial standard errors of τ were in most case smaller for the modified Elliott–Green than the Bird–Leider model (Table 1).

The parameter G^* correlated with the transient shear stress value at the 5% significance level in emulsion I, but the correlation was not significant

Table 1

Means of the parameters describing the curves and the mean standard errors ($s_{\tau t}$) of the estimate of shear stress

Model and salt type	Emulsion I						
	τ_m	G or G^*	$G\eta^{-1}$ or k $\times 10^3$	τ_e	$s_{\tau t}$ (N m^{-2})		
	(N m^{-2})	(N m^{-2})	(s^{-1})	(N m^{-2}	total	$t \leq t_m$	$t > t_m$
Elliott–Green							
NaCl	20.5	0.37	17	12.8	1.3	2.6	0.2
Morton Salt	25.2	0.47	26	14.9	1.1	2.3	0.3
Mineral Salt	28.6	0.47	22	17.5	1.3	2.6	0.3
LSD	3.2	n.s.	n.s.	2.3			
Bird–Leider							
NaCl		0.45	43	14.0	1.6	3.1	0.7
Morton Salt		0.48	44	14.6	2.4	4.3	1.4
Mineral Salt		0.54	37	17.7	2.4	4.1	1.4
LSD		0.05	n.s.	n.s.			

Model and salt type	Emulsion II						
	τ_m	G or G^*	$G\eta^{-1}$ or k $\times 10^3$	τ_e	$s_{\tau t}$ (Nm^{-2})		
	(N m^{-2})	(N m^{-2})	(s^{-1})	(N m^{-2})	total	$t \leq t_m$	$t > t_m$
Elliott–Green							
NaCl	160	6.3	32	132	10.3	19.7	0.8
Morton Salt	228	7.4	35	161	6.4	10.8	1.7
Mineral Salt	153	6.7	22	122	6.4	12.4	0.6
LSD	24	n.s.	9	25			
Bird–Leider							
NaCl		7.9	45	127	8.6	14.6	4.9
Morton Salt		8.0	34	155	17.2	23.5	12.9
Mineral Salt		7.4	42	120	8.6	14.7	4.8
LSD		n.s.	8	20			

G or G^* and $G\eta^{-1}$ or k: moduls and decay rate constants for the models
τ_e: limit of model equation at a particular combination of the other parameters
τ_m: transient shear stress value
LSD: least significant difference ($p \leq 0.05$)
n.s.: non significant

in emulsion II. A 23% increase in the transient shear stress value was observed when Morton Salt replaced NaCl in emulsion I; a 40% increase was observed with Mineral Salt (Table 1). Mineral Salt did not markedly change the rheological parameter values in emulsion II, but a 42% increase in the transient shear stress value was observed in the case of Morton Salt (Table 1). These results point to a possibility of modifying the textural properties of some emulsions by the addition of salt mixtures. This conclusion was not reached in earlier studies on mayonnaise and two other food emulsions (LAHTINEN, 1984; 1986).

Literature

BENNINGTON, R. (1981): — in: HEILBORN, J. (Ed.) *Science and engineering programs. Apple.* 2. ed. Osborne/McGraw-Hill, Inc., Berkeley, pp. 19–22.

DEMAN, J. M. (1969): Effect of mechanical treatment on the hardness of margarine and butter. *J. Texture Studies, 1,* 109–113.

DICKIE, A. M. & KOKINI, J. L. (1982): Use of the Bird–Leider equation in food rheology. *J. Fd Process Engng., 5,* 157–174.

DICKIE, A. M. & KOKINI, J. L. (1983): An improved model for food thickness from non-Newtonian fluid mechanics in the mouth. *J. Fd Sci., 48,* 57–61, 65.

ELLIOTT, J. H. & GANZ, A. J. (1971): Modification of food characteristics with cellulose hydrocolloids. — Part I. Rheological characterization of an organoleptic property (unctuousness). *J. Texture Studies, 2,* 220–229.

ELLIOTT, J. H. & GREEN, C. E. (1972): Modification of food characteristics with cellulose hydrocolloids. — Part II. The modified Bingham body — a useful rheological model. *J. Texture Studies, 3,* 194–205.

FIGONI, P. I. & SHOEMAKER, C. F. (1983): Characterization of time dependent flow properties of mayonnaise under steady shear. *J. Texture Studies, 14,* 431–442.

HOLDSWORTH, S. D. (1971): Applicability of rheological models to the interpretation of flow and processing behaviour of fluid food products. *J. Texture Studies, 2,* 393–418.

KIOSSEOGLOU, V. D. & SHERMAN, P. (1983): The rheological conditions associated with judgement of pourability and spreadability of salad dressings. *J. Texture Studies, 14,* 277–282.

KOKINI, J. L. & DICKIE, A. M. (1981): An attempt to identify and model transient viscoelastic flow in foods. *J. Texture Studies, 12,* 539–557.

KOKINI, J. L. & DICKIE, A. M. (1982): A model of food spreadability from fluid mechanics. *J. Texture Studies, 13,* 211–227.

LAHTINEN, S. (1984): Effects of dietary salts on the rheological properties of food emulsions. *Lebensm. Wiss. + Technol., 17,* 299—301.

LAHTINEN, S. (1986): Physical effects of salt mixtures in mayonnaise. *J. Fd Quality, 9,* 1–10.

LEIDER, P. J. & BIRD, R. B. (1974): Squeezing flow between parallel disks. — Part I. Theoretical analysis. *I & EC Fundamentals, 13,* 336–341.

MASON, P. L., BISTANY, K. L., PUOTI, M. G. & KOKINI, J. L. (1982): A new empirical model to simulate transient shear stress growth in semi-solid foods. *J. Fd Process Engng., 6,* 219–233.

STEEL, R. G. B. & TORRIE, J. H. (1960): *Principles and procedures of statistics.* McGraw-Hill, Inc., New York, p. 109.

VAN DEN TEMPEL, M. (1980): — in: WALTERS, K. (Ed.) *Rheometry: Industrial applications.* John Wiley & Sons, Letchworth, pp. 179–207.

Acta Alimentaria, Vol. 17 (3), pp. 227–237 (1988)

THE APPLICATION OF PHOTOACOUSTIC SPECTROSCOPY IN FOOD INVESTIGATIONS

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(Received: 13 May 1987; accepted: 30 September 1987)

The new sensing technique "photoacoustics" (PA) has some advantages over the conventional optical spectroscopic detection methods. It permits simple sample pretreatment and it is independent of the opaqueness and scattering of the sample. Finally it permits depth profiling of the sample.

First, principles of photoacoustic spectroscopic (PAS) measurement technique is summarized. To show its applicability for investigation of food samples photoacoustic spectra of paprika powder and fresh paprika were measured and evaluated including depth profile measurements on the fresh sample.

In order to support practical applicability of the technique a new simple contact probe was developed that permits measurement to be carried out without any sample pretreatment.

PA spectra of paprika and carbon powder were measured both with a conventional gas-microphone cell and the new contact probe.

Keywords: photoacoustic spectroscopy, paprika, food analysis

Photoacoustic spectroscopy (PAS) is a new technique for determining the structure, composition, phase, colour or other molecular parameters of liquids, solids and gases. In a photoacoustic spectroscope the sample is placed in a sealed cell (Fig. 1). During the illumination of the sample by periodically modulated light there is an acoustic signal measured with a microphone placed in the cell. Changing the wavelength of the incident light and measuring the acoustic signal the photoacoustic spectrum of the sample can be detected.

Several reviews have been published on the method in general (ADAMS et al., 1976; ROSENCWAIG, 1980; TAM, 1986) and its application in biology (CAHEN et al., 1980; BUSCHMANN et al., 1984) and for investigation of foods (HENDERSON & BRYANT, 1980).

The technique provides the following main advantages over the conventional types of optical spectroscopy:

— It permits characterization and analysis of highly scattering or opaque materials.

— It gives information not only on the absorption but on the thermal properties of the sample.

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— It permits depth profile analysis.

— It gives information about the non-radiative deexcitation processes occourring within the molecules of the sample.

In order to study applicability of PA technique in the investigation of food samples, the following questions were raised: What information can be gained using PAS in the case of powdered food materials? Can depth profile analysis be carried out on vegetables or fruits? How can the detection methods be simplified for powder or liquid samples?

1. Materials and methods

1.1 Principle of PA effect

The PA effect (sound generation during periodic light illumination) was first detected by BELL, 1880. The signal in the case of the gas-microphone cell (Fig. 1) is a result of four basic physical processes:

— Periodic light absorption which excites the molecules of the sample.

— Yield of non radiative deexcitation processes occurring after absorption. A part of the absorbed light energy is periodically emitted as heat.

— As a result of the periodic heat emission thermal waves are generated at the location of light absorption. While propagating, these thermal waves are damped along a very short pathway. This so called thermal diffusion length μ_s (cm) depends on the period of the excitation. It is given by the following equation:

$$\mu_s = \sqrt{\frac{2\alpha}{\omega}} \text{ (cm)}$$

where α: $\frac{\varkappa}{\varrho c}$ — thermal diffusivity ($cm^2\ s^{-1}$)

ω: angular frequency of excitation (rad s^{-1})

$\varkappa$: thermal conductivity of the sample (J cm^{-1} $sec^{-1}K^{-1}$)

ϱ: density of the sample (g cm^{-3})

c: specific heat of the sample (J $g^{-1}K^{-1}$)

Only those thermal waves can reach the surface of the sample, that are generated in the region near the surface not deeper than μ_s.

— The periodic heat transport (carried by the thermal waves) causes the periodic expansion of the gas layer adjacent to the sample surface, and it can be detected as periodic pressure variation by a microphone.

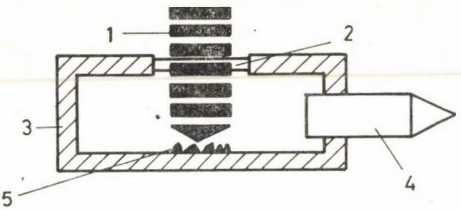

Fig. 1. Photoacoustic effect in a closed cell. 1: chopped light; 2: window; 3: cell wall; 4: microphone; 5: sample

1.2. The spectroscope

The schematic diagram of the photoacoustic spectroscope can be seen in Fig. 2.

For excitation a high-power (500W) Xenon-lamp with grating-monochromator Jobin-Yvon H-20 or 35, for detection

— a commercial available Princeton Applied Research, Type: 6003 photoacoustic cell, and a

— contact PAS detector built at TU Bp. (see para. 1.3) were used.

The signal of a detector was fed into a lock-in amplifier. To eliminate intensity variation of the incident light on the sample due to changing the wavelength, the light intensity was monitored using a pyroelectric detector.

Detailed description of the spectroscopes used in the following experiments can be found in: KOCSÁNYI and co-workers (1986), NAGEL and co-workers (1987).

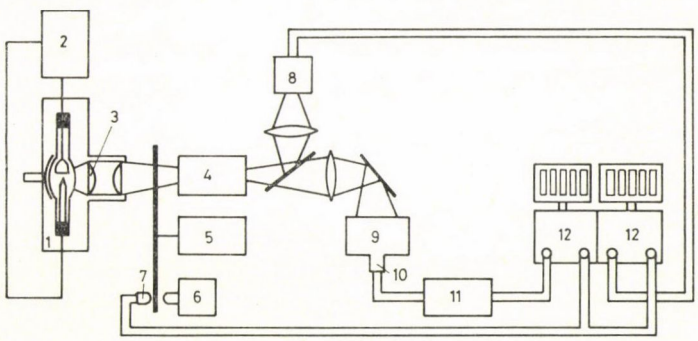

Fig. 2. Schematic diagram of a photoacoustic spectrometer 1: xenon-arc lamp; 2: lamp power supply; 3: condenser; 4: monochromator; 5: lightchopper; 6–7: reference signal sender; 8: pyroelectric detector; 9: PA-cell; 10: microphone; 11: amplifier; 12: lock-in amplifier

1.3. The contact PAS detector

An advantage of the PAS technique is that it can be used without sample preparation, however the fact that the sample must be placed in a small closed acoustically isolated cell makes PAS acceptable for laboratory measurements only.

The aim of designing a contact detector was to simplify PA detection. It has the perspective of making PAS detection applicable among industrial circumstances, as well.

The principle of the contact cell operation is the following (the structure of the cell can be seen in Fig. 3).

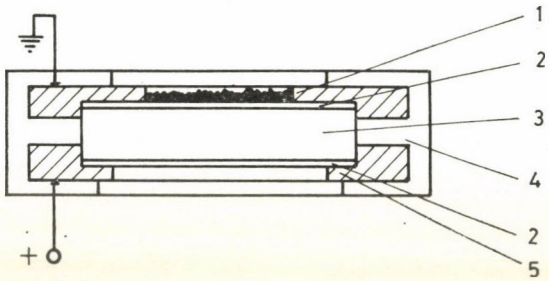

Fig. 3. Cross section of the contact detector. 1: sample; 2: electrodes: 3: piezoelectric material; 4: isolating holders; 5: metal contact

Instead of placing the sample into a closed cell it is simply brought into contact with a transparent piezoelectric detector. The periodic illumination occurs through the detector (Fig. 3). The detector absorbs only a negligible part of the light energy relative to that absorbed by the sample.

The basic physical processes in this case are different from that of the gas microphone cell. Steps I and II are the same, but the thermal waves generated in the sample heat the detector, resulting in a periodic charge devision on the electrodes according to the principle of pyroelectricity. The detector is connected to a current controlled amplifier, which can be directly connected to the input of the lock-in amplifier. The exact theoretical treatment of this cell will be published elsewhere.

1.4. Material and measuring circumstances

Red ground paprika (Ostmann, Bielefeld FRG "edelsüss") and fresh paprika fruits *(Capsicum annuum)* were taken as samples for the photoacoustic study.

The spectra were measured between 380 and 720 nm in steps of 5 nm with waiting times of 10 s for the powder and 30 s for the fruit. The excitation

light was applied with a modulation frequency of 22 Hz, 88 Hz and 515 Hz, respectively. The time constant of the two lock-in amplifiers was set to 3 s for the powder and to 10 s for the fruit. The slit width of the monochromator was 1.75 mm corresponding to 5.25 nm spectral dispersion. The spectra were normalized by dividing with the spectra of carbon black of the according chopping frequency.

Here it has to be pointed out that PAS is always a relative measuring method. A normalized PAS spectrum corresponds to a signal originating from the sample normalized to the signal originating from a reference material. The absolute spectrum of carbon black in the visible and NIR range is proportional to the lamp power spectrum, so it is preferably chosen as reference material.

For the investigation of the applicability of the contact detector absolute spectra of carbon black and paprika powder of Kalocsa (Hungary) were used.

2. Results

2.1. Measurements with the closed PA cell

The photoacoustic spectra of the red paprika powder are similar for all the three chopping frequencies of the excitation light (Fig. 4). They are characterized by a broad and high maximum between 420 and 550 nm. The spectra

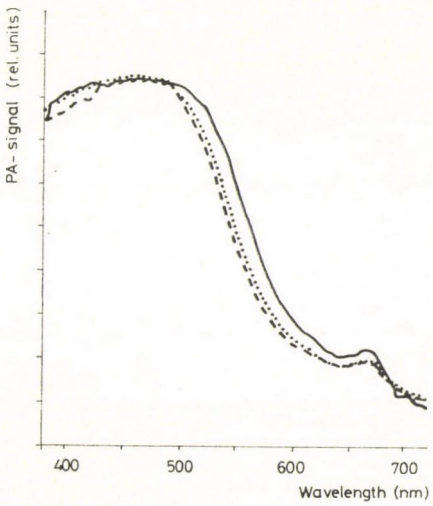

Fig. 4. Photoacoustic spectra of red paprika powder taken at 22 Hz (———) 210 Hz (. . . .) and 515 Hz (– – – –) modulation frequency of the excitation light. The spectra were normalized to the same height at 465 nm

decline towards longer wavelengths and show a small maximum at about 665 nm. With increasing chopping frequency the broad maximum in the blue light region becomes narrower.

The spectra taken at 22 Hz modulation frequency of the excitation light are similar for fruit and powder (Fig. 5). The main difference is that the

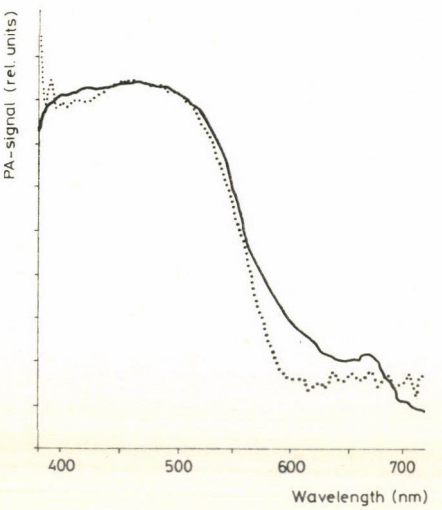

Fig. 5. Photoacoustic spectra of red paprika powder (———) and a red paprika fruit (. . . .) taken at 22 Hz modulation frequency of the excitation light. The spectra were normalized to the same height at 465 nm

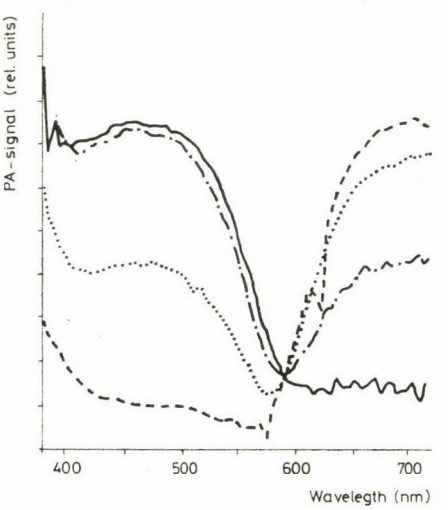

Fig. 6. Photoacoustic spectra of a red paprika fruit taken at 22 Hz (———), 88 Hz ((-·-·-), 210 Hz (. . . .) and 515 Hz (- - - -) modulation frequency of the excitaton light. The spectra were normalized to the same height at 590 nm

spectrum of the fruit has no maximum at 665 nm and its amplitude is lower by a factor of seven compared to the spectrum of the powder. In the photoacoustic spectrum of the fruit the maximum between 420 and 550 nm becomes smaller when increasing the chopping frequency (Fig. 6). In parallel the signal above 650 nm and below 420 nm rises considerably, which appears to be due the increasing contribution of the epidermis.

After removing the epidermis the spectrum taken at 22 Hz modulation frequency remains unchanged compared to the intact fruit (Fig. 7). It is however narrower and shows a decline in the region below 420 nm. Compared to the spectrum at 22 Hz, the spectrum at 515 Hz shows a steep rise above 570 nm (Fig. 8). This rise is also visible in the spectrum of the paprika fruit with epidermis, at 515 Hz (confer Figs. 6 and 8). However the removal of the epidermis leads to a broad maximum around 465 nm, which can also be detected in the spectrum taken at 22 Hz.

2.2. Measurements with the contact detector

To compare the contact PA detector and the gas-microphone cell measurements were made on "carbon black" in the visible region at 22 Hz (Fig. 9). For the exact comparison the signal at the pyroelectric detector was registered, too. The spectra can be seen in Fig. 10.

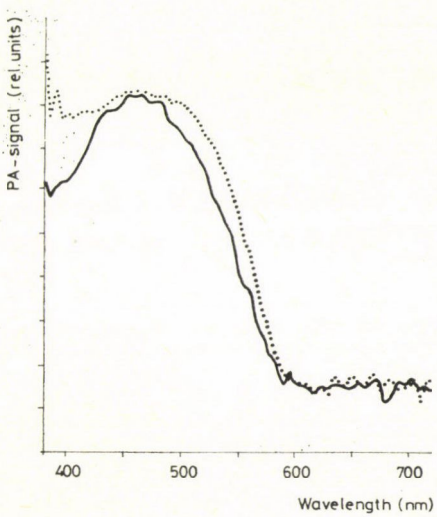

Fig. 7. Photoacoustic spectra of a red paprika fruit before (....) and after (———) removing the epidermis taken at 22 Hz modulation frequency of the excitation light. The spectra were normalized to the same height at 465 nm

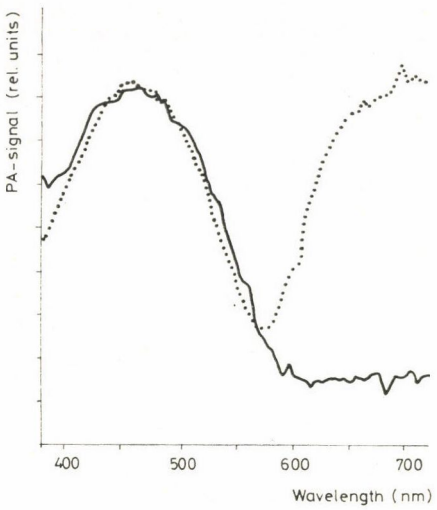

Fig. 8. Photoacoustic spectra of a red paprika fruit after removing the epidermis. The spectra were taken at 22 Hz (———) and 515 Hz (....) modulation frequency of the excitation light. They were normalized to the same height at 465 nm

We can conclude that both detectors give the same PA spectrum and that they are the same as the power spectrum of the xenon lamp taken with a pyroelectric detector.

Normalized PA spectra were taken at 22 Hz on paprika powder samples of Kalocsa using the contact detector. The spectrum can be seen in Fig. 11. A good correlation can be observed between the spectra taken with the closed cell and those taken with the contact detector.

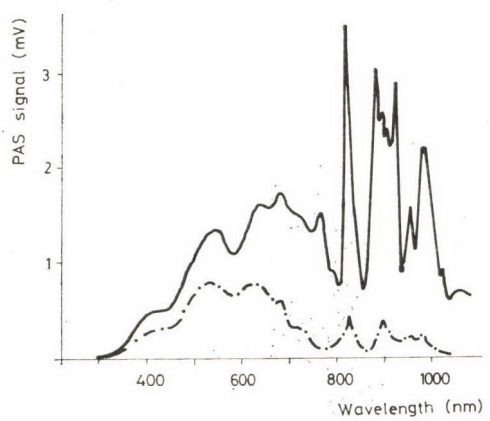

Fig. 9. Absolute photoacoustic spectrum of carbon black at 22 Hz measured by the contact detector (———). Absolute photoacoustic spectrum of red paprika powder of Kalocsa measured by the contact detector (–·–·–)

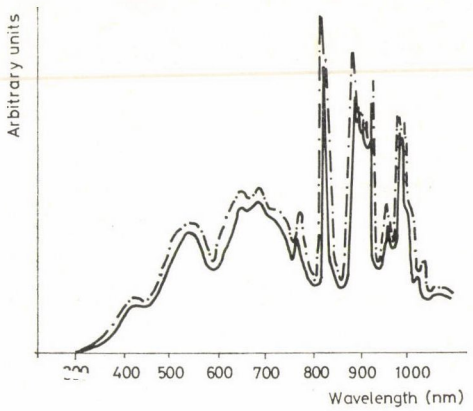

Fig. 10. Photoacoustic spectrum of carbon black at 22 Hz measured by a closed gas-microphone cell ((–·–·–). Lamp power spectrum measured by the pyroelectric detector (———)

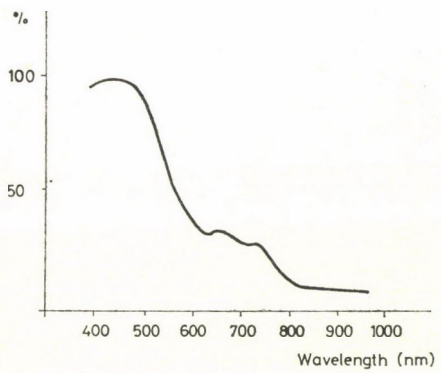

Fig. 11. Normalized photoacoustic spectrum of red paprika powder of Kalocsa measured by the contact detector at 22 Hz chopping frequency

3. Discussion

The photoacoustic spectra of red paprika powder and of the fresh paprika fruit are determined by the absorption maxima of carotenoids in the blue light region around 465 nm (Figs. 4 and 5). According to the literature red paprika fruit contains as main carotenoid components in per cent of total pigments: 31.7% capsanthene, 12.3% β-carotene, 9.8% violaxanthin, 9.2% antheraxanthin, 7.8% β-cryptoxanthin, 7.5% capsorubin, 6.5% zeaxanthin (GOODWIN, 1980).

By changing the modulation frequency of the excitation light one can distinguish photoacoustic spectra emanating from different depth of the

sample. According to the theory of Rosencwaig (ROSENCWAIG, 1973 and ROSENCWAIG & GERSHO, 1976) the photoacoustic signal is detected from deeper inside the sample when the frequency is decreased and thus can be used for a depth profile analysis of inhomogeneous biological materials.

The well ground powder lacks inner cells structure, thus the photoacoustic spectrum does not change very much when applying different chopping frequencies of the excitation light. Because of the saturation effect, which must be taken into account in strongly absorbing samples, the maxima become broader with decreasing chopping frequency (PERKAMPUS, 1982).

The small maximum around 665 nm, which is visible in the powder but does not appear in the fruit, is due to little amounts of chlorophylls. Obviously the fruits used for the preparing of the powder were not fully ripened or were harvested with the stalk attached. The chlorophyll is not in its physiological condition as the maximum position is shifted towards shorter wavelengths compared to an intact green tissue (BUSCHMAN & PREHN, 1981, NAGEL et al., 1987) and thus points to a ruptured chlorophyll–protein complex.

The photoacoustic signal of the powder is seven times higher than the signal of the fruit. This is due to the lack of the heat damping epidermis and especially to the larger surface of the powder which leads to a better transfer from the sample to the gas above the sample (ADAMS et al., 1976).

The photoacoustic signal between 400 and 600 nm decreases with increasing chopping frequency (Fig. 6) as the heat emanates more from the outer parts of the sample (ROSENCWAIG & GERSHO, 1976). Carotenoids, which determine the spectrum in the region, are located in the chromoplasts of the subepidermal layers, the contribution of which becomes smaller, when the modulation frequency is increased. This depth profiling effect has been demonstrated for leaves of Tradescantia, which posses anthocyanins in the epidermis (BUSCHMANN & PREHN, 1983).

In contrast to the expectation the signal in the region between 650 and 720 nm increases with increasing chopping frequency of the excitation light. This effect, which is visible especially at a modulation frequency of 515 Hz, was also demonstrated with needles (NAGEL et al., 1987) and was explained by an enhanced signal emanating from the epidermis after absorption of light reflected from the inner tissue. Reflection is highest in the long wavelength region, where only small amounts of chlorophylls could be absorbing. This absorption of reflected light from deeper inside the tissue is much lower after remowing the epdermis (Fig. 8) than before the removal (Fig. 6; 515 Hz).

The rise of the photoacoustic signal below 380 nm in the spectrum of the fruit, which is visible expecially at higher chopping frequencies (Fig. 6; 210 Hz and 515 Hz) must be explained by an absorption of substances contained in the epidermis or in the cuticula. This becomes also apparent by the lack of this rise in the fruit with the epidermis removed (Fig. 8; 515 Hz).

Finally, the good correlations between the spectra received with the contact detector and the closed gas-microphone cell show that the contact detector for photoacoustic spectroscopic investigation gives simila rresults on powdered material as the closed gas-microphone cell, however, due to the fact that it can be manipulated easier and quicker than the closed PA cell it shows distinct advantages in case of practical testing powdered materials.

*

The authors express their sincere thanks to Prof. PÉTER BIACS and DR. KÁROLY KAFFKA (Central Food Research Institute, Hungary) and Prof. JÁNOS GIBER (Technical University, Department of Atomic Physics, Budapest) for their continuous support in the project "the application of photoacoustic spectroscopy in food science".

Literature

ADAMS, M. J., KING, A. A. & KIRKBRIGHT, G. F. (1976): Analytical optoacoustic spectrometry. — Part I. Instrument assembly and performance characteristics. *Analyst, 101,* 73–85.

BELL, A. G. (1880): Upon the production and reproduction of sound by light. *Telegr. Engineers,* London, *9,* 404–426.

BUSCHMANN, C. & PREHN, H. (1981): In vivo studies of radiative and non-radiative deexcitation processed of pigments in Raphanus by photoacoustic spectroscopy. *Photobiochem. Photobiophys., 2,* 209—215.

BUSCHMANN, C. & PREHN, H. (1983): In vivo photoacoustic spectra of Raphanus and Tradescantia leaves taken at different chopping frequencies of the excitation light. *Photobiochem. Photobiophys., 5,* 63–69.

BUSCHMANN, C., PREHN, H. & LICHTENTHALER, H. K. (1984): Photoacoustic spectroscopy (PAS) and its application in photosynthesis research. *Photosynth. Res., 5,* 29–46.

CAHEN, D., BULTS, G., GARTY, H. & MALKIN, S. (1980): Photoacoustics in life sciences. *J. biochem. biophys. Methods, 3,* 292–298.

GOODWIN, T. W. (1980): *The biochemistry of the carotenoids.* Vol. 1, Plants. Chapman and Hall, New York.

HENDERSON, G. & BRYANT, M. F. (1980): Comparison of near infrared reflectance and photoacoustic spectra of grain samples. *Analyt. Chem., 52,* 1787–1789.

KOCSÁNYI, L., RICHTER, P. & KAFFKA, K. (1986): A fotoakusztikus spektroszkópia (PAS) és alkalmazása biológiai anyagokon. (Photoacoustic spectroscopy and its application with biological substances.) *Finommechanika-Mikrotechnika, 25.* 192–195.

NAGEL, E. M., BUSCHMANN, C. & LICHTENTHALER, H. K. (1987): Photoacoustic spectra of needles as an indicator for the activity of the photosynthetic apparatus of healthy and damaged conifers. *Physiologia Pl.* (in press)

PERKAMPUS, H. H. (1982): Photo-Akustik-Spektroskopie an festen Stoffen. *Naturwissenschaften, 69,* 162–172.

ROSENCWAIG, A. (1973): Photoacoustic spectroscopy of solids. *Optics Commun., 7,* 305–308.

ROSENCWAIG, A. (1980): *Photoacoustics and photoacoustic spectroscopy in chemical analysis.* Vol. 57. John Wiley & Sons, London,

ROSENCWAIG, A. & GERSHO, A. (1976): Theory of the photoacoustic effect with solids. *J. appl. Phys., 47,* 64–69.

TAM, A. C. (1986): Applications of photoacoustic sensing techniques. *Rev. mod. Phys., 58,* 381–420.

Acta Alimentaria, Vol. 17 (3), pp. 239–244 (1988)

PIGMENT EXTRACTION FROM ALFALFA PROTEIN CONCENTRATES

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(Received: 21 May 1987; accepted: 8 September 1987)

The increasing demand for food-grade proteins makes the use of leaf protein concentrates (LPC) an interesting approach. LPC have good amino acid profiles but their organoleptic characteristics, above all the green colour and the grassy flavour, strongly limit their use.

Nine pure solvents and seven solvent mixtures were tested in order to verify their suitability for improving the organoleptic characteristics of LPC. Sensory evaluation was carried out on LPC after a solvent extraction period of 6, 12 and 18 h.

Polar solvents, especially acetone and propane-2-ol, gave the best results, both for colour and flavour, but none of them was able to reduce the negative characteristics to such an extent that could make possible the use of LPC in human nutrition.

Keywords: alfalfa, leaf protein concentrates, pigments, solvent extraction

One of the main aims of food research is to satisfy the present and foreseeable protein demand by incrasing and improving vegetable protein production. For this reason much research has also been carried out on photosynthetic tissues (leaves)), whose protein fraction is characterized by a good essential amino profile (Gerloff et al., 1965; Anelli & Galoppini, 1974).

Leaf protein extraction technologies are based on wet green crop fractionation. By squeezing the grassy material, it is possible to obtain a juice with more than 10% of dry matter and a very high protein content (30–40% dry basis), thus allowing it for the preparation of leaf protein concentrates (LPC).

As far as their amino acid profile is concerned, LPC could be used as a source of protein in human nutrition, but the organoleptic characteristics (mainly colour and flavour) limit their use. Because of the pigment content (chlorophylls and carotenoids) and products formed by polyphenol oxidation and/or by Maillard reaction, the LPC colour goes from deep green to black, while its strong grassy flavour is closely correlated to the lipid fraction and to products formed during the oxidation of natural unsaturated fatty acids (Bray et al., 1978).

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Practical use of LPC for human consumption is therefore strictly dependent on the reduction of the negative organoleptic properties. Even if good laboratory results are obtained, problems still arise in industrial production of food-grade LPC. This is mainly due to the lack of suitable and economical processes for obtaining inexpensive final products having the desired organoleptic properties.

Because of rigorous legislation on food additivies at international level, the food industry is deeply concerned with the use of natural pigments instead of artificial ones. The recovery and commercialization of the pigments contained in LPC could be helpful in solving the economic problems arising in LPC production.

At the Istituto di Industrie Agrarie of the University of Pisa current research is tackling these problems, using both traditional and unusual solvents, such as near-critical and supercritical gases. This note reports the results obtained in preliminary tests carried out using traditional organic solvents.

1. Materials and methods

Taking into account results obtained by other authors (BRAY & HUMPHRIES, 1978; BRAY et al., 1978), 5 polar, 4 non-polar organic solvents and 7 mixtures of them were chosen in order to evaluate their extraction efficiency (Table 1).

LPC were prepared from alfalfa (*Medicago sativa* L.) harvested in preflowering. After washing in a 0.1% sodium metabisulfite solution, the grass was chopped and squeezed in a pilot plant (FIORENTINI & GALOPPINI, 1981) and the collected juice centrifuged (4200 × G, retention time 15 s) in order to separate any remaining fibre particles (LENCIONI et al., 1984). The juice was then heated to 85–90 °C by steam injection to obtain a protein coagulum

Table 1

Tested solvents and mixtures

Polar	Non-polar	Mixtures (v/v)
Acetone	Chloroform	Acetone/Chloroform (1 : 1)
Methanol	Hexane	Acetone/Hexane (1 : 1)
Ethanol	Ethyl acetate	Ethanol/Chloroform (1 : 1)
Propane-2-ol	Diethyl ether	Ethanol/Diethyl ether (3 : 1)
Butane-1-ol		Propane-2-ol/Chloroform (1 : 1)
		Propane-2-ol/Hexane (1 : 1)
		Propane-2-ol/Diethyl ether (3 : 1)

Table 2

Terms used in the sensory evaluation and associated number

Colour		Grassy flavour	
Term	Number	Term	Number
White	0	Absent	0
Pale white-gray	1	Weak	1
White-gray	2	Moderate	2
Pale green-gray	3	Strong	3
Pale green	4	Very strong	4
Green	5		
Deep green	6		

that was recovered by centrifugation (2000×G, retention time 5 min) and stored at −20 °C.

Extraction trials were performed using a Soxhlet apparatus at the boiling point of the pure solvents or mixtures. Total extraction time was 18 h, but after 6 and 12 h 10 g of wet LPC were sampled and the solvent renewed. Samples were dried overnight under vacuum (20 °C, 600 Pa) and stored at −20 °C.

The solvent efficiency was assessed by sensory evaluation of colour and flavour of the extracted LPC (AMERINE et al., 1965; ANELLI et al., 1984). Six panelists had the choice of 5 adjectives for flavour and 7 for colour (Table 2). The most frequent adjective chosen by the panel was selected as the colour or flavour of the sample and described by an arbitrary number shown in the histograms.

2. Results and discussion

Sensory evaluation data for pure solvents and mixtures are reported in Figs. 1–3. Each set of histograms represents the attribute score of the sample with regard to its colour (white histograms) and flavour (dashed histograms), after an extraction period of 6, 12 and 18 h, respectively.

As far as mixtures are concerned only those containing propane-2-ol and/or diethyl ether gave a good decrease both in grassy flavour and green colour (Fig. 1). Propane-2-ol and diethyl ether mixture (3 : 1 v/v) gave the best results after 12 h of extraction. The use of other mixtures was generally unsatisfactory because appreciable decolourization was not achieved even if some of them induced a substantial decrease in grassy flavour.

Pure non-polar solvents showed a similar extraction activity with no or very slight decolourization and a relatively high score for flavour. Only hexane after 18 h of extraction had stronger effects (Fig. 2).

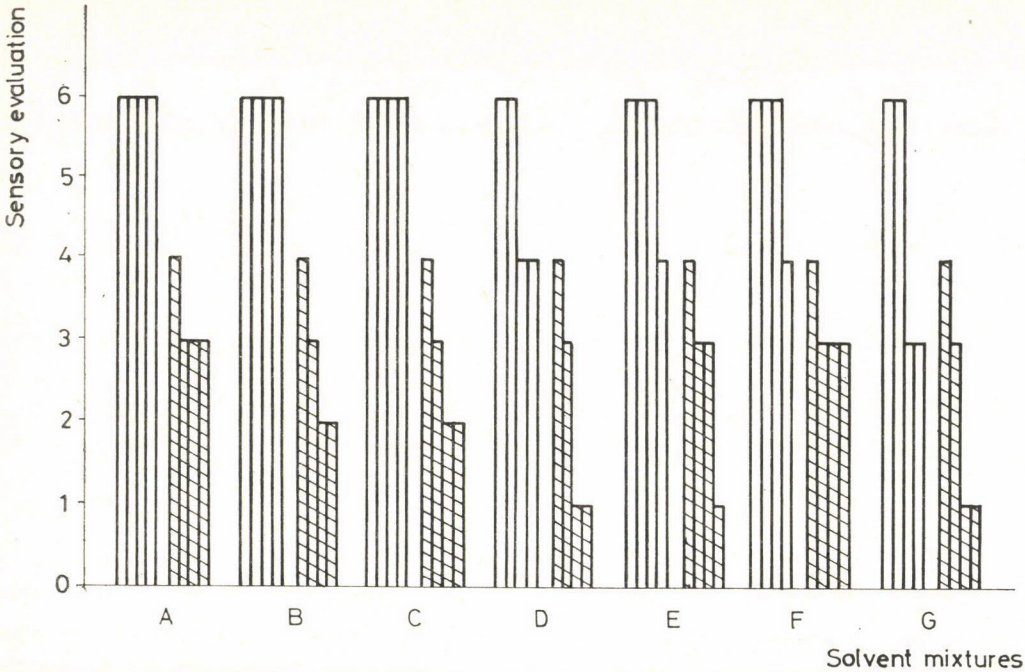

Fig. 1. Extraction efficiency of mixtures (A = acetone/chloroform 1 : 1; B = acetone/hexane 1 : 1; C = ethanol/chloroform 1 : 1; D = ethanol/diethyl ether 3 : 1; E = propane-2-ol/chloroform 1 : 1, F = propane-2-ol/hexane 1 : 1; G = propane-2-ol/diethyl ether 3 : 1). Data are referred to colour (white histograms) and flavour (dashed histograms) sensory evaluation after 0, 6, 12 and 18 h of extraction. For sensory evaluation description see text and Table 2

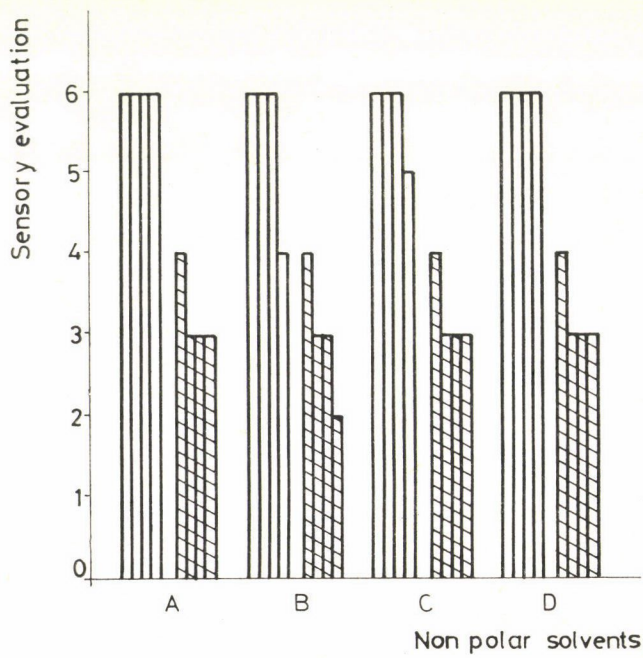

Fig. 2. Extraction efficiency of non-polar solvents (A = chloroform; B = hexane; C = ethyl acetate; D = diethyl ether). Data are referred to colour (white histograms) and flavour (dashed histograms) sensory evaluation after 0, 6, 12 and 18 h of extraction. For sensory evaluation description see text and Table 2

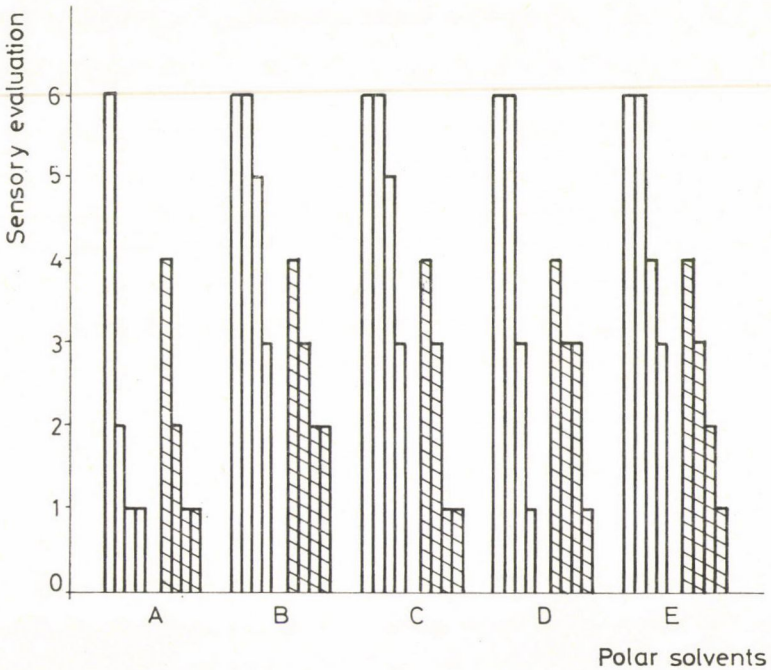

Fig. 3. Extraction efficiency of polar solvents (A = acetone; B = methanol; C = ethanol; D = propane-2-ol; E = butane-1-ol). Data are referred to colour (white histograms) and flavour (dashed histograms) sensory evaluation after 0, 6, 12 and 18 h of extraction. For sensory evaluation description see text and Table 2

Polar solvents gave the best results both for colour and flavour (Fig. 3). Within an 18 h extraction period the flavour was strongly reduced to "weak" and using only methanol it was evaluated as "moderate". Colour was at least reduced to the best values obtained with non-polar solvents and mixtures (pale green-grey). The highest amount of decolourization was achieved using acetone and propane-2-ol (score 1: pale white-grey), but comparing the data, a different behaviour between the two solvents can be seen. Acetone induced the greatest decrease in colour and flavour in the shortest time, reaching the best values in only 12 h.

These results are in accord with similar tests carried out by other authors (BRAY, 1976; BRAY et al., 1978), and sensory evaluation tests show that with organic solvents it is possible to obtain appreciable reduction of the green colour and grassy flavour of LPC. However, these organoleptic characteristics are still too strong to allow a large scale use of LPC in food formulation, especially in western countries.

Literature

AMERINE, A. M., PANGBORN, R. M. & ROESSLER, E. B (1965): *Principles of sensory evaluation of food*. Academic Press, New York.

ANELLI, G., FAVATI, F., FIORENTINI, R. & MENCARELLI, F. (1984): Maturazione e qualita' dei prodotti ortofrutticoli. — in: POMA TRECCANI, C. FIORENTINI, R. (Eds) *Ortofrutticoli freschi*. Monografia n. 2. C.N.R., Roma, pp. 11–43.

ANELLI, G. & GALOPPINI, C. (1974): Proteine dalle erbe. *Riv. It. Sost. Grasse, 51*, 208–211.

BRAY, J. W. (1976): The processing of leaf protein to obtain food-grade products. — in: WILKINS, R. J. (Ed.) *Green crop fractionation. Proc. Symp.* Harrogate, pp. 107–116.

BRAY, J. W. & HUMPHRIES, C. (1978): Solvent fractionation of leaf juice to prepare green and white protein products. *J. Sci. Fd Agric., 29*, 839–84.

BRAY, J. W., HUMPHRIES, C. & INERITEI, M. S. (1978): The use of solvents to decolories leaf protein concentrates. *J. Sci. Fd Agric., 29*, 165–171.

FIORENTINI, R. & GALOPPINI, C. (1981): Pilot plant production of an edible alfalfa protein concentrate. *J. Fd Sci., 46*, 1514–1520.

GERLOFF, E. D., LIMA, I. H. & STAHMAN, M. A. (1965): Amino acid composition of leaf protein concentration *J. agric. Fd Chem., 13*, 139–143.

LENCIONI, L., FIORENTINI, R. & GALOPPINI, C. (1984): Caseificazione di latte di pecora miscelato con succo di erba medica. *Ind. Aliment., 23*, 106–111.

Acta Alimentaria, Vol. 17 (3), pp. 245—255 (1988)

CHARACTERIZATION OF EXTRUDED FULL FAT SOY FLOUR FOR HUMAN CONSUMPTION

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(Received: 12 June 1987; accepted: 23 October 1987)

Full fat soy flour was prepared from dehulled soybean (Glycine max) by extrusion used in the industry (Insta-Pro Extruder made in Hungary). It was characterized by different chemical and biochemical methods in order to determine the possibilities of its application in food products.

The main components of the extruded soy flour was the following: water content: 5.8 g per 100 g matter; protein content: 37.3 g per 100 g dry matter; oil content: 24.6 g per 100 g dry matter.

The activities of trypsin inhibitor, lipoxygenase and peroxidase in extruded soy flour are significantly lower than in soybean, most of them being destroyed by the high temperature (140 °C) of extrusion.

In the course of extrusion peroxide and acid values increased, but this did not affect negatively the shelf life of extruded full fat soy flour.

Water and fat binding capacity are higher, emulsification properties and Nitrogen Solubility Index (NSI) are lower in extruded soy flour than in soybean. Heat treatment denatures soy proteins, and the number of hydrogen and hydrophobic bonds increases. This is demonstrated by different solubilities of soy proteins in different solvents and buffers.

After extrusion lysine and sulfuric amino acid contents decreased, in vitro protein digestibility increased. The biological value of full fat soy flour characterized by NPU and NPR is higher than the biological value of dehulled soybean.

Keywords: soybean, extruded full fat soy flour, extrusion, solubility of soy protein, biological value

Different soy products (grits, flours, concentrates, texturized products, isolates, etc.) are becoming more and more important protein sources for direct human consumption all over the world.

Research work has been done first of all on soybean, soy concentrates and isolates. Nevertheless, full fat soy flour can also be taken into consideration as an economical and widely available source of protein and energy (MUSTAKAS et al., 1970).

Full fat soy flour beside some of its disadvantages (relatively poor shelf life, stability, raffinose and stachyose content) is of advantageous quality having high vitamin, fibre, mineral and unsaturated fatty acid contents (LORENZ et al., 1980).

LAM-SANCHEZ and co-workers (1985) also recommended the extrusion process to make high quality full fat soybean flour. Three cone openings and

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three initial moisture content values were used to evaluate their effects on the protein quality of full fat soy flour, extruded in a Brady Crop Cooker. They concluded that to destroy the antiphysiological factors in whole soybeans with the extruder used by them, soy flour has to be processed at a higher temperature with low initial moisture content.

In a Hungarian factory in Törökszentmiklós full fat soy flour is being processed for food purposes by extrusion in an Insta-Pro extruder (USA). The parameters of temperature, moisture and retention time are constant. This extrusion process offers many unique features enabling it to become important in converting soybeans into food products. Characterization of this extruded full fat soy flour in a complex way is needed to determine the applicability of it in food production.

In this work quality differences between dehulled soybean and extruded soy flour were examined in order to determine changes caused in soy flour by extrusion processing, as the result of high pressure and temperature.

Chemical composition, functional properties, enzyme activities, peroxide and acid values, protein solubility and biological value are presented in this paper.

1. Materials and methods

1.1. Soy samples

Soybeans were steamed (127 °C, 3 min), dehulled, and passed through an air flow to remove seed coats.

The initial moisture content of soybeans before extrusion was: 12.5% (w/w).

Dehulled soybeans were extruded in an Insta-Pro extruder. Parameters of the extruder:

Rotation of screw per min: 500 r.p.m.
Diameter of screw: 95 mm
Peripherical speed of screw: 2.5 m sec^{-1}
Temperature of extrusion: 140 °C
Average residence time: 30 s
Extruded soybeans were cooled and ground to full fat flour (0–600 μm).

1.2. Analytical methods

1.2.1. Determination of chemical composition. Moisture content was determined by drying the samples at 105 °C to constant weight. Protein content ($N \times 6.25$) was measured with an automatic Kjell-Foss equipment.

Oil content was determined by petroleum ether extraction in a Soxhlet apparatus.

Ash content was determined by incinerating the samples at 550 °C to constant weight.

1.2.2. Determination of trypsin inhibitor, lipoxygenase and peroxidase activities. The trypsin inhibitor activity was determined according to the method of KAKADE and co-workers (1974), adapted by PETRES and KÁRPÁTI 1981).

The peroxidase activity was determined by the method of MIHÁLYI and VÁMOS-VIGYÁZÓ (1975), the lipoxygenase activity by the method of TAPPEL and co-workers (1952).

1. 2. 3 Determination of peroxide and acid values. Peroxide and acid values were measured according to Hungarian Standard (1978). Acid value: mg KOH needed to neutralize 1 g oil. Peroxide value: cm^3 of 1 mol dm^{-3} $Na_2S_2O_3$ solution equivalent to iodine selected by 1000 g oil.

1.2.4. Determination of functional properties. Functional properties were analyzed by the folllowing procedures. Water binding capacity by the method of SOSULSKI (1962), fat binding capacity by the method of LIN and co-workers (1974), emulsification activity and emulsion stability by the method of YASUMATSU and co-workers (1972). Nitrogen Solubility Index (NSI) by the method of SMITH and CIRCLE (1972).

1. 2. 5. Extraction and fractionation of soy proteins. The fat free soy samples were extracted by the OSBORNE method (1924).

The fat free soy samples were also extracted with 0.5 mol dm^{-3} Na_2HPO_4 (pH = 7.8) containing either 5% (w/v) sodiumdodecyl sulfate (SDS) or 5% (w/v) SDS and 1% (w/v) 2-mercapto-ethanol (2-ME).

1.2.6. Determination of amino acid content. Amino acid values were obtained by analysis of the acid hydrolysates of the proteins with amino acid analyzer Biotronik LC 2000 (FRG).

Tryptophan was determined using the method of RÉKÁSI-SZOMBATI and co-workers (1979).

1.2.7. Determination of in vitro biological values. In vitro protein digestibility was measured by the method of AKESON and STAHMAN (1964).

Chemical Score (CS) values were calculated by the method of BLOCK and MITCHELL (1946) using FAO/WHO (1973) amino acid patterns as reference.

Computed Protein Efficiency Ratio (C-PER) values were calculated by the method of SATTERLEE and co-workers (1979).

1.2.8. Determination of in vivo biological values. Net Protein Ratio (NPR) and Net Protein Utilization (NPU) values were determined in rat feeding experiments.

2. Results and discussion

2.1. Chemical composition

Chemical composition of dehulled soybean and extruded full fat soy flour (EFFSF) is given in Table 1.

Table 1

Chemical composition of dehulled soybean and extruded full fat soy flour

	Dehulled soybean		Extruded full fat soy flour	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Water content (g per 100 g matter)	8.3	0.1	5.8	0.1
Protein content (g per 100 g dry matter)	37.6	0.3	37.3	0.3
Oil content (g per 100 g dry matter)	25.3	0.1	24.6	0.1
Ash content (g per 100 g dry matter)	5.3	0.1	5.3	0.1

$\bar{x}$: mean value of three measurements
$\pm s$: standard deviation

During extrusion the moisture content decreased because of the high temperature applied. Protein, oil and ash contents of the samples are given related to dry matter. Protein ($N \times 6.25$) and ash contents of soy did not change during extrusion. The oil content of soy flour decreased because some of the free oil was expelled from the soybean during extrusion. Our results agree with those obtained by other workers (HARPER & DEL VALLE, 1979).

2.2. Tripsin inhibitor, lipoxygenase and peroxidase activities

Heat treatment of soybeans is necessary for the destruction of trypsin inhibitor as the main antinutritive factor in raw beans, for inactivation of the enzymes lipoxygenase and peroxidase in order to increase storage life, for removal of the raw, bitter and beany flavour of raw beans (KOUZEH-KANANI et al., 1981).

Table 2 shows the activity of trypsin inhibitor, lipoxygenase and peroxidase in soybean and the corresponding full fat flour.

EFFSF shows a decrease in these activities. These enzymes and inhibitor are thermolabile and the extrusion temperature destroyed the greater part of them. Lipoxygenase and peroxidase activities are two important factors that affect organoleptic qualities as well (RACKIS et al., 1975). They can cause

Table 2

Trypsin inhibitor, lipoxygenase and peroxidase activities dehulled soybeans and extruded full fat soy flour

	Trypsin inhibitor activity (TIU mg^{-1} dry matter)		Lypoxygenase activity (1000 U mg^{-1} dry matter)		Peroxidase activity (1000 U mg^{-1} dry matter)	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Dehulled soybean	90.7	7.3	1786.0	44.0	67.3	1.5
Extruded full fat soy flour	31.6	3.0	4.0	0.3	5.5	0.2

$\bar{x}$: mean value of three measurements
$\pm s$: standard deviation

oxidation of lipids, and off-flavour and bad smell. In this case, however, these enzymes are inactivated, and EFFSF has no off-flavour.

The trypsin inhibitor activity is also decreased and this improves the nutritional quality of EFFSF.

2.3. Peroxide and acid values

During extrusion oxidative processes can take place in soybean. This is indicated by an increase in the acid and peroxide values of EFFSF. Heat treatment can cause autooxidation and destruction of natural antioxidant present in soybeans (MUSTAKAS et al., 1970). Peroxide and acid values of samples are summarized in Table 3.

Our results show that oxidative processes caused by heat did not reach the level where they could affect negatively the organoleptic and storage quality of EFFSF (HORVÁTH et al., 1986).

Table 3

Peroxide and acid values of dehulled soybean and extruded full fat soy flour

	Dehulled soybean		Extruded full fat soy flour	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Acid value (mg KOH, required for neutralization of 1 g oil)	1.3	0.1	3.2	0.4
Peroxide value (cm^3 of 1 mol dm^{-3} $Na_2S_2O_3$ equivalent to iodine selected by 1000 g oil)	0		3.4	0.3

$\bar{x}$: mean value of three measurements
$\pm s$: standard deviation

2.4. *Functional properties*

Applicability of different soy products in food processing could be characterized by their functional properties. Functional properties of the samples are presented in Table 4.

Table 4

Functional properties of dehulled soybean and extruded full fat soy flour

	Water-binding capacity		Fat-binding capacity		Emulsification activity		Emulsion stability		Nitrogen Solubility Index (NSI)	
	(g per 100 g sample)				(%, v/v)				(%, w/w)	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Dehulled soybean	157.8	1.5	106.8	5.9	55.9	7.4	67.7	3.3	40.6	0.3
Extruded full fat soy flour	210.4	9.3	124.3	3.2	45.8	0.2	54.3	6.9	24.2	2.5

$\bar{x}$: mean value of three measurements
$\pm s$: standard deviation

After extrusion the water and fat binding capacity of soy increased, while emulsification properties and Nitrogen Solubility Index (NSI) decreased. CUMMING and co-workers (1973) have shown that extrusion causes a dissociation followed by an aggregation of the water-soluble protein. Decrease of NSI was observed by other workers, as well (BOOKWALTER et al., 1971). It can be concluded from the results that functional properties of EFFSF make it applicable in different food products.

2.5. *Solubility and fractionation of soy proteins*

First the solubility of soy proteins was characterized by the Osborne method. By this method the following fractions were separated: albumins (H_2O-soluble), globulins (NaCl-soluble), prolamins (C_2H_5OH-soluble) and gluteins (KOH-soluble). The resulsts are shown in Table 5.

It is evident that the quality of soluble proteins was reduced and the proportion of fractions was changed by extrusion. After extrusion the proportion of water-soluble proteins decreased while that of NaCl-, and KOH-soluble proteins increased.

Only a part of soy proteins could be extracted by the Osborne-method, so various buffers were also used to extract proteins from dehulled soybean and EFFSF. JEUNINK and CHEFTEL (1979) suggested, that extensive solubilization could be achieved by buffers containing sodium dodecyl sulfate (SDS), an agent known to disrupt non-covalent interactions and dithiothreitol, an agent known to reduce disulfide bonds. According to CLATTERBUCK and co-

Table 5

Fractionation of proteins of dehulled soybean and extruded full fat soy flour by Osborne-method

Dissolved protein fraction	Dehulled soybean (% of total protein)		Extruded full fat soy flour	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Total dissolved protein	37.5	1.5	19.9	1.1
	(% of dissolved protein)			
Water-soluble	58.45	4.0	26.5	1.5
NaCl-soluble	32.7	0.8	50.0	2.6
C_2H_5OH-soluble	3.5	0.2	6.4	1.1
KOH-soluble	7.3	0.3	17.2	2.9

$\bar{x}$: mean value of three measurements
$\pm s$: standard deviation

workers (1980) the presence of urea and/or 2-mercaptoethanol (2-ME) in the buffer causes higher increase in solubility in the fully heat-treated flour than in the minimal heat treated flours. This suggests that heat treatment increased disulfide and hydrogen, and/or hydrophobic bonds in soy flours.

In our work 0.5 mol dm^{-3} Na_2HPO_4 (pH = 7.8) buffer was used containing either 5% SDS (buffer 1) or 5% SDS and 1% 2-ME (buffer 2). The results are shown in Table 6.

Table 6

Solubility of soy proteins in different buffers

Buffers	Dehulled soybean		Extruded full fat soy flour	
	(% of total dissolved protein)			
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Buffer 1				
0.5 mol dm^{-3} Na_2HPO_4 (pH = 7.8) containing 5% SDS				
Extraction 1	22.3	6.1	36.2	6.2
Extraction 2	14.9	6.2	15.5	8.1
Total extraction	37.3	12.1	51.8	2.3
Buffer 2				
0.5 mol dm^{-3} Na_2HPO_4 (pH =7.8) containing 5% SDS + 1% 2-ME				
Extraction 1	72.7	16.6	83.1	4.8
Extraction 2	12.5	7.9	7.2	5.9
Total extraction	85.2	9.6	90.3	6.5

$\bar{x}$: mean value of three measurements
$\pm s$: standard deviation

The total protein extracted by buffer from EFFSF was higher than that extracted from dehulled beans. If the buffer contained 2-ME beside SDS more protein was extractable. With buffer 2 a greater part of proteins could be extracted in the first fraction from both samples.

These findings do not agree with the results obtained by BURGESS and STANLEY (1976). Their data show that urea, SDS and 2-ME are less effective with textured soy than with soy grits. But they used a higher extrusion temperature (178 °C) and only 1.5% SDS in the buffer solution.

Our results as shown in Tables 5 and 6 prove that the structure of soy proteins had changed during extrusion as an effect of heat treatment. The quantity of hydrogen and hydrophobic bonds increased and special detergents and disulfide cleaving agents are needed to disrupt these bonds. This is in agreement with the results reported by HAGER (1984).

2.6. Essential amino acid composition of soy proteins

Essential amino acid contents of samples are presented in Table 7.

Table 7

Essential amino acid[a] profile of proteins of dehulled soybean and extruded full fat soy flour

	LYS	THR	SULP	VAL	ILE	LEU	AROM	TRP
	(g amino acid 100 g^{-1} protein)							
Dehulled soybean	6.8	4.5	3.2	4.8	4.7	7.7	7.3	1.3
Extruded full fat soy flour	6.7	4.4	2.9	4.8	4.6	7.6	7.2	1.3

[a] Abbrevations for amino acids: LYS: lysine, THR: threonine, SULP: methionine + cystine' VAL: valine, ILE: isoleucine, LEU: leucine, AROM: phenylalanine + tyrosine, TRP: tryptophan

The high temperature of extrusion caused some changes in the essential amino acid profile of soy proteins. The content of lysine and sulfuric amino acids (methionine + cystine) decreased. This is unfavourable, because, as it is known, soy protein is deficient in sulfuric amino acids and its relatively high lysine content enables the complementation of cereal proteins with soy proteins (HOPKINS & STEINKE, 1981).

The content of other essential amino acids did not change.

2.7. Biological values of soy proteins

According to results reported in the literature (MUSTAKAS et al., 1970; BOOKWALTER et al., 1971; LORENZ et al., 1980) extruder processing improves the nutritional quality of soybean. Protein Efficiency Ratio (PER) increases

Table 8

Biological values of protein of dehulled soybean and extruded full fat soy flour

	Dehulled soybean	Extruded full fat soy flour
In vitro biological values		
chemical score (%)	90.9	83.4
limiting amino acid	SULP	SULP
computed PER (g per g)	2.1	2.3
in vitro protein digestibility (%)	71.9	85.5
In vivo biological values		
NPU (%)	50.9	62.7
NPR (g per g)	2.6	3.3

progressively. In vitro and in vivo nutritive values of our samples are shown in Table 8.

In vitro protein digestibility of EFFSE is higher than in vitro protein digestibility of dehulled soybeans. The reason for this may be found in inactivation of the trypsin inhibitor and the better digestibility of denatured soy proteins (BOONVISUT & WHITAKER, 1976).

In vitro biological values: Chemical Score (CS) and Computed Protein Efficiency Ratio (C-PER) were calculated on the basis of the essential amino acid content. On calculating C-PER the in vitro protein digestibility is taken into consideration as well.

CS of the protein in EFFSF is lower than CS of protein in dehulled soybean, because of the lower lysine, methionine, and cystine contents. At the same time the C-PER value of EFFSF is higher than the C-PER of dehulled soybean.

In vivo biological experiments also support the higher biological value of extruded soy flour. Both Net Protein Utilization (NPU) and Net Protein Ratio (NPR) of EFFSF are higher than NPU and NPR of dehulled bean. These results prove that all factors affecting nutritive value could be taken into consideration in a complex way.

3. Conclusion

Our results show that a good quality full fat soy flour applicable in food production can be produced by extrusion-cooking in an Insta-Pro extruder, under parameters as used in the factory for extrusion. The biological value of soy protein increased after extrusion in spite of the decreasing quantity of lysine and sulfuric amino acid.

The protein digestibility of soy also incresed in consequence of partial inactivation of the trypsin inhibitor and protein denaturation. The soy protein was denatured because of the effect of high temperature. Thus the functional properties of soy also changed, particularly NSI decreased after extrusion. The solubility of soy protein in water was limited. Detergents and reducing agents are needed to solubilize soy proteins.

The activities of lipoxygenase and peroxidase in extruded soy flour are significantly lower than in the soybean, and this improved the flavour of soy and made the product suitable for use in human foods.

*

The authors wish to thank A. MARTIN, Enterprise for Production of Feeds from Animal By-products, Budapest for amino acid analysis, K. CZULEK, Department of Enzymology, Central Food Research Institute, Budapest for the analysis of lipoxygenase and peroxidase activity, and M. HEGEDÜS, Department of Animal Nutrition, University of Veterinary Science, Budapest for determination of in vivo biological values.

Literature

AKESON, W. R. & STAHMAN, M. A. (1964): A pepsin pancreatin digest index of protein quality evaluation. *J. Nutr., 83,* 257–261.

BLOCK, R. I. & MITCHELL, H. H. (1946): The correlation of the amino acid composition of proteins with their nutritive value. *Nutr. Abstr. Rev., 16,* 240–278.

BOOKWALTER, G. N., MUSTAKAS, G. C., KWOLEK, W. F., MCGHEE, J. E. & ALBRECHT, W. J. (1971): Full fat soy flour extrusion cooked: properties and food uses. *J. Fd Sci., 36,* 5–9.

BOONVISUT, S. & WHITAKER, J. R. (1976): Effect of heat, amylase, and disulfide bond cleavage on the in vitro digestibility of soybean proteins. *J. agric. Fd Chem., 24,* 1130.

BURGESS, L. D. & STANLEY, D. W. (1976): A possible mechanism for thermal texturization of soybean protein. *Can, Inst. Fd Sci. Technol. J., 9,* 228–231.

CLATTERBUCK, K. L., KEHRBERG, N. L. & MARABLE, N. L. (1980): Solubility and in vitro digestibility of soy flours, concentrates and isolates. *J. Fd Sci., 45,* 931–935.

CUMMING, D. B., STANLEY, D. W. & DE MAN ,J. M. (1973): Fate of water soluble soy protein during thermoplastic extrusion. *J. Fd Sci., 38,* 320.

FAO/WHO (1973): *Energy and protein requirements.* Report of a Joint FAO/WHO ad hoc Expert Committee on Energy and Protein Requirements. WHO, Geneva.

HAGER, D. F. (1984): Effects of extrusion upon soy concentrate solubility. *J. Agric. Fd Chem., 32,* 293–296.

HARPER, J. & DEL VALLE, F. R. (1979): Mexican soy plant. *Fd Engng. Int.,* (July) 22–23.

HOPKINS, D. T. & STEINKE, F. H. (1981): Uses of soy protein in mixed protein systems to meet nutritional needs. *J. Am. Oil Chem. Soc.,* 452–455.

HORVÁTH, E., PETRES, J. & CZUKOR, B. (1986): Szója eltarthatóságával kapcsolatos vizsgálatok (I). Teljes olajtartalmú extrudált szójaliszt eltarthatóságának vizsgálata. (Essay into the storability of soybeans. — Part I. Storage stability of extruded full fat soy flour.) *Gabonaipar, 33,* 7–12.

HUNGARIAN STANDARD (1978): Takarmányok tápláló értékének megállapítása. Peroxidszám, savszám meghatározása. (Animal feeding stuff. Determination of nutritive value. Determination of peroxide value and acidity). MSz 6830/11-78.

JEUNINK, J & CHEFTEL, J. C. (1979): Chemical and physicochemical changes in field bean and soybean proteins texturized by extrusion. *J. Fd Sci., 44,* 1322–1325, 1328.

KAKADE, M. L., RACKIS, I. J., MCGHEE, J. E. & PUSKI, G. (1974): Determination of trypsin inhibitor activity of soy products. A collaborative analysis of an improved procedure. *Cereal Chem., 51,* 376–382.

KOUZEH-KANANI, M., VAN ZUILICHEM, D. J., ROOZEN, J. P. & PILNIK, W. (1981): A modified procedure for low temperature infrared radiation of soybeans. — Part I. Improvement of nutritive quality of full-fat flour. *Lebensm. Wiss. Technol., 14,* 242–244.

LAM-SANCHEZ, A., BRESSANI, R., MOLINA, M. R., ELIAS, L. G., GONZALEZ, J. M. & DURIGAN, J. F. (1985): Effects of cone-opening, initial moisture content and multiplex extrusion on the protein quality of extruded soybean using the Brady Crop Cooker. *Archos. lat.-am. Nutr., 35,* 447–457.

LIN, M. J. Y., HUMBERT, E. S., & SOSULSKI, F. W. (1974): Certain functional properties of sunflower meal products. *J. Fd Sci., 39,* 368–370.

LORENZ, K., JANSEN, G. R. & HARPER, J. (1980): Nutrient stability of full fat soy flour and corn-soy blends produced by low-cost extrusion. *Cereal Foods Wld, 25,* 161–162, 171–172.

MIHÁLY, K. & VÁMOS-VIGYÁZÓ, L. (1975): Determination, localization and heat inactivation of peroxidase in some vegetables. *Acta Alimentaria, 4,* 291—308.

MUSTAKAS, G. C., ALBRECHT, W. J., BOOKWALTER, G. N., MCGHEE, J. E., KWOLEK, W. F & GRIFFIN, E. L. (1970): Extruder-processing to improve nutritional quality, flavour and keeping quality of full fat soy flour. *Fd Technol., 24,* 1290–1296.

OSBORNE, T. B. (1924): *The vegetable proteins.* 2. ed. Longmans & Green Co. London.

PETRES, J. & KÁRPÁTI, GY. (1981): Szójatermékek tripszininhibitor aktivitásának meghatározása. (Determination of the trypsin inhibitor activity in soy products.) *Élelmiszerv. Közl., 27,* 179–186.

RACKIS, J. J., MCGHEE, J. E. & HONIG, D. H. (1975): Processing soybeans into foods. Selected aspects of nutrition and flavor. *J. Am. Oil Chem. Soc.,* 249–253.

RÉKÁSI-SZOMBATI, É., CABALLERO, O. P., MIHÁLYI-KENGYEL, É., & KÖRMENDY, L. (1979): Triptofántartalom meghatározása húsban és húskészítményekben. (Determination of the tryptophan content in meat and meat products). *Élelmiszerv. Közl., 25,* 17–26.

SATTERLEE, L. D., MARSHALL, H. F. & TENNYSON, J. M. (1979): Measuring protein quality. *J. Am. Oil Chem. Soc., 56,* 103–109.

SMITH, A. K. & CIRCLE, S. J. (1972): *Soybeans chemistry and technology.* Vol. I. O.C.S. Method Ba 11–65. AVI Publishing Company, Westport, Connecticut, pp. 451–452.

SOSULSKI, F. W. (1962): The centrifuge method for determining flour absorption in hard red spring wheats. *Cereal Chem., 39,* 344–350.

TAPPEL, A. L., BOYER, P. D. & LUNDBERG, W. O. (1952): The reaction mechanism of soybean lipoxidase. *J. Biol. Chem., 199,* 267.

YASUMATSU, K., SAWADA, K., MORITAKA, S., MISAKI, M., TODA, J. WADA, T. & ISHII, K. (1972): Whipping and emulsifying properties of soybean products. *Agric. biol. Chem., 36,* 719–729.

Acta Alimentaria, Vol. 17 (3), pp. 257–264 (1988)

MICROBIOLOGICAL INVESTIGATIONS OF PAPRIKA AND PEPPER WITH SPECIAL REGARD TO SPORE FORMERS INCLUDING *B. CEREUS*

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(Received: 13 July 1987; accepted: 22 October 1987)

From microbiological investigations of 64 spice samples (ground pepper and paprika) it is concluded that the dominant role in the microbiology of spices is played by aerobic spore formers, including thermophiles. Determination of the numbers of coliforms as fecal indicators must be substituted by determination of the numbers of the Enterobacteriaceae family since the former occurs in very small numbers in these products. Since the occurrence of the Enterobacteriaceae family in the products is not rare, the presence of Salmonella must be expected, on occasion. In the case of the detection of the presence of Salmonella, the antimicrobial effect of the spices, as well as their microecological conditions makes it necessary to use resuscitation of samples in large quantities of medium.

With due consideration to the fact that in spices, as much as 50% *B. cereus* contamination may be expected, their direct detection is considered a necessity in those food products where multiplication may occur.

According to the results of a comparative study mannitol–phenol red–egg yolk–polymixin agar may be substituted with a simple egg yolk agar for the detection of *B. cereus* in spices.

Keywords: microbiological contamination, spices, Enterobacteriaceae

Spices as additives in foods, play a very important role both in the food industry and in domestic sectors in two ways:

— With their content of essential oils they have an antimicrobial effect (PIVNICK, 1980). On the other hand, in meat products with decreased rH they act as antioxidants to increase shelf life (GERHARDT & BÖHM, 1980).

— If contaminated with microbes, their addition to foods may cause spoilage or decrease of shelf life. In addition to the potential for spoilage, the potential health hazard should not be ignored if we consider the conditional presence of bacteria, such as *B. cereus*, Salmonella, etc. causing food poisoning.

Their microbial contamination is of special importance where some treatment is applied to destroy microbes. Resistant spore formers may survive pasteurizing, canning, etc. and the number of survivors has a significant influence on the end product. It is well known, that the predominant microbes in spices are the aerobic spore formers (FARKAS et al., 1973, SEENAPPA & KEMPTON, 1981) but less resistant groups of microbes are encountered, too.

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That is why it was found necessary to obtain data on microbial contamination of the two most often used spices, pepper and paprika and at the same time to gain more information on some methodological problems.

Our investigations were carried out in the framework of the Balkan and Danubian Subcommission of ICMSF.

1. Materials and methods

We carried out microbiological analysis of 49 ground paprika and 15 black pepper samples.

The sampling was based partly on the 5 samples procedure (three class attributes plans), and partly on the random sampling approach. The spices were of heterogeneous origin, coming at times from the industry, from commercial networks, and even at even at times from the private sectors.

Five g samples were thoroughly stirred into suspension in 45 cm^3 of 0.2% peptone containing physiological sodium chloride solution and decimal dilution series were made.

Detection of coliforms were made by determination of titers in brilliant green bile lactose broth incubated at 30 °C for 48 h, on the basis of gas production. The titers of Enterobacteriaceae contamination were determined by breakdown of glucose in buffered brilliant-green-bile-glucose broth. After incubation for 24 h at 37 °C, the positive tubes were subcultured on violet-red-bile-glucose agar under similar culturing conditions, and typical colonies were selected for the following confirmation tests: glucose fermentation in semisolid glucose agar containing bromcresol-red indicator, nitrate reduction in potassium nitrate containing broth, as well as the oxidase test. For the determination of total counts the nutrient agar pour plate procedure was applied in two replicates and was covered with so-called water agar (Blanche agar).

For control, titer values were determined in nutrient broth media. Both the plates and the controls were incubated at 30 °C for 72 h.

Titer values of mesophilic sulfite-reducing Clostridia were determined in semisolid agarmedium containing sodium sulfite and ferric citrate incubated at 37 °C for 72 h. For thermophilic aerobic bacteria nutrient broth was used, and for the anaerobic ones, the above mentioned semisolid agar. All were incubated at 58 °C for 72 h after which period titer values were taken.

For the detection of spores, the initial suspension was heat treated at 72 °C for 30 min. (The majority of foods received a heat treatment at pasteurization level. According to MOSSEL (1977) 30 min at 65 °C, 20 min at 72 °C are equivalent to a heat treatment of 1 min at 80 °C. This heat treatment is effective to kill vegetative cells so that only spores survive. For aerobic spores the method used was similar to that described for the determination of meso-

philic counts. For anaerobic spore formers titer values were determined on the basis of sulfite reduction in semisolid agar medium.

For the detection of Salmonella 25 g of the sample were resuscitated in 225 cm^3 of buffered peptone water for 6 h, after which 10 cm^3 were transferred into 90 cm^3 of tetrathionate enrichment broth and incubated at two different temperatures (37 °C and 43 °C) for 24 h, after which loop transfers were surface plated on brilliant green phenol red agar and incubated at 37 °C for 24 h.

Moulds were demonstrated in 0.1 cm^3 sample for surface plating on maltose agar, pH 5.6, incubated at 22 °C for 3–5 days.

For *B. cereus* counts mannitol, phenol red, egg yolk polymixin agar (MYP) (MOSSEL et al., 1967) was used and incubated at 30 °C for 24 h. Parallel with it simple egg yolk agar containing egg yolk without the inhibitor, carbohydrate and indicator, were used. To make a comparison between the two media, in respect of applicability, the sensitivity of the two procedures was evaluated by methods of mathematical statistics. For comparison of averages t (Students) test was used to check whether the differences were significant. Previously we applied F test for examination of mean values. The lecithinase positive colonies were checked by Voges-Proskauer test, nitrate reduction and breakdown of carbohydrates.

2. Results

The results of the investigations are presented in Tables 1 and 2. The average levels of different groups of microbes are demonstrated in Fig. 1.

Table 1

Orders of magnitude and frequencies of occurrence of the different microbial groups in ground paprika

Microbe or microbial group	No. of samples	Microbial numbers per gram								
		$<10^1$	10^1	$<10^2$	10^2	10^3	10^4	10^5	10^6	10^7
Coliform group	49	35	8		5	1				
Family of Enterobacteriaceae	25	20			2	2	1			
Standard plate count	41					3	15	21	1	1
Sulfite-reducing clostridia	49	39	3		4	3				
Moulds	49			16	12	12	7	2		
B. cereus	48			28	16	4				
Thermophilic aerobic bacteria	6				2	1	2		1	
Thermophilic aerobic spores	6		1			1	2	2		
Thermophilic anaerobic bacteria	6	6								
Thermophilic aerobic spores	6	6								
Mesophilic aerobic spores	35					5	12	12	6	
Mesophilic anaerobic spores	49	39			6	4				

Table 2

Orders of magnitude and frequencies of occurrence of the different microbial groups in ground pepper

Microbe of microbial group	No. of samples	Microbial numbers per gram								
		$<10^1$	10^1	$<10^2$	10^2	10^3	10^4	10^5	10^6	10^7
Coliform group	15	10	2		3					
Family of Enterobacteriaceae	5	3			1	1				
Standard plate count	15						6	4	5	
Sulfit-reducing clostridia	15	8	2		3	2				
Moulds	15			5	1	3	3	2	1	
B. cereus	15			4	9	1	1			
Thermophilic aerobic bacteria	3					1	1		1	
Thermophilic aerobic spores	3				1	1			1	
Thermophilic anaerobic bacteria	3	2			1					
Thermophilic anaerobic spores	3	3								
Mesophilic aerobic spores	15					2	2	2	6	3
Mesophilic anaerobic spores	15	7	2		2	2	2			

It can be seen from the data, that the frequency and order of magnitude of coliform contamination was very low, around 10^1–10^2. Similar observations were valid for the Enterobacteriaceae family as well, even though the values here were around 10^2–10^3. This is not expected from the method, since the breakdown of glucose was carried out by the whole family. Extreme values

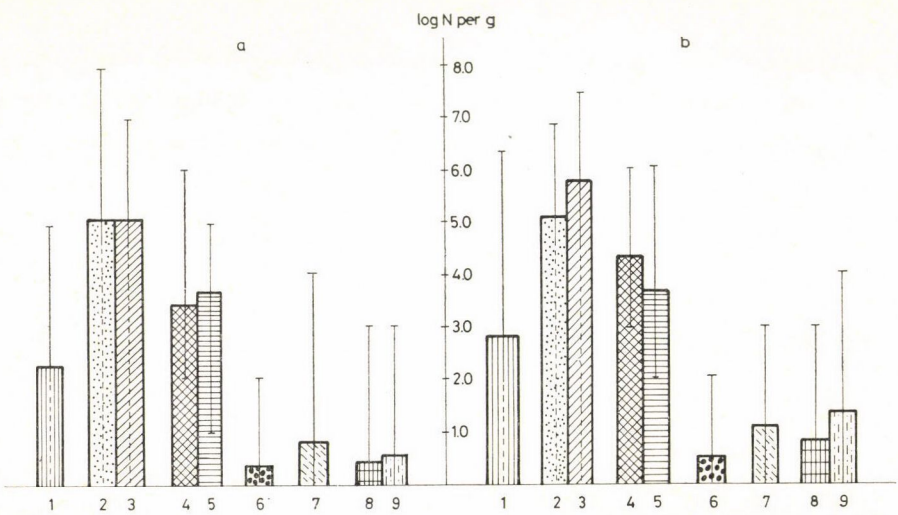

Fig. 1. Microbiological contamination of ground paprika (a) and pepper (b)
1: thermophilic aerobic bacteria; 2: aerobic plate count; 3: mesophilic aerobic spores; 4: thermophilic aerobic spores; 5: moulds; 6: coliforms; 7: Enterobacteriaceae; 8: mesophilic clostridia; 9: mesophilic anaerobic spores; I: extreme values

of 10^4 indicate that relatively great orders of magnitude may be encountered at irregular times. (Deviations in the numbers of samples were caused by the fact that in the course of the investigations, the scope was widened to cover Enterobacteriaceae, too, after it had become clear that the incidences and orders of coliforms were very low.)

Contamination orders of sulfite-reducing clostridia were observed to be similar to that of the Enterobacteriaceae, which indicates that the micro-ecology of the spices (atmosphere, a_w, pH, etc.) do not provide favourable conditions for existence and multiplication of anaerobes. When the numbers of spores are compared with those of vegetative cells, the conclusion can be drawn that the former have higher values, since heat treatment stimulated activation of the spores.

Attention must be drawn to the observation that in the case of spices, in the lower dilution regions, microbiological investigations may not lead to correct results, because the antimicrobial effect of the high essential oil content may often cause incorrect results even around 10^0 and 10^1 dilutions. This is exactly why in the case of the former groups of microbes, the low order of contamination can be said unconditionally to be true, but the frequencies cannot always be said to be reliable.

Mesophilic counts depicted higher orders of magnitude of 10^4–10^6 and there were even extreme values of 10^7. After heat treatment, counts of spore formers approached, and in certain cases exceeded the counts of vegetative cells, indicating that the dominating microbial forms in the spices are the spores. One procedural problem was encountered in the course of the investigations. In the case of the spices where the majority of microbial contamination comprised aerobic spore forming bacteria spreading colonies often made counting difficult, and at times impossible, even when cover layers were used on the pour plates. Thus, during the course of our investigations as much as 20% of samples could not be estimated. This is why the introduction of broth series, which could be used as an auxiliary control or used to replace the pour plate results, is advisable.

Mould contamination was generally 10^2–10^3 and the extreme value of 10^6 reflects on the deficiencies of storage conditions. Considering the fact that counts of the thermophiles were low, they can only be assessed on informative basis. All the same, it is clear that their presence must always be presumed. Extreme values in the million orders of magnitude, indicate that their presence cannot be simply overlooked, since among them the, "flat sour" bacteria may play a negative role in the canning industry. Like mesophilic contamination, the anaerobes occurred in small numbers, and clostridia could be demonstrated in only a single case at a magnitude of 10^2.

In agreement with several reports the spices were contaminated with *B. cereus* at the following frequencies: paprika 42%, pepper 73%. The contam-

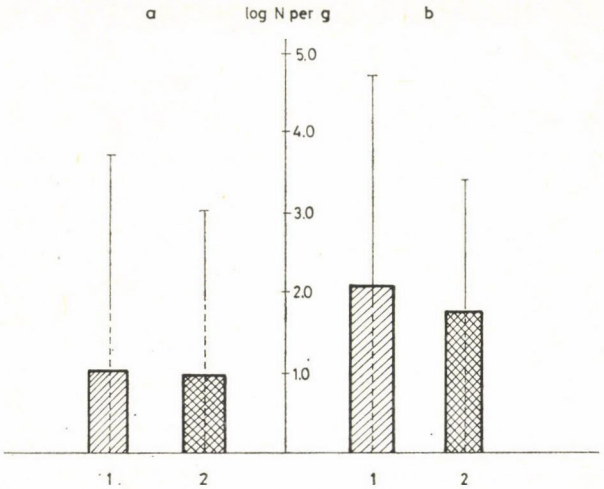

Fig. 2. Comparative study on egg yolk and mannitol–egg yolk–polymixine agar for detection of *B. cereus* from spices (a: paprika; b: pepper). 1: egg yolk agar; 2: MYP; I: extreme values

ination (Fig. 2) level was on the average 10^1–10^2 g^{-1}, but extreme values showed higher orders of about 10^3–10^4 g^{-1}.

Concerning the detection of *B. cereus* it is known from several reports and from the ISO standard, as well that if samples contain numerous mannitol-fermenting microorganisms leading to production of acid, then the characreristic pink colour of *B. cereus* colonies on MYP agar, may be reduced or disappear entirely. Furthermore the use of the supernatant liquid of egg yolk instead of the whole, no zone of precipitation, but a clear halo appears around the *B. cereus* colonies, making recognition impossible. That is why we made comparative study on the effectiveness of the two media. According to the t test there is no significant difference between the two methods ($t_{calculated} < t_{tabulated}$ viz. in the case of paprika $t_c = 1.75\, t_t = 1.99$ with $F_c = 1.21$ $F_t = 1.6$; in the case of pepper $t_c = 0.72$ $t_t = 2.06$ $F_c = 1.15$ $F_t = 2.6$ at a significance level of 5%). Moreover the recovery of *B. cereus* was higher with simple egg yolk agar than with MYP.

Salmonella results were negative for all samples investigated.

3. Conclusion

In conclusion it can be inferred that coliforms, as fecal indicators, are not very applicable in spices owing to their occurrence in small numbers and the antimicrobial effect of the spices (PIVNICK, 1980). Instead, the detection of the Enterobacteriaceae family for fecal indication, is more effective. These

conclusions are in agreement with data reported by other authors (SCHWAB et al., 1982; BAXTER & HOLZAPFEL, 1982).

In view of the above mentioned antimicrobial effects of these materials as well as the prevailing microecological conditions, the direct detection of Salmonella is well emphasized, in which procedure, a pre-enrichment resuscitation is necessary with the use of relatively large quantities of medium.

PRUTHI (1980) states that bacterial counts of various spices may vary from several thousands to several millions per gram. The variations in the microbial count may be ascribed to the source of the spices, the method they have been grown and harvested, the existing sanitary conditions of processing and storage (JULSETH & DEIBEL, 1974).

Aerobic spore formers constitute the most important microflora of spices (PIVNICK, 1980; SEENAPPA & KEMPTON 1981; BAXTER & HOLZAPFEL, 1982; FÁBRI et al., 1982). This includes the members of the genus Bacillus, whereas anaerobic spore formers, thermophilic aerobs and anaerobs may be detectable occasionally in various number. As for aerobic spore formers the determination of their numbers is often impeded in the pour plate procedure by spreading colonies, and in view of this it is recommended that investigations be always supplemented with the determination of titer values as controls. The microbiological condition of spices must always be controlled by carefully selected methods. Thus for products intended for the canning industry the scope of determinations must also cover thermophiles.

Since — in agreement with POWERS and co-workers (1976) — in spices as much as 50% *B. cereus* contamination may be encountered, in those cases where multiplication of these spore formers is possible — as for instance, in ready-to-eat products — direct demonstration is advised (BECKER, 1976; ELLIOTT, 1980).

On the basis of the results of the comparative study on the sensitivity of MYP and simple egg yolk agar, there is no significant difference between the two media. Moreover with the simple egg yolk agar we gain a slightly higher average, than with MYP. This is a consequence of the characteristic of spices where the predominant microflora, the aerobic spore formers, overcrowding in lower dilutions inhibit the development of typical colony forms of *B. cereus*. For this reason, in case of spices, for detection of *B. cereus* the simple egg yolk agar may be recommended.

Literature

BAXTER, R. & HOLZAPFEL, W. H. (1982): A microbial investigation of selected spices, herbs and additives in South Africa. *J. Fd Sci., 47*, 570–574.

BECKER, H. J. (1976): The bacteriological quality of boiled and fried rice from Chinese restaurants in relation to *Bacillus cereus* food poisoning. *Archiv Lebensmittelhyg., 27*, 66–68.

ELLIOTT, R. P. (1980): Cereals and cereal products. — in: *Microbial ecology of foods.* Vol. 2. Food commodities. International Commission on Microbiological Specifications for Foods, p. 669.

FÁBRI, I., NAGEL, V., TABAJDI-PINTÉR, V., ZALAVÁRI, ZS., SZABAD, J. & DEÁK, T. (1982): Qualitative and quantitative analysis of aerobic spore forming bacteria in Hungarian paprika. — in: DRING, G. J., ELLAR, D. J. & GOULD, G. V. (Eds.) *Fundamental and applied aspects of bacterial spores.* Academic Press, London. pp. 455–462.

FARKAS, J., BECZNER, J. & INCZE, K. (1973): Feasibility of irradiation of spices with special reference to paprika. — in: *Radiation preservation of food.* IAEA, Vienna, p. 389.

GERHARDT, U. & BÖHM, TH. (1980): Das Redox Verhalten von Gewürzen Fleischerzeugnissen. *Fleischwirtschaft, 60*, 1523.

JULSETH, R. M. & DEIBEL, R. H. (1974): Microbial profile of selected spices and herbs at import. *J. Milk Fd Technol., 37*, 414. — ref.: BAXTER, R. & HOLZAPFEL, W. H. (1982).

MOSSEL, D. A. A. KOOPMAN, M. J. & JONGERIUS, E. (1967): Enumeration of *Bacillus cereus* in foods. *Appl. Microbiol., 15*, 650–653.

MOSSEL, D. A. A. (1977): *Microbiology of foods.* Occurrence, prevention and monitoring of hazards and deterioration. The University of Utrecht, Faculty of Veterinary Medicine.

PIVNICK, H. (1980): Spices. — in: *Microbial ecology of foods.* Vol. 2. Food commodities. International Commission on Microbiological Specificactions for Foods, p. 731.

POWERS, E. M., LATT, T. G. & BROWN, T. (1976): Incidence and levels of *Bacillus cereus* in processed spices. *J. Milk Fd Technol., 39*. 668–670. — ref. BAXTER, R. & HOLZAPFEL, W. H. (1982).

PRUTHI, J. S. (1980): Spices and condiments: Chemistry, microbiology, technology. *Adv. Fd. Res.*, Supp. No. 4. Academic Press, New York. — ref. BAXTER, R. & HOLZAPFEL, W. H. (1982).

SCHWAB, A. H., HARPESTAD, A. D., SWARZENTRUBER, A., LANIER, J. M., WENTZ, B. A., DURAN, A. P., BARNARD, R. J. & READ, R. B. (1982): Microbiological quality of some spices and herbs in retail markets. *Appl. environm. Microbiol., 44*, 627–630.

SEENAPPA, M. & KEMPTON, A. G. (1981): Note on the occurrence of *Bacillus cereus* and other species of bacillus in Indian spices of export quality. *J. appl. Bact., 50*, 225.

Acta Alimentaria, Vol. 17 (3), pp. 265–270 (1988)

CHANGE OF DEGREE OF HYDROLYSIS IN THE COURSE OF ENZYMATIC PEPTIDE MODIFICATION

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(Received: 4 September 1987; accepted: 12 November 1987)

Hydrolysis and synthesis of peptide bonds as consequences of enzymatic peptide modification (EPM) have been studied by determination of the degree of hydrolysis (DH) using the TNBS-method. Enzymatic hydrolysates of casein were used as substrates. All enzyme-catalyzed reactions were studied without and with the addition of L-methionine ethyl ester.

Correlations between DH and reaction time could be described by power curves. In the case of EMP without amino acid addition, a moderately increasing curve while in the case of EPM with Met-addition, a slightly decreasing power curve was found to fit in satisfactorily with the experimental values.

The results of experiments confirm the fact, that new covalent bonds were formed in the course of the enzymatic peptide modification.

Keywords: degree of hydrolysis, transpeptidation, EPM

Enzymatic Peptide Modification (EPM) (HAJÓS, 1986) proved to be suitable to obtain protein products of designed amino acid composition.

The ability of proteinases to catalyze peptide bond synthesis has a historical background (SAWJALOW, 1907; FRUTON, 1949; WASTENEYS & BORSOOK, 1930) and a renewed interest in recent years, too (FUJIMAKI et al., 1977; ANDERSON & LUISI; 1979, MORIHARA & OKA, 1981; ASHLEY et al. 1983; ASO et al. 1985).

YAMASHITA and co-workers (1973) supposed that condensation was the main force in the enzyme-catalyzed protein-modification process. TSAI and co-workers (1974) found a significant increase in the molecular mass of the enzymatically modified protein, as a result of polycondensation reactions.

HOFSTEN and LALASIDIS (1976) were of the opinion that covalent forces do not play a role in these reactions. HOROWITZ and HAUROWITZ (1959) and FUJIMAKI and co-workers (1977) presumed transpeptidation in the course of this enzymatic process. NOAR and SHIPE (1984) concluded that the incorporation of methionine by a one-step enzymatic process (YAMASHITA et al., 1979) was due to the formation of hydrophobic bonds.

Our earlier studies confirmed the fact of covalent amino acid incorporation and of transpeptidation in the course of the enzymatic peptide modifica-

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tion (HAJÓS & HALÁSZ, 1982; DELINCÉE & HAJÓS, 1984; HAJÓS & SZARVAS, 1986).

In this study we followed the EPM reaction by measuring the quantity of free amino groups and by determining the degree of hydrolysis in the case of two different casein hydrolysates.

1. Materials and methods

Casein (Reanal, Hungary), papain (Sigma, FRG), α-chymotrypsin (Sigma), L-methionine (Reanal) and trinitrobenzene sulfonic acid (TNBS) (Serva, FRG) were used.

1.1. Preparation of the substrate

Casein was hydrolysed with α-chymotrypsin (pH: 7.5) and papain (pH: 6.0), respectively. The enzyme–protein ratio was 1 : 100. The incubation was carried out at 37 °C for 4 h. The reaction mixture was stirred during hydrolysis.

1.2. Preparation of methionine ethyl ester

L-Methionine was esterified with ethanol and anhydrous hydrogen chloride and the reaction product was recrystallized from ethanol–ether to obtain L-methionine ethyl ester hydrochloride.

1.3. Enzymatic peptide modification

Substrate (5 g), Met-ethyl ester (1 g) and enzyme (0.05 g) were mixed to give a 25% (w/v) substrate concentration at pH 6.0. The reaction temperature was 37 °C.

1.4. Determination of the degree of hydrolysis (DH)

The TNBS-method (ADLER-NISSEN, 1979) was used to determine the free amino groups in the reaction mixture. Photometric determination of free amino acids was carried out in solution with sodium dodecyl sulfate (SDS). The degree of hydrolysis (DH) is defined (ADLER-NISSEN, 1982) as the percentage of peptide bonds cleaved:

$$\mathrm{DH}\% = \frac{\text{number of free amino groups}}{\text{total number of amino acid residues}} 100$$

The total number of amino acid residues was experimentally determined from the hydrochloric acid hydrolysates of the samples.

2. Results and discussion

The change in DH as a function of time is shown in Figs. 1 and 2. The EPM reaction was carried out starting from an α-chymotryptic hydrolysate of casein in the presence of α-chymotrypsin (Fig. 1) and a papain hydrolysate of casein in the presence of papain (Fig. 2) with and without addition of Met-ethyl ester.

The statistical results of curve fitting are summarized in Table 1. All relations between DH (y) and time (x) can be described with the aid of power curves ($y = ax^b$). The results show regression equations to be highly significant for all the correlations between DH and time.

The slight increase in the number of free amino groups in samples 1 and 3 (Figs. 1 and 2) reveals that the enzyme-catalyzed equilibrium process (EPM) has slightly been shifted in the direction of hydrolysis, apart from the fact that the main force of EPM is transpeptidation (DELINCÉE & HAJÓS, 1984; HAJÓS et al. 1987).

In the case of samples 2 and 4 (Figs. 1 and 2), the high starting DH values are due to the great amount of amino acid derivative added to the reaction mixture.

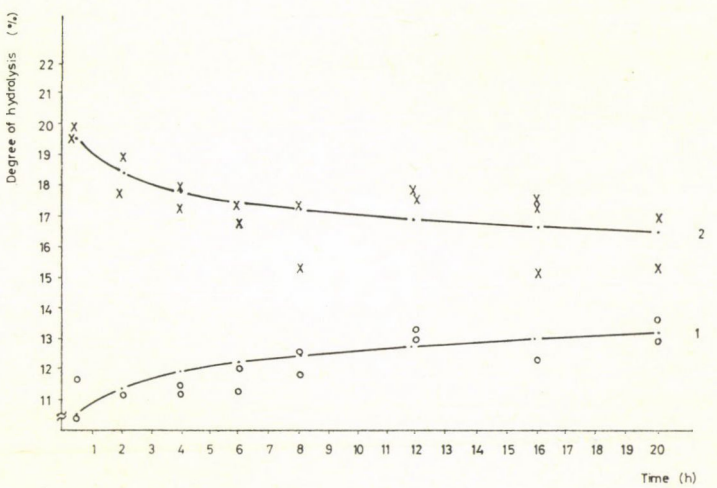

Fig. 1. The change in degree of hydrolysis (DH) as a function of time in the course of EPM. The substrate was an α-chymotryptic hydrolysate of casein. The enzyme used for EPM was α-chymotrypsin. 1(○): without, 2(x): with L-methionine ethyl ester addition, values calculated from the equation (○)

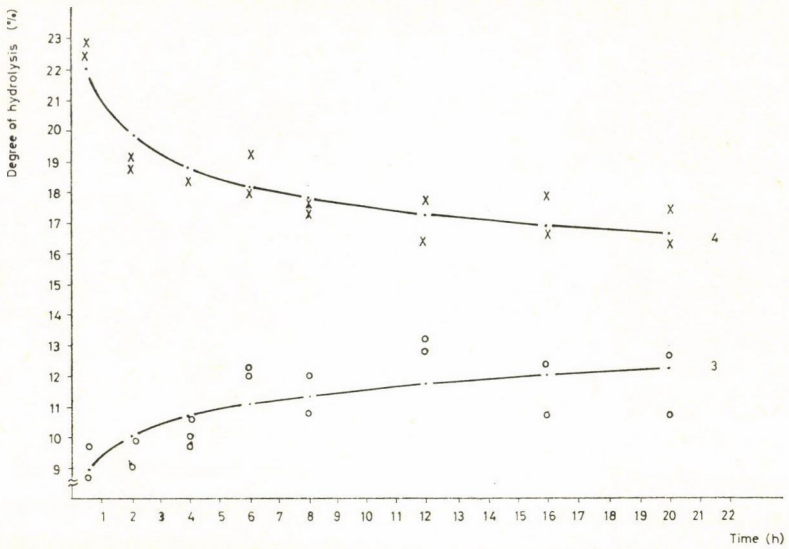

Fig. 2. The change of DH as a function of time during EPM. Substrate: papain hydrolysate of casein, enzyme: papain; 3(○): without, 4(x): with L-methionine ethyl ester, addition values calculated from the equation (○)

Table 1

The statistical results of power curve ($y = a\,x^b$) fittings

Sample	Regression coefficient a	Regression coefficient b	Correlation coefficient (*r*)
1	10.9855	0.0615	0.7635*
2	18.8878	—0.0444	0.7433*
3	9.5373	0.0841	0.7552*
4	20.8106	—0.0750	0.9181*

* Significant at $P < 0.01$ probability level (n=16)

One reason for the decrease of DH is that a portion of Met has been incorporated into the peptide chain resulting in new peptide bonds. Our earlier results, however, revealed an amino acid incorporation to a 3 % extent under these reaction conditions of EPM. Therefore this observed decrease in DH cannot be explained solely by methionine incorporation. Probably, condensation between peptides promoted by the presence of the great amount of free amino acids takes also place.

Even the highest DH values of samples 1 and 3 (without amino acid addition) do not exceed the lowest DH values of samples 2 and 4 (with amino

acid addition). This finding may well be explained by the amount of non-incorporated amino acids still present in the mixtures in samples 2 and 4.

Since the decrease of DH was always observed in these experiments in the presence of SDS which strongly promotes dissociation, the formation of covalent bonds in agreement with our earlier findings, is supposed to be highly probable.

The determinations of the degree of hydrolysis in the course of the enzymatic reaction reveal that transpeptidation is the major process in the enzymatic peptide modification.

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The excellent experimental performance of Ms. IDA ÉLIÁS and Mr. JÓZSEF FEHÉR is gratefully acknowledged.

Literature

ADLER-NISSEN, J. (1979): Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulphonic acid. *J. agric. Fd Chem., 27*, 1256–1262.

ADLER-NISSEN, J. (1982): Limited enzymic degradation of proteins: A new approach: industrial application of hydrolases. *J. chem. Technol. Biotechnol., 32*, 138–156.

ANDERSON, G. & LUISI, P. L. (1979): Papain-induced oligomerisation of α-amino acid esters. *Helv. chim. Acta, 62*, 488–493.

ASHLEY, D. V. M., TEMLER, R., BARCLAY, D., DORMOND, C. A. & JOST, R. (1983): Amino acid-enriched plasteins: A source of limiting amino acids for the weanling rat. *J. Nutr., 113*, 21–27.

ASO, H., KIMURA, H., WATANABE, M. & &ARAI, S. (1985): Chemical properties of enzymatically modified proteins produced from soy protein by covalent attachment of methionine. *Agric. & biol. Chem., 49*, 1649–1654.

DELINCÉE, H. & HAJÓS, GY. (1984): Investigation of plasteins by SDS polyacrylamide gel electrophoresis. *Acta Alimentaria, 13*, 309–313.

FRUTON, J. S. (1949): The synthesis of peptides. *Adv. Protein Chem., 5*, 1–82.

FUJIMAKI, M., ARAI, S. & YAMASHITA, M. (1977): Enzymatic protein degradation and resynthesis for protein improvement. *Adv. Chem. Ser., 160*, 156–184.

HAJÓS, GY. (1986): Incorporation of essential amino acids into protein by enzymatic peptide modification (EPM). *Nahrung, 30*, 418–419.

HAJÓS, GY & HALÁSZ, A. (1982): Incorporation of L-methionine into casein hydrolysate by enzymatic treatment. *Acta Alimentaria, 11*, 189–197.

HAJÓS, GY. & SZARVAS, T. (1986): Metionin-metilészter-hidroklorid-($^{14}CH_3$) beépítése fehérje proteolitikus hidrolizátumába enzimes eljárással. (Methionine-methylester-hydrochloride-($^{14}CH_3$) incorporation into the proteolytic hydrolysate of protein by an enzymatic process.) *Izotóptechnika, 29*, 166–170.

HAJÓS, GY., ÉLIÁS, I. & HALÁSZ, A. (1987): Methionine-enrichment of milk protein by enzymatic peptide modification. *J. Fd Sc. 53*, 739—742.

HOFSTEN, B. V. & LALASIDIS, G. (1976): Protease catalyzed formation of plastein product and some of their properties. *J. agric. Fd Chem., 24*, 460–465.

HOROWITZ, J. & HAUROWITZ, F. (1959): Mechanism of plastein formation. *Biochem. biophys. Acta, 33*, 231–238.

MORIHARA, K. & OKA, T. (1981): Peptide bond synthesis catalyzed by subtilisin, papain and pepsin. *J. Biochem., 89*, 385–395.

NOAR, S. R. & SHIPE, W. F. (1984): A study of the forces involved in the incorporation of L-methionine into soy protein by the one-step plastein-like process. *J. Fd Sci., 49*, 1316–1318.

SAWJALOW, W. W. (1907): Über das Plastein. *Z. physiol. Chem., 54*, 119–150.

TSAI, S. J., YAMASHITA, M., ARAI, S. & FUJIMAKI, M. (1974): Polyacrylamide gel electrophoresis of plasteins. *Agric. & biol. Chem.*, *38*, 641–644.

WASTENEYS, H. & BORSOOK, H. (1930): The enzymatic synthesis of protein. *Physiol. Rev.*, *10*, 110.

YAMASHITA, M., ARAI, S., TANIMOTO, S. & FUJIMAKI, M. (1973): Condensation reaction occurring during plastein formation by α-chymotrypsin. *Agric. & biol. Chem.*, *37*, 953–954.

YAMASHITA, M., ARAI, S., IMAIZUMI, Y., AMANO, Y. & FUJIMAKI, M. (1979): A one-step process for incorporation of L-methionine into soy protein by treatment with papain. *J. agric. Fd Chem.*, *27*, 52–56.

RECENTLY ACCEPTED PAPERS

Comparative effect of ethylene oxide and gamma irradiation on the chemical, sensory and microbial quality of ginger, cinnamon, fennel and fenugreek
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Sodium dodecyl sulphate polyacrylamide gel electrophoresis of seed proteins as a test for screening high cooking quality durum wheat strain
TAHA, S. A. & SÁGI, F.

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International Biochemical Symposium on Food Proteins, Balatonszemes, 1987

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ACTA ALIMENTARIA

VOLUME 17 No. 3 — 1988

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Index: 26.039

ACTA

ALIMENTARIA

An International Journal of Food Science

Editor

J. HOLLÓ

Volume 17 **December 1988** **Number 4**

Akadémiai Kiadó
Budapest

Kluwer Academic Publishers
Dordrecht/Boston/London

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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For all other countries:

Kluwer Academic Publishers
P.O. Box 17, 3300 AA Dordrecht, Holland
and 101 Philip Drive, Norwel, MA 02061 U. S. A.

Publication programme, 1988: Volume 17 (4 issues).
Subscription prices per volume: Dfl. 276,—/$ 130.00 including postage.

Acta Alimentaria is published 4 times per annum: March, June, September and December

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.

ACTA ALIMENTARIA

AN INTERNATIONAL JOURNAL OF FOOD SCIENCE

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Acta Alimentaria, Vol. 17 (4), pp. 271—281 (1988)

COMPARATIVE EFFECT OF ETHYLENE OXIDE AND GAMMA IRRADIATION ON THE CHEMICAL, SENSORY AND MICROBIAL QUALITY OF GINGER, CINNAMON, FENNEL AND FENUGREEK

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(Received: 2 December 1985; revision received: 28 January 1988; accepted: 2 February 1988)

The effect of gamma irradiation and ethylene oxide fumigation on the microbiological and sensory properties of ground cinnamon, ginger, fennel and fenugreek, and on the volatile oil content of ground cinnamon, ginger and fennel was investigated.

It was found that for cinnamon and ginger a 6 kGy dose, for fennel 6–10 kGy and for fenugreek 6–8 kGy dose were equal in microbial effects to the commercially established fumigation process.

No significant change in the volatile oil contents of the fumigated or irradiated cinnamon and fennel has been observed. Irradiation of ginger with a dose of 5 kGy resulted in a slight decrease of 14%, while fumigated ginger showed no significant loss in volatile oil content.

Between the untreated, irradiated or fumigated spices no major differences in sensory properties were found.

Keywords: ethylene oxide treatment, irradiation, spices

Spices and herbs are used to flavour the foods in most categories of the food industry such as meat, fish, vegetable products, convenience foods and bakery products. Spices are an important commodity in international trade, which is still growing. Production of most spices is concentrated in tropical and subtropical countries with the exception of Hungary.

Spices like other agricultural commodities, are known to be contaminated with spoilage organisms such as bacteria, moulds and yeast, in the range of 10^3–10^8 organism per gram. The majority of the microbial flora of spices consists of aerobic spore-forming bacteria (FARKAS, 1983).

Sometimes spices contain in addition microorganisms of health significance such as *Bacillus cereus*, *Clostridium perfringens*, mycotoxin producing moulds, *Staph. aureus*, *Salmonella spp.* and *Shigella spp.* Relative high incidence of toxigenic moulds has been found in spices and aflatoxin was detected in a range of spices, although the levels of aflatoxins recorded was generally low (FLANNIGAN & HUI, 1976).

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Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

A number of decontamination methods have therefore been applied in the past and some are still employed to spices used in the food industry. Methods based upon heat are not very suitable since an increase in temperature is accompained by loss of volatile contents and reduction in quality (THIESSEN, 1970). The UV irradiation as a means of sterilizing spices is not effective because of its low penetrating power (BERGMAN, 1955). Microwave irradiation is not effective enough to reduce bacterial population during the treatment (VAJDI & PEREIRA, 1973).

Chemical treatment is widely used for reducing bacterial count or for sterilizing. Fumigation with ethylene oxide (EO) or propylene oxide (PO) is used commercially in several countries to kill insect pests in seasonings and to reduce the viable cell count. WESLEY and co-workers (1965) pointed out that during treatment with EO in the presence of water and inorganic chlorides, chlorohydrin is formed while PO treatment produces chloropropanol isomers both of which are persistent toxic compounds.

Gamma irradiation of spices is very promising for reasons of great efficiency in the inactivation of infective micro-organisms and almost no loss of flavour quality (FARKAS, 1983). This method makes it possible to treat spices in whole or broken form, in packages of any size from different materials especially thermoplastic polymers, which protect the product from secondary infection.

In addition fumigation may result in reduction of volatile oil content of spices, while irradiation does not reduce the volatile oil content of most of the spices up to a dose level of 10 kGy or higher (FARKAS, 1981; 1984). Although sterilizing doses of 15–20 kGy slightly change the flavour of some spices, but the 3–10 kGy doses, sufficient for pasteurization, do not influence the colour and flavour of the spices (FARKAS, 1984).

In this study the effect of irradiation and fumigation on the microbiological, chemical (volatile oil) and sensory quality of four different spices, namely ginger, cinnamon, fennel and fenugreek was determined. These spices were chosen, because according to the literature study of FARKAS (1984) not all chemical, microbiological and sensory aspects were covered by earlier investigations.

1. Materials and methods

Ground ginger, cinnamon, fennel and fenugreek were obtained from a Dutch trade company, ginger originated from China, cinnamon was produced in Indonesia and fennel and fenugreek were produced in India. The samples were ground by the trade company, and divided in three parts. One part was fumigated by the trade company at ambient temperature during eight hours with ethylene oxide (EO) at a concentration of 1500 g m^{-3}.

The second part was gamma irradiated at room temperature in aluminium/polyethylene bags at the 40 kCi cobalt-60 research source of the Pilot Plant for Food Irradiation in Wageningen. The untreated, fumigated and irradiated samples were stored in gas-impermeable packages.

1.1. Microbiological examination

Ten to 20 gram of samples were mixed with a nine-fold quantity of peptone physiological saline to make dilutions from 1 : 10 to 1 : 100 000. From these dilutions, duplicate plates were prepared with the following media:

– *Aerobic colony count:* pour plates on tryptone soya broth (Oxoid-CM 131) + 3 g yeast extract + 12 g agar (TSBYA), incubated for 3 days at 30 °C.

– *Pseudomonas:* surface plates on Pseudomonas agar (Oxoid CM 559), incubated for 48 h at 30 °C.

– *E. coli:* enrichment in lauryl sulphate tryptose broth for 24 h at 37 °C, followed by EC broth for production of gas and tryptone water for production of indol according to ISO 7251.

– *Enterobacteriaceae:* poured plates (double layer) on Violet red bile glucose agar (Oxoid CM 107), incubated for 24 h at 37 °C.

– *Lactobacillus:* poured plate on Rogosa agar (Oxoid PM 221), incubated in anaerobic jar at 37 °C for 3 days.

– *Lancefield D streptococci:* poured plates on Kanamycin Aesculin Azide agar (Oxoid CM 481), incubated for 24 h at 37 °C.

– *Moulds and yeasts:* poured plates on Oxytetracycline glucose yeasts extract agar (Oxoid CM 545), incubated with daily reading for 3–5 days at 25 °C.

– *Mesophilic aerobic spores:* poured plates on TSBYA and incubated for 3 days at 30 °C.

– *Clostridium spp.:* poured plates on Differential reinforced clostridium agar (Merck), incubated in anaerobic jar at 30 °C for 3 days.

– *Thermophilic aerobic spores:* poured plates on TSBYA, incubated at 55 °C for 3 days.

– *Thermophilic flat-sour spores:* poured plates on Dextrose tryptone agar (Oxoid CM 75), incubated at 55 °C for 3 days.

– *Thermophilic anaerobic spores:* poured plates on Schaedler agar (Oxoid CM 437), incubated at 55 °C for 3 days in anaerobic jar.

– *Clostridium thermosaccharolyticum:* Liver broth (Oxoid CM 77) incubation at 55 °C for 3–5 days.

– *Clostridium nigrificans:* poured plates on Differential reinforced clostridium agar (Merck) for 3–5 days at 55 °C in anaerobic jar.

For enumeration of mesophilic spores the dilutions were heated for 10 min at 80 °C and for the enumeration of thermophilic spores 30 min at 100 °C.

1.2. Volatile oil content

Volatile oil content was determined according to ISO document, No. 6571: Spices, condiments and herbs — Determination of volatile oil content. The spices were distilled with water by using the oil trap to collect volatile oil. For ginger 17.0 g of sample was dissolved in 500 cm^3 of water and distilled for 5 h in 130 °C. For cinnamon 20.0 g in 400 cm^3 of water and 25.0 g of fennel in 300 cm^3 of water. The content of volatile oil is expressed as a percentage of volume to weight (% v/w).

1.3. Sensory evaluation

Sensory evaluation was carried out according to the method of paired comparisons as described by EDWARDS (1960), with a panel of twenty members.

Assessors are asked to select one of two coded samples with the most characteristic flavor for the offered spices. In this method three or four samples, fumigated and irradiated with doses of 0, (5) and 10 kGy were tested in one session. For each session three or six pairs were given to the panel in a randomized order. The samples were kept in small closed plastic boxes.

2. Results

2.1. Microbiological effects of fumigation and gamma irradiation

Results of the comparative microbiological study of fumigation and irradiation on the microbiological quality of cinnamon, ginger, fennel and fenugreek are summarized in Tables 1–4. Microbial counts are expressed in colony forming units (cfu) per gram.

The aerobic mesophilic colony count of cinnamon reached 1.5×10^4 cfu g^{-1} and consisted mainly of bacterial spores. Moulds were present at 5.1×10^3 per gram. No *Pseudomonas spp.*, *E. coli*, *Lactobacillus spp.*, or Lancefield D streptococci were detected in cinnamon. Fumigation resulted in more than 4 log cycles reduction of the aerobic mesophilic colony count and the aerobic spore count. Irradiation with 6 kGy gave an effect similar to the treatment with ethylene oxide.

The aerobic mesophilic colony count of ginger was 6.5×10^6 cfu g^{-1}, the aerobic mesophilic spore count 3.5×10^6 per gram and the mould count 3.4×10^3 per gram. In addition, *Pseudomonas spp.*, *E. coli*, *Enterobacteriaceae* and Lancefield D streptococci were present, varying from 10^3 to 10^4 cfu g^{-1}. Fumigation and irradiation with 6 kGy resulted in a ca 3.5 log cycles reduction of the aerobic mesophilic colony count and the aerobic mesophilic spore count.

No *Pseudomonas spp.*, *E. coli*, *Enterobacteriaceae* or Lancefield D streptococci were found after fumigation or after irradiation.

The aerobic mesophilic colony count and the aerobic mesophilic spore count of fennel was 4.1×10^5, 1.8×10^4 cfu g^{-1}, respectively. Fumigation and irradiation with 6 kGy caused a reduction of 3–4 orders of magnitude in the mesophilic aerobic spore count. It was found that a reduction of 3.5 log cycles of the aerobic mesophilic colony count could be achieved by fumigation. However, a radiation dose of 10 kGy was required for a 3 log cycles reduction of the aerobic mesophilic colony count. This dose was also required for the elimination of *Enterobacteriaceae* in fennel. Moulds were counted in fumigated fennel.

The aerobic mesophilic colony count of fenugreek was 8.0×10^4 cfu g^{-1}, and consisted mainly of bacterial spores. Fumigation and irradiation with 6 kGy resulted over 4 log cycles reduction of the aerobic mesophilic spore count. Fumigation resulted in 4–5 log cycles reduction of the aerobic mesophilic colony count. In case of irradiation a dose of 8 kGy might be necessary.

Table 1

The comparative effect of ethylene oxide and gamma irradiation on the microbial quality of cinnamon

Organisms	Colony forming (cfu g^{-1})				
	Untreated	Fumigated	2 kGy	4 kGy	6 kGy
Aerobic colony count	1.5×10^4	10	4.9×10^3	2.7×10^2	100
Pseudomonas	10	10	—	—	—
E. coli	0.3	0.3	—	—	—
Enterobacteriaceae	4.5×10^1	10	—	—	—
Lactobacillus	10	10	—	—	—
Lancefield D streptococci	10	10	—	—	—
Moulds	5.2×10^3	10	10	10	10
Yeasts	10	10	10	10	10
Mesophiles					
Aerobic spores	1.1×10^4	10	3.1×10^3	5.0×10^1	10
Anaerobic spores	2.9×10^4	10	8.1×10^2	10	10
Clostridia	10	10	—	—	—
Thermophiles					
Aerobic spores	10	10	—	—	—
Flat sour spores	1.1×10^2	10	—	—	—
Anaerobic spores	100	10	—	—	—
C. thermosaccharolyticum	1	1	—	—	—
C. nigrificans	10	10	—	—	—

—: no growth

Irradiation: at the 40 kCi cobalt-60 research source of the Pilot Plant for Food Irradiation in Wageningen.

Date of irradiation: March 1985

Fumigation: with ethylene oxide (EO) at a concentration of 1500 g m^{-3} during eight hours at ambient temperature.

Date of fumigation: February, 1985

2.2. Effect of fumigation and irradiation on the volatile oil content

Table 5 summarizes the comparative effect of fumigation and gamma irradiation on the volatile oil content of cinnamon, ginger and fennel. The volatile content of fenugreek is nil.

It was found that fumigation with ethylene oxide and irradiation with a dose of 10 kGy did not affect significantly the volatile oil content of cinnamon and fennel. The volatile oil content of ginger was affected by gamma irradiation, while no change in the volatile oil content of ginger was observed after fumigation. Irradiation at a level of 5 kGy resulted in a slight decrease of 14% in the yield of volatile oils and 10 kGy in a 34% loss.

FARKAS (1984) reported for other spices than cinnamon, ginger and fennel that no substantial changes were found in the volatile oil content of most spices when they were treated with doses up to 10–15 kGy. VAJDI and PEREIRA (1973) found that the volatile oil content of black pepper and allspice were reduced by more than half after fumigation, while gamma irradiated spices indicated no change in volatile oils after irradiation.

Table 2

The comparative effect of ethylene oxide and gamma irradiation on the microbial quality of ginger

Organisms	Colony forming (cfu g^{-1})				
	Untreated	Fumigated	2 kGy	4 kGy	6 kGy
Aerobic colony count	6.5×10^{6}	1.3×10^{3}	4.0×10^{5}	1.4×10^{4}	4.3×10^{3}
Pseudomonas	5.5×10^{4}	100	1.5×10^{2}	1.0×10^{2}	10
E. coli	5.0×10^{3}	0.3	3	0.3	0.3
Enterobacteriaceae	7.7×10^{4}	10	5.5×10^{3}	3.0×10^{2}	10
Lactobacillus	1.7×10^{2}	10	—	—	—
Lancefield D streptococci	3.5×10^{3}	10	2.5×10^{1}	2.5×10^{1}	10
Moulds	3.4×10^{3}	10	1.6×10^{2}	1.5×10^{1}	10
Yeasts	10	10	10	10	10
Mesophiles					
Aerobic spores	3.5×10^{6}	5.6×10^{2}	3.0×10^{5}	1.8×10^{4}	9.3×10^{2}
Anaerobic spores	5.5×10^{5}	1.0×10^{2}	1.1×10^{4}	3.1×10^{2}	100
Clostridia	10	10	—	—	—
Thermophiles					
Aerobic spores	100	10	—	—	—
Flat sour spores	1.1×10^{2}	10	10	10	10
Anaerobic spores	100	10	—	—	—
C. thermosaccharolyticum	1	1	—	—	—
C. nigrificans	10	10	—	—	—

—: no growth

Irradiation: at the 40 kCi cobalt-60 research source of the Pilot Plant for Food Irradiation in Wageningen

Date of irradiation: March 1985

Fumigation: with ethylene oxide (EO) at a concentration of 1500 g m^{-3} during eight hours at ambient temperature

Date of fumigation: February, 1985.

2.3. Sensory evaluation

The results of sensory evaluation of the spices are given in Fig. 1.

The scale values of a psychological continuum is got after the z score transformation (EDWARDS, 1960). In this case a preference scale is given, with, at the right, the most preferred samples. On each separate scale the distance between the samples may be compared with each other and it is not allowed to compare a distance between two samples on different scales.

The overall results indicate that the panel is not able to distinguish among the several treatments. There are two possibilities which can explain these results, one because of the slight difference between the not treated and the several times treated spices and the second one is that the panel is not able to distinguish between samples because of adaptation effects of taste.

Table 3

The comparative effect of ethylene oxide and gamma irradiation on the microbial quality of fennel

Organisms	Colony forming (cfu g^{-1})						
	Untreated	Fumigated	2 kGy	4 kGy	6 kGy	8 kGy	10 kGy
Aerobic colony count	4.1×10^5	1.2×10^2	6.1×10^4	1.7×10^4	8.5×10^3	1.5×10^3	4.9×10^2
Pseudomonas	4.7×10^2	10	100	10	10	—	—
E. coli	15	0.3	0.3	0.3	0.3	—	—
Enterobacteriaceae	9.4×10^4	10	1.3×10^4	7.3×10^3	2.9×10^3	4.5×10^2	100
Lactobacillus	100	10	—	—	—	—	—
Lancefield D streptococci	10	10	—	—	—	—	—
Moulds	1.1×10^2	6.5×10^1	1.6×10^2	100	10	—	—
Yeasts	10	10	10	10	10	—	—
Mesophiles							
Aerobic spores	1.8×10^4	100	8.4×10^2	100	10	—	—
Anaerobic spores	1.3×10^4	100	4.1×10^3	10	10	—	—
Clostridia	10	10	—	—	—	—	—
Thermophiles							
Aerobic spores	7.5×10^1	10	—	—	—	—	—
Flat sour spores	2.0×10^1	10	—	—	—	—	—
Anaerobic spores	1.1×10^2	10	—	—	—	—	—
C. thermosaccharolyticum	1	1	—	—	—	—	—
C. nigrificans	10	10	—	—	—	—	—

—: no growth

Irradiation: at the 40 kCi cobalt-60 research source of the Pilot Plant for Food Irradiation in Wageningen
Date of irradiation: April 1985
Fumigation: with ethylene oxide (EO) at a concentration of 1500 g m^{-3} during eight hours at ambient temperature
Date of fumigation: February, 1985.

Table 4

The comparative effect of ethylene oxide and gamma irradiation on the microbial quality of fenugreek

Organisms	Colony forming (cfu g^{-1})				
	Untreated	Fumigated	2 kGy	4 kGy	6 kGy
Aerobic colony count	8.0×10^4	10	4.2×10^3	2.4×10^3	1.9×10^2
Pseudomonas	5.0×10^2	10	—	—	—
E. coli	0.3	0.3	—	—	—
Enterobacteriaceae	5.9×10^2	10	100	10	10
Lactobacillus	10	10	—	—	—
Lancefield D streptococci	10	10	—	—	—
Moulds	3.1×10^2	10	4.0×10^1	10	10
Yeasts	10	10	10	10	10
Mesophiles					
Aerobic spores	8.0×10^4	10	2.5×10^3	100	10
Anaerobic spores	3.0×10^4	10	4.7×10^2	100	10
Clostridia	10	10	—	—	—
Thermophiles					
Aerobic spores	4.0×10^1	10	—	—	—
Flat sour spores	4.0×10^1	10	—	—	—
Anaerobic spores	10	10	—	—	—
C. thermosaccharolyticum	1	1	—	—	—
C. nigrificans	10	10	—	—	—

—: no growth

Irradiation: at the 40 kCi cobalt-60 research source of the Pilot Plant for Food Irradiation in Wageningen
Date of irradiation: April 1985
Fumigation: with ethylene oxide (EO) at a concentration of 1500 g m^{-3} during eight hours at ambient temperature
Date of fumigation: February, 1985

Table 5

Effect of fumigation and irradiation on the volatile oil content of cinnamon ginger and fennel

	Volatile oil content (% v/w)							
	Untreated		Fumigated		5 kGy		10 kGy	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Cinnamon	1.02	0.03	0.88	0.03	—		0.99	0.04
Ginger	1.40	0.02	1.40	0.03	1.21	0.03*	0.92	0.02*
Fennel	1.02	0.02	1.07	0.02	—		1.00	0.005

* Significant at = 5% probability level

Irradiation: at 40 kCi cobalt-60 research source of the Pilot Plant for Food Irradiation in Wageningen
Date of irradiation: April 1985
Fumigation: with ethylene oxide (EO) at a concentration of 1500 g m^{-3} during eight hours at ambient temperature
Date of fumigation: February, 1985

Several factors influence the results, for example fenugreek was not known by the panel as a common spice. In addition, it is more difficult to distinguish among odours than among tastes because of the already mentioned adaptation effect of the smell of spices.

More studies are necessary to find the best concentration to do the experiment and to find the best test procedure. Also, the results indicate that there could be some difference between the wet and dry state of samples.

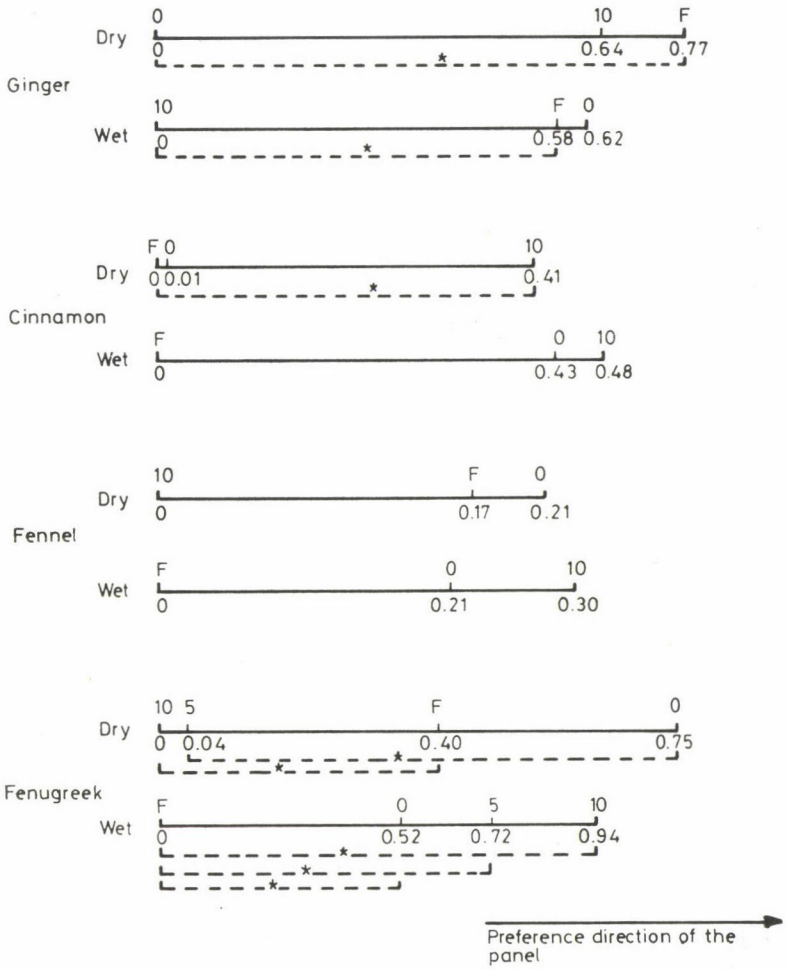

Fig. 1. Effect of gamma radiation and fumigation upon the sensory quality of some spices. The scale values of the preference on a psychological continuum was used. The most preferred samples are at the right of the scale. The irradiated (5–10 kGy) and fumigated (F) and control samples (O) were tested by a panel of 20 members. The samples evaluated in dry and wet state. For ginger, cinnamon and fennel a suspension of 2 g l^{-1} of water and for fenugreek 5 g l^{-1} of water at 60 °C were prepared. The original spices (10 g) were tested at room remperature.
* Significant at P = 0.5% probability level

Perhaps in wet state after making the solution, a layer of oil content covers the solution and makes it difficult to distinguish the original characteristics of the spices.

It may be helpful to order the panel to rest during the tests for at least five minutes in order to get more information about the level of differences between the treatments.

ZEHNDER and ETTEL (1981) reported that irradiation up to a dose of 10 kGy did not affect significantly the flavour of cinnamon. Cinnamon was tested by the panelists in tubes, containing a dry sample of 0.5 gram.

VAN KOOIJ (1967) found that a radiation dose of 5 kGy did not affect the sensory quality of ginger. TJABERG and co-workers (1972) reported that irradiation with doses of 0 to 4.5 Mrad did not significantly alter the taste or odour of ginger.

PADWAL-DESAI and co-workers (1985) reported for cinnamon and dry ginger that the aroma characteristics of the 10 kGy irradiated spices were comparable to the unirradiated ones.

According to ZEHNDER and ETTEL (1981) the aroma of fenugreek powder was significantly damaged by a dose of 5 and 10 kGy. They tested the fenugreek powder in a spice-carrier (skim milk quark, 1% NaCl, 0.5% fenugreek).

3. Conclusions

It was found that for cinnamon and ginger a 6 kGy dose, for fennel 6–10 kGy dose and for fenugreek 6–8 kGy dose were equal in microbial effect to the commercially established fumigation process.

No substantial changes were determined in the volatile oil contents of cinnamon, ginger and fennel treated with ethylene oxide or in the volatile oil contents of cinnamon and fennel treated with ionizing radiation up to 10 kGy. Ginger was sensitive to irradiation, a slight decrease in the volatile oil content was observed after irradiation with a dose of 5 kGy.

In the sensory test used no major differences in sensory characteristics between irradiated, fumigated and untreated spices were found. However, it is not absolutely impossible that using another sensory evaluation method slight differences in some sensory properties can be observed.

From this investigation it can be concluded that irradiation of cinnamon, fennel and fenugreek with doses up to 10 kGy and ginger up to 5–6 kGy is technologically feasible.

*

We would like to express our appreciation to Dr. A. B. CRAWINCKEL, Ms. D. M. VAN MAZIJK for the sensory evaluation of the spices and Dr. OOSTERHEERT, director of IFFIT for correcting the manuscript.

Literature

BERGMAN, C. (1955): Ein Beitrag zur Frage des Keimgehaltes der Gewürze und seiner Abtötung mit der Asta Analysen Quarzlampe. *Mhft. Vet. Med.*, 301–307.

EDWARDS, A. L. (1960): *Techniques of attitude scale construction.* Appleton–Century–Crofts, Inc., New York, pp. 19–52.

FARKAS, J. (1981): *Irradiation of spices and condiments.* IFFIT Report No. 20, IFFIT, Wageningen, The Netherlands.

FARKAS, J. (1983): Radurization and radicidation of spices. — in: JOSEPHSON, E. S. & PETERSON, M. S.: *Preservation of food by ionizing radiation.* CRC Press, Inc. Boca Raton, Vol. III, pp. 109–128.

FARKAS, J. (1984): Radiation processing of dry food ingredients. A review paper. *5th International Meeting on Radiation Processing*, San Diego, USA.

FLANNIGAN, B. & HUI, S. C. (1976): The occurrence of an aflatoxin-producing strains of *Aspergillus flavus* in the mould floras of ground spices, *J. appl. Bact.*, *41*, 411–420.

PADWAL-DESAI, S. R., MUNASIRI, M. A. & GHANKAR, A. S. (1985): The improvement of quality of spices by gamma-irradiation. Poster IAEA-SM-271/23p. *International Symposium on Food Irradiation Processing*, Washington D. C.

THIESSEN, F. (1970): Effect of heat on spices. *Fleischerei*, *22*, (11), 15–18.

TJABERG, T. B. UNDERDAL, B. & LUNDE, G. (1972): The effect of ionizing radiation on the microbial content and volatile constituents of spices. *J. appl. Bact.*, *35*, 473–479.

VAJDI, M. & PEREIRA, R. R. (1973): Comparative effects of ethylene oxide, gamma irradiation and microwave treatments on selected spices. *J. Fd Sci.*, *38*, 893–895.

VAN KOOIJ, J. G. (1967): Neuere Erkentnisse über die Bestrahlung von Früchten und Gemüsen. — in: SOMOGYI J. C. (Ed.) *Nahrungsmittelbestrahlung.* Forster Verlag A. G. Zurich, pp. 44–57.

WESLEY, F., ROUREE, B. & PARBISHIRE, O. (1965): The formation of persistent toxic chlorohydrins in foodstuffs by fumigation with ethylene oxide and with propylene oxide, *J. Fd Sci.*, *30*, 1037–1043.

ZEHNDER, H. J. & ETTEL, W. (1981): Zur Keimzahlverminderung in Gewürzen mit Hilfe von ionisierenden Strahlen. *Alimenta*, *20*, 95–100.

Acta Alimentaria, Vol. 17 (4), pp. 283—289 (1988)

TLC ANALYSIS OF THE 2,4-DINITROPHENYLHYDRAZONE DERIVATIVES OF THE CARBONYL COMPOUNDS OF COOKED, MATURE TUBERS OF WHITE YAM *(DIOSCOREA ROTUNDATA POIR)*

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(Received: 5 January 1987; revision received: 26 November 1987; accepted: 11 January 1988)

The hexane extractable carbonyl compounds of cooked mature tubers of white yam *(Dioscorea rotundata poir)* were precipitated as the 2,4-dinitrophenylhydrazone (2,4-DNPH-one) derivatives. TLC was used to analyse the derivatives. A total of eight carbonyl compounds (four aldehydes, three ketones and one keto-acid) were identified.

Keywords: carbonyl, TLC, white yam

Yams (white yam, yellow yam and water yam) rank with cassava as the staple food of many inhabitants of tropical regions of the world particularly the West African sub-region. Of the three common edible varieties mentioned above, the white yam is the most popular. It is eaten either in the cooked, roasted or fried form with or without vegetable soup, meat or fish.

Like its other edible varieties, white yam is rich in starch but very low in protein content — less than 1% on wet weight basis — (KAY, 1973; OPUTE & OSAGIE, 1978). OSAGIE and OPUTE (1981) reported that lipids comprise 0.42% of the white yam tissue dry weight. Of the fatty acids content, linoleic, palmitic and linolenic acids constitute 47.7%, 32.5% and 8.1%, respectively. Others are pentadecanoic, stearic and archidic acids which constitute 1.9%, 1.6% and 0.6% respectively.

There has been no report as yet, on the carbonyl content of white yam. However, GRAMSHAW and OSINOWO (1982) using GC-MS characterised some of the volatile components of water yam *(Dioscorea alata)*. They identified, among other components, eight aldehydes and four ketones.

Volatile carbonyl compounds, even though usually present in very small amounts, are important flavour components in foodstuff. Of recent, industries producing dehydrated cooked yam powder and flakes are springing up. Some of the food items prepared from such powders and flakes have aroma characteristics not as rich as in those prepared directly from the yam.

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This study is part of a continuing effort of characterizing the flavour pattern of white yam. Apart from its fundamental interest, such knowledge could become very useful in the improvement of the flavour of foods made from processed white yam.

1. Materials and methods

1.1. *Materials and preparation*

Mature tubers of white yam (10.4 kg) were bought from the local market (New Benin). They were peeled, chopped into small pieces and washed with distilled water. The yam was cooked in three batches of about 3.5 kg each in 2 dm^3 of distilled water in 5 dm^3 flat-bottomed flask, mounted with a reflux condenser. After two hours of cooking, the contents of the flask was homogenized into a pasty mash in a cutterblender. This was then extracted batch-wise with 2 dm^3 of hexane previously rendered carbonyl free by the method of HORNSTEIN and CROWE (1962) by continuous shaking for 6 h in a bottle shaker. After filtration, the hexane extract was separated by decantation in a separating funnel. It was then concentrated to about 30 cm^3 in a rotary evaporator. Next it was stirred mechanically for 6 h with twice its own volume of methanolic solution of 2,4-dinitrophenylhydrazine reagent (VOGEL, 1968). The solution was then left undisturbed for 24 h during which time precipitate of the 2,4-dinitrophenylhydrazone (2,4-DNPH-one) derivative of the carbonyl content was formed. The precipitate was filtered, washed with $2N$ H_2SO_4 and then in distilled water. It was purified by recrystallization from dilute methanol. A yield of 0.02% on wet weight basis of the white yam was obtained.

1.2. *TLC analysis*

1.2.1. Separation into classes. The 2,4-DNPH-one derivatives of the carbonyl content of white yam obtained were separated into three classes based on the structures of the parent carbonyl compounds: aliphatic monocarbonyls, aromatic monocarbonyls and bis-derivative of dicarbonyls according to the method of BEYER and KARGL (1972).

A methanolic solution of the 2,4-DNPH-one derivatives of white yam (A) was spread as a streak (using standard TLC micropipette) on standard TLC plates (20 × 20 cm), 2 cm from the base. The plates were coated (0.75 mm thick) with $ZnCO_3$, analar grade, from B. D. H. Chemicals Limited, (sieved to 80 mesh), by using the slurry of the $ZnCO_3$ and distilled water (1 : 2, w/w). Just before use, the coated plates were activated by drying in the oven at 130 °C for 30 min. Spotted alongside (A) as reference samples for each class

were methanolic solution of 2,4-DNPH-one derivatives of acetaldehyde and acetone for aliphatic monocarbonyls; benzaldehyde for aromatic monocarbonyls and diacetyl for bis-derivative of dicarbonyls.

The plates were developed in a Shandon chromatographic tank fitted with filter paper on two sides to ensure saturation of the air within the tank with the vapour of the developing solvent system.

The developing solvent system was pyridine – water (99 : 1, v/v). The atmosphere in the tank was allowed to equilibrate for one hour before the plates were put in. The developing solvent system was allowed to ascend a distance of 15 cm from the base of the plates before the plates were removed. Three bands were obtained. The bands were scraped from the plates and eluted with methanol. Each eluent was filtered and concentrated on water bath to about 1.0 cm^3.

1.2.2. Analysis of the classes. The analysis of the three classes by TLC for the individual carbonyls was done according to the method of DHONT and DIJKMAN (1967; 1969). Standard TLC plates (20×20 cm) were coated with Silica gel G (0.25 mm thick). The plates were activated for 1 h at 120 °C and then cooled in a dessicator. Just before use, the plates were placed on the bench and allowed to equilibrate with the atmosphere for about 30 minutes. In each of the classes, four separate chromatographic runs were done. The mean of the R_f values of each of the spot obtained (R_{fm}) was determined. The standard deviation of the R_{fm} value for each spot was calculated. It was not greater than ± 0.014 for any of the spots. The colours of the spots obtained after development were self-indicating. Spraying with 95% ethanolic solution of 5% aqueous KOH intensified the colours.

1.2.2.1. Aliphatic monocarbonyls — The eluent of the band of R_f value 0.75 to 0.95 (Table 1), corresponding to the 2,4-DNPH-one derivatives of aliphatic monocarbonyls was spotted on the equilibrated TLC plates, 2 cm from the base using two types of adsorbents: Silica gel G (L_1) and Alumina G type 60/E (L_2). Spotted alongside were 2,4-DNPH-one derivatives of acetaldehyde, propanal, n-butanal, iso-butanal, hexane-2-one, heptane-2-one, pentanal, hexanal, heptanal, octanal, acetone, butanone and pentane-2-one. The plates were developed using three solvent systems (Table 2):

— benzene – hexane (1 : 1, v/v)
— CCl_4 – cyclohexane – ethyl acetate (10 : 2 : 1, v/v/v)
— petroleum ether (bp 60–80 °C) – ethyl acetate (7 : 1, v/v).

In each case, the solvent front was allowed to ascend a distance of 15 cm from the spot.

1.2.2.2. Aromatic monocarbonyls — The eluent of the second band of (A) of R_f 0.35 to 0.50 (Table 1), corresponding to the 2,4-DNPH-one derivatives of aromatic monocarbonyls was spotted on the equilibrated TLC plates alongside the 2,4-DNPH-one derivatives of phenylacetaldehyde, benzalde-

hydes, furfural and benzophenone as reference compounds. Two developing solvent systems were used:

— petroleum ether (bp 60–80 °C) – benzene (1 : 1, v/v)
— petroleum ether (bp 60–80 °C) – benzene – ethyl acetate (2 : 1 : 1, v/v/v).

The R_{fm} values of the spots obtained are shown in Table 3.

1.2.2.3. Other carbonyls — The eluent of the last band of (A) of R_f 0.00 to 0.20 (Table 1) was spotted on Silica gel G coated TLC plates (20×20 cm and 0.25 cm thick) and activated at 100 °C for 2 h just before use. Spotted alongside as reference samples were the bis-2,4-DNPH-one derivatives of glyoxal, methylglyoxal, diacetyl and furfural. Two developing solvent systems were used:

— toluene – ethyl acetate (3 : 1, v/v)
— toluene – ethyl acetate (1 : 1, v/v).

Finally, a portion of the original 2,4-DNPH-one derivative of white yam (A) (Table 5) was extracted three times over with 50% aqueous solution of sodium carbonate by vigorous shaking. The carbonate extract was acidified with 2*N* HCl and then extracted with carbonyl free hexane. The hexane layer was concentrated to about 0.5 cm^3 (A_4). The TLC of the concentrate (A_4) was then developed on a Silica gel G plate activated for 30 min at 120 °C and using as developing solvent system: petroleum ether (bp 60–80 °C) — ethyl formate — propionic acid (26 : 14 : 3, v/v/v). Spotted on the plate as reference samples were the 2,4-DNPH-one derivatives of pyroracemic acid, acetoacetic acid, laevulic acid and glyoxylic acid.

2. Results

Three bands of (A) of R_f values 0.75 to 0.95 (A_3); 0.35 to 0.50 (A_2); and 0.00 to 0.20 (A_1) were obtained. These bands correspond to three classes of carbonyl compounds: aliphatic monocarbonyls (A_3); aromatic monocarbonyls (A_2), and bis-derivatives of dicarbonyls (A_1). They are presented in Table 1.

The compounds identified in (A_3), (A_2) and (A_1) are shown in Tables 2, 3 and 4, respectively.

An attempt was made to identify other carbonyl components of (A) — mainly the aldehydic and ketonic acids — not belonging to any of the three classes enumerated above. The result of this attempt is shown in Table 5.

In all, eight carbonyl compounds (four aldehydes, three ketones and one ketoacid) were identified among the hexane extractable carbonyl compounds of cooked white yam. They are: isobutanal, hexanal, phenylacetaldehyde, benzaldehyde, butanone, hexane-2-one, heptane-2-one and pyroracemic acid.

Five other carbonyl compounds were also detected in the extract. Two of these are aliphatic monocarbonyls which are likely to contain not more than four carbon atoms per molecule, as concluded from their R_f values. Two others are likely to be aromatic monocarbonyls or aliphatic monocarbonyls with an aromatic substituent. The last component is likely to belong to the class of bis-derivative of dicarbonyls.

Table 1

The TCL analysis of 2,4-DNPH-one derivatives of white yam (A) and reference compounds

	R_f value	Colour	Class
Diacetyl	0.17	dark blue	bis derivative of dicarbonyls
Sample (A) Band No. 1 (A_1)	0.00—0.20	dark blue	
Benzaldehyde	0.40	purple	aromatic monocarbonyls
Sample (A) Band No. 2 (A_2)	0.35—0.50	purple	
Acetone	0.80	yellow	aliphatic monocarbonyls
Acetaldehyde	0.85	yellow	
Sample (A) Band No. 3 (A_3)	0.75—0.95	yellow	

Adsorbent: $ZnCO_3$
Developing solvent system: pyridine–water (99 : 1, v/v)

Table 2

Analysis by TLC of A_3 (Eluent of R_f value 0.75–0.95 of A)

Sample A_3	L_1S_1		L_1S_2		L_2S_3		Components identified
Spot number	R_{fm} values	colour	R_{fm} values	colour	R_{fm} values	colour	
1	0.15	dirty yellow	0.19	yellow	0.48	yellow	—
2	—	—	0.25	yellow	—	—	—
3	0.38	yellow	0.52	deep yellow	0.54	deep yellow	butanone
4	0.42	yellow	0.55	yellow	—	—	iso-butanal
5	0.46	yellow	0.60	yellow	0.57	brownish yellow	hexanal
6	0.49	orange	0.65	orange	0.60	orange	hexane-2-one
7	0.53	orange	0.68	orange	0.63	orange	heptane-2-one

Adsorbents
L_1: Silica gel G.
L_2: alumina G (Type 60/E)
Developing solvent systems
S_1: benzene–hexane (1 : 1, v/v)
S_2: CCl_4–cyclohexane–ethyl acetate (10 : 2 : 1, v/v/v)
S_3: petroleum ether (bp 60–80 °C)–ethyl acetate (7 : 1, v/v)

Table 3

Analysis by TLC of A_2 (Eluent of R_f value 0.35–0.50 of A)

Sample A_2 spot number	L_1S_1		L_1S_2		Components identified
	R_{fm} values	colour	R_{fm} values	colour	
1	0.25	purple	0.34	purple	—
2	0.33	lilac	0.48	lilac	phenylacet-aldehyde
3	0.38	purple	0.57	purple	benzaldehyde
4	0.44	purple	0.67	purple	—

Adsorbent
L_1: Silica gel G
Developing solvent systems
S_1: petroleum ether (60–80 °C)–benzene (1 : 1, v/v)
S_2: petroleum ether (60–80 °C)–benzene–ethylacetate (2 : 1 : 1, v/v/v)

Table 4

Analysis by TLC of A_1 (Eluent of R_f value 0.00–0.20 of A)

Sample A_1	L_1S_1		L_1S_2		Component identified
	R_{fm}	colour	R_{fm}	colour	
1	0.64	purple	0.55	purple	—

Adsorbent
L_1: Silica gel G
Developing solvent systems
S_1: toluene–ethyl acetate (1 : 1, v/v)
S_2: toluene–ethyl acetate (3 : 1, v/v)

Table 5

The TLC analysis of the ketoacids of the 2,4-DNPH-one derivatives of white yam

Sample A_4 spot number	L_1S_1		Components identified
	R_{fm}	colour	
1	0.22	violet	pyroracemic acid

Adsorbent
L_1: Silica gel G
Developing solvent system
S_1: petroleum ether (60–80 °C)–ethyl formate–propionic acid (26 : 14 : 3, v/v/v)

3. Conclusions

Identification of the compounds and classification of the carbonyl components were facilitated by the colours of the TLC spots which were made more intense by spraying with the alcoholic potassium hydroxide indicator.

The thin-layer-chromatographic technique is a proven and well characterized method of analysing the constituents of a complex mixture of chemical compounds. It is simple, fast and reliable. While the technique cannot by itself give a full range of all the components of a complex chemical mixture like the one under investigation, the major components (of relatively high concentration) can be identified. The limitation of the technique notwithstanding, this study may be seen as a beginning of an effort to analyse and characterize the flavour pattern of white yam.

Work is still in progress at identifying more of the compounds that constitute the carbonyl fraction of cooked white yam.

*

The author is grateful to Miss VICTORIA BABATUNDE who helped in the preparation of the 2,4-dinitrophenylhydrazone derivative of cooked white yam.

Literature

BAYER, C. F. & KARGL, T. E. (1972): Separation of 2,4-dinitrophenylhydrazone into classes by thin layer chromatography on zinc carbonate. *J. Chromatogr., 65,* 435–438.

DHONT, J. H. & DIJKMAN, G. J. C. (1967): The qualitative analysis of complex carbonyl mixtures by thin-layer chromatography. *Analyst, 92,* 431–435.

DHONT, J. H. & MULDERS—DIJKMAN, G. J. C. (1969): The resolution of mixtures of aliphatic and terpene-type 2,4-dinitrophenylhydrazones by thin-layer chromatography on silica gel. *Analyst, 94,* 1090–1094.

GRAMSHAW, J. W. & OSINOWO, F. A. O. (1982): Volatile components of cooked tubers of the water yam *(Dioscorea alata). J. Sci. Fd Agric., 33,* 71–80.

HORNSTEIN, I. & CROWE, P. F. (1962): Carbonyl removal from nonoxygenated solvents. *Anal. Chem., 34,* 1037–1038.

KAY, D. E. (1973): *Root crops.* Tropical Products Institute HMSO, London. – ref.: OPUTE & OSAGIE (1978).

OPUTE, F. I. R. & OSAGIE, A. U. (1978): Identification and quantitative determination of the lipids of *Alocasia macrorrhiza* tubers. *J. Sci. Fd Agric., 29,* 1002–1006.

OSAGIE, A. U. & OPUTE, F. I. (1981): Major lipid constituents of *Dioscorea rotundata* tuber during growth and maturation. *J. Exp. Bot., 32,* 737–740.

VOGEL, A. I. (1968): *A textbook of practical organic chemistry.* E. L. B. and Longmans, Green and Co. Ltd. London, p. 1061.

Acta Alimentaria, Vol. 17 (4), pp. 291—297 (1988)

SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS OF SEED PROTEINS AS A TEST FOR SCREENING HIGH COOKING QUALITY DURUM WHEAT STRAINS

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(Received: 13 April 1987; revision received: 8 January 1988; accepted: 13 January 1988)

An improved method of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate the glutenin subunits of seven durum wheat varieties previously evaluated for quality and gliadin composition.

Varieties studied differ in the presence or the absence of two medium molecular weight glutenin subunit bands denoted as MI and MII. Band MI appears to be linked with γ-gliadin 45 band and strong gluten plus good cooking quality, and band MII linked with γ-gliadin 42 band and weak gluten plus poor cooking quality.

The described method can be used within a breeding program as a screening test for gluten strength and cooking quality in the early generations of durum wheat.

Keywords: SDS-PAGE method, durum wheat, glutenin, gliadin

Protein quantity and quality of durum wheat (*Triticum durum* Desf.) are decisive factors of pasta cooking quality. High-protein durum wheat varieties with strong gluten and high elastic recovery exhibit good cooking quality, whereas low-protein and weak gluten varieties with low elastic recovery are poorer in this respect (FEILLET et al., 1977). It is known that gliadins and glutenins interact to form the gluten complex, and provide special characteristics: gliadins with relatively low molecular weights (25 000–100 000) extensibility, and glutenins with high molecular weights (100 000–3 000 000) elasticity (SARKKI, 1980; FEILLET, 1980).

In the last decade, more attention has been paid to specific gliadin polypeptides and their relationships with the elastic recovery and pasta cooking quality. It was found that γ-gliadin 42 is associated with poor elastic recovery and γ-gliadin 45 with high elastic recovery (DAMIDAUX et al., 1978, 1980; KOSMOLAK et al., 1980; DUCROS et al., 1982; TAHA & SÁGI, 1987). In contrast, the relationship between glutenin subunits and pasta quality parameters deserved limited attention. WASIK and BUSHUK (1975) observed that the amount of some medium molecular weight glutenin subunits are virtually

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related to the spaghetti-making quality. In spite of it, AUTRAN (1981) and DUCROS and co-workers (1982) found that the highest molecular weight glutenin subunits do not have any specific effects and could act only as a bulk fraction relative to other protein fractions. Nevertheless, some glutenin subunits generally linked to γ-gliadin 45 (AUTRAN, 1981; PAYNE et al., 1984) seem to affect the cooking quality of pasta products beneficially. More recently, DUCROS (1987) found that the quantity of low molecular-weight glutenin polypeptides is highly correlated to gluten strength, whereas the high-molecular weight glutenins are generally poor indicators of the viscoelastic properties.

In the present study, an improved method of discontinuous SDS-PAGE standardized for screening high cooking quality durum wheat strains in the early generations within a breeding program was used to examine the glutenin subunits as possible indicators of pasta quality.

1. Materials and methods

Seven durum wheat varieties, four Hungarian (GK Minaret, GK Basa, GK Madur, SZ D 8), two Austrian (Miradur, Attila) and one Egyptian (Stork-s), grown in the nursery of the Cereal Research Institute (Szeged, Hungary) previously evaluated for quality and gliadin proteins from crop years 1983, 1984 and 1985 (TAHA & SÁGI, 1987) were used in this study.

Mixing properties (farinograph and mixograph), gluten strength (SDS sedimentation test), firmness of normally cooked pasta and tolerance to over-cooking (aleurograph test) and gliadin protein composition (AL-PAGE) were assessed as described previously (TAHA & SÁGI, 1987).

For the SDS-PAGE of proteins, an improved method of discontinuous buffer system was used by the combination of the methods of LAEMMLI (1970) and PAYNE and CORFIELD (1979) with some modifications (Fig. 1).

Total proteins were extracted for 2 h at room temperature from 0.1 g flour with 2 cm^3 of extracting solution, containing 0.0625 M TRIS–HCl (pH 6.8), 2% SDS, 10% glycerol, 5% 2-mercaptoethanol and 0.01% pyronine G (LAEMMLI, 1970), followed by centrifugation for 10 min at 10 000 r.p.m. Then the supernatant was poured off and the residue was immersed for 3 min in boiling water.

A vertical slab gel electrophoresis apparatus consisting of 17×20 cm glass plates, 1.5 mm spacers, 9 well combs and two buffer reservoirs of 250 cm^3 and 500 cm^3 was used, with a power supply unit EPS 500/400 from Pharmacia.

The required stocks for gel mixtures and reservoir buffer were prepared as described in Table 1. The resolving and stacking gels of 12% and 3.75% acrylamide, respectively, were prepared as listed in Table 1, and polymerized

in a gel former of 180×150×1.5 mm as described by HAMES and RICKWOOD (1981).

Aliquots (70 μl) of each sample extract were carefully loaded into the gel sample wells using a Hamilton microliter syringe. A protein mixture of molecular weight (MW) range between 30 000 and 94 000 from Pharmacia was used.

In a cooled room (ca 7 °C) the electrophoresis apparatus was connected to the power supply unit with the anode (+) connected to the bottom reservoir,

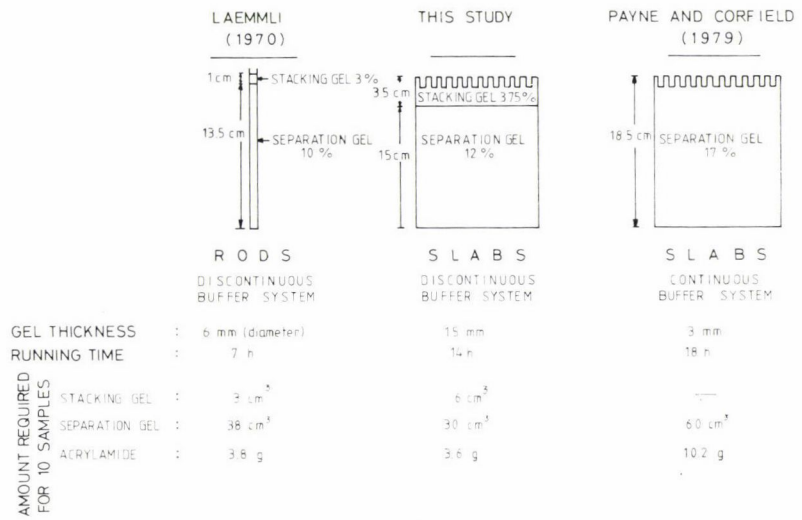

Fig. 1. Comparison of the SDS-PAGE method used in this study with those of LAEMMLI (1970) and PAYNE and CORFIELD (1979)

Table 1

Protocol of gel preparation for SDS-discontinuous buffer system

Stock solution	Stacking gel 3.75% per one gel (cm³)	Resolving gel 12% per one gel (cm³)	Reservoir buffer per two gels (cm³)
Acrylamide–bisacrylamide (30 : 0.8)	1.25	12.00	—
Stacking gel buffer stock (0.5 mol l^{-1} TRIS-HCl, pH 8.8)	2.50	—	—
Resolving gel buffer stock (3.0 mol l^{-1} TRIS-HCL, pH 8.8)	—	3.75	—
Reservoir buffer stock (0.25 mol l^{-1} TRIS, 1.92 mol l^{-1} glycine, 1% SDS, pH 8.3)	—	—	100
10% SDS	0.10	0.30	—
1.5% ammonium persulphate	0.50	1.50	—
Water	5.65	12.45	900
TEMED (Trimethyl ethylenediamine)	0.015	0.015	—
Total volume	10.00	30.00	1000

and the cathode (—) to the upper. The current was maintained at 10 mA per gel and electrophoresis was performed for about 14 h when the tracking dye moved 1 cm from the bottom of the gel.

After electrophoresis, the gels were loosened from the glass plates by forcing water under the gel using a syringe. Then the proteins were fixed in the gel with 14% trichloroacetic acid (200 cm^3) for 5 h with continuous swirling on a shaker. The gels were stained according to PAYNE and CORFIELD (1979) with 0.5% (w/v) Coomassie Brilliant Blue R 250 in water–methanol–acetic acid (53 : 25 : 7, v/v/v) overnight, with continuous stirring and destained with several changes of water–methanol–acetic acid (68 : 25 : 7, v/v/v) until obtaining a clear background. Records were made by a Joyce-Loebl densitometer.

2. Results

The SDS-PAGE patterns of reduced total protein extracted from flours of the seven durum wheat varieties studied are shown in Fig. 2. The areas of the high molecular weight (HMW, $>$ 60 000), medium molecular weight (MMW, 40 000–60 000) and low molecular weight (LMW, $<$ 40 000) gluten bands are marked on Fig. 2.

The most important observation is that the studied varieties differ by the presence or the absence of two MMW glutenin subunit bands in the top half of the gel between 45 000 and 60 000 MW (denoted by MI and MII in the Figs. 2 and 3). According to the presence or absence of these bands, the 7 durum wheats examined could be classified into three groups. In group I (lane 4) band MII is present and band MI is absent. In group II (lanes 2, 3) both MI and MII bands are present. In group III (lanes 1, 5, 6, 7) band MI is definitely present and band MII is absent.

Based upon the previous quality evaluation of the investigated varieties from crop years 1983, 1984 and 1985 for mixing properties, gluten strength and cooking quality (firmness and tolerance to overcooking) as well as the examination of glidin protein composition by AL-PAGE (TAHA & SÁGI, 1987), it can be stated that the varieties of high gluten strength and good cooking quality with γ-gliadin 45 band (i.e. GK Minaret, Miradur, SZ D 8 and Stork-s) also contain a strong glutenin subunit MI, but not MII (Fig. 2, lanes 1, 5, 6 and 7, respectively), whereas the variety of the lowest gluten strength and poorest mixing properties with strong γ-gliadin 42 band and without γ-gliadin 45 band (Attila) contains MII glutenin subunit without MI (lane 4). The varieties of relatively low gluten strength and poor cooking quality with both γ-gliadins 42 and 45 (GK Madur and GK Basa) also contain the MII band associated with MI (lanes 2, 3).

The HMW glutenin subunit bands at the top of the gel and LMW bands at the bottom half of the gel are virtually not related with the quality of durum wheat or pasta products. However, the pattern differences in these band groups can be used for cultivar identification. See, for instance, HMW and MMW bands denoted by asterisks in Fig. 2.

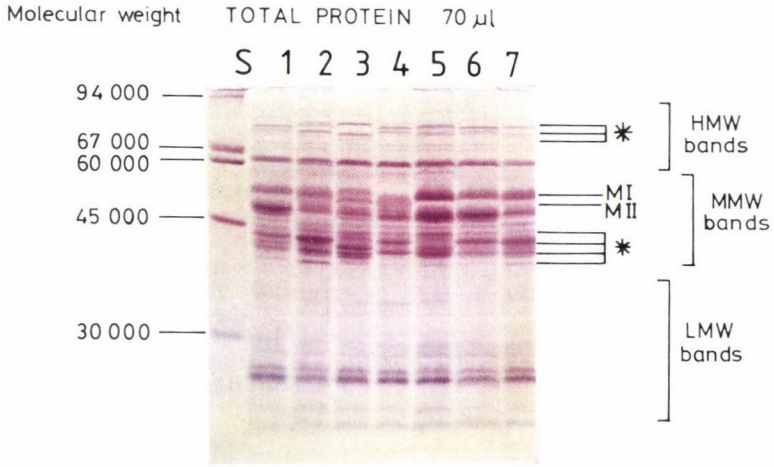

Fig. 2. Discontinuous SDS-PAGE patterns of reduced proteins extracted from the flour of some durum wheats. S: Standard; 1: GK Minaret 2: GK Madur; 3: GK Basa 4: Attila; 5: Miradur; 6: SZ D 8; 7: Stork-s. For technical details, see Fig. 1.

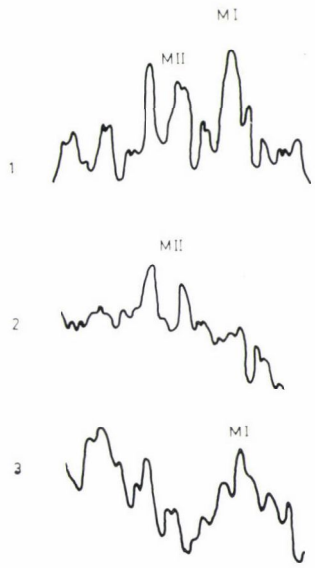

Fig. 3. Densitometer tracings of the MI–MII region of flour protein electrophoregrams obtained by SDS-PAGE. 1: GK BASA, 2: ATTILA, 3: MIRADUR

3. Conclusions

By the combination of the results of this study with that of obtained from the evaluation of gluten strength, cooking quality and gliadin composition for the seven durum wheat varieties under investigation (TAHA & SÁGI, 1987), it can be suggested that glutenin subunit MI appears to be linked with γ-gliadin 45 and strong gluten plus good cooking quality, while glutenin subunit MII linked with γ-gliadin 42 and weak gluten plus poor cooking quality.

For the durum wheat breeders and processors, it can be recommended to use the SDS-PAGE of total protein extracts additionally with AL-PAGE of gliadins as a good screening test for gluten strength and cooking quality. The SDS-PAGE requires only a small number of seeds, thus it can be easily used for screening early generations of durum wheat when the amount of seed is limited.

URIEL (1966) suggested that to obtain an optimal electrophoretic separation, an electrophoresis apparatus should have three attributes:

— ability to maintain the gel at a uniform thickness throughout;

— the same materials and conditions in contact with all of the gel solution during polymerization;

— a cover for the gel before and during electrophoresis to prevent the gel surface from drying out.

In addition, the gel should be maintained at a constant temperature below room temperature. The vertical slab gel former apparatus used and the method described here meet all of these requirements, and offer definite advantages over the method of LAEMMLI (1970) and PAYNE and CORFIELD (1979). Beside the reduction of running time and saving of chemicals required (Fig. 1), higher volumes of protein extract can be used and sharper bands can be obtained because of stacking effect and thinner gels used (Fig. 2).

Literature

AUTRAN, J. C. (1981): Recent data on the biochemical basis of durum wheat quality. — in: CHARALAMBOUS, G. & INGLETT, G. (Eds.) *The quality of food and beverages.* Vol. 1. *Chemistry and technology.* Academic Press, New York, p. 257.

DAMIDAUX, R., AUTRAN, J. C., GRIGNAC, P. & FEILLET, P. (1978): Mise en évidence de relations applicables en sélection entre l'électrophorégramme des gliadines et les propriétés viscoélastiques du gluten de *Triticum durum* DESF. *C. r. hebd. Acad. Sci.*, Paris, *Sér. D., 287*, 701.

DAMIDAUX, R., AUTRAN, J. C. & FEILLET, P. (1980): Gliadin electrophoregrams and measurements of gluten viscoelasticity in durum wheats. *Cereal Fds Wld, 25*, 754.

DU CROS, D. L. (1987): Glutenin proteins and gluten strength in durum wheat. *J. Cereal Sci., 5*, 3–12.

DU CROS, D. L., WRIGLEY, C. W. & HARE, R. A. (1982): Prediction of durum wheat quality from gliadin protein composition. *Aust. J. agric. Res., 33*, 429.

FEILLET, P. (1980): Wheat proteins. Evaluation and measurements of wheat quality. — in: INGLETT G. E. & MUNCK, L. (Eds.) *Cereals for foods and beverages.* Academic Press, New York, p. 183.

FEILLET, P., ABECASSIS, J. & ALARY, R. (1977): Description d'un nouvel appareil pour mesurer les propriétés viscoélastiques des produits céréaliers. Application à l'appréciation de la qualité du gluten, des pâtes alimentaires et du riz. *Bull. ENSMIC, 273*, 97.

HAMES, B. D. & RICKWOOD, D. (1981): *Gel electrophoresis of proteins: A practical approach.* IRL Press Ltd., London.

KOSMOLAK, F. G., DEXTER, J. E., MATSUO, R. R., LEISLE, D. & MARCHYLO, B. A. (1980): A relationship between durum wheat quality and gliadin electrophoregrams. *Can. J. Pl. Sci., 60*, 427–432.

LAEMMLI, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, 227*, 680–685.

PAYNE, P. I. & CORFIELD, K. G. (1979): Subunit composition of glutenin proteins, isolated by gel filtration in a dissociating medium. *Planta, 145*, 83–88.

PAYNE, P. I., JACKSON, E. A. & HOLT, L. N. (1984) The association between γ-gliadin 45 and gluten strength in durum wheat varieties: A direct causal effect or result of genetic linkage. *J. Cereal Sci., 2*, 73–81.

SARKKI, M. L. (1980): Wheat gluten. — in: INGLETT, G. E. & MUNCK, L. (Eds.) *Cereals for food and beverages*. Academic Press, New York, p. 155.

TAHA, S. A. & SÁGI, F. (1987): Quality of durum wheat (*Triticum durum* Desf.): Grouping of varieties according to their gluten strength, cooking behavior and gliadin composition. *Cereal Res. Communs, 15*, 281–288.

URIEL, J. (1966): Méthode d'électrophorèse dans les gels d'acrylamide-agarose. *Bull. Soc. Chim. biol., 48*, 969.

WASIK, R. J. & BUSHUK, W. (1975): Relation between molecular weight distribution of endosperm proteins and spaghetti-making quality of wheats. *Cereal Chem., 52*, 322–327.

Acta Alimentaria, Vol. 17 (4), pp. 299—307 (1988)

COMPARATIVE BIOCHEMICAL STUDY ON THE EFFECT OF DRYING TEMPERATURE ON MACARONI QUALITY

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(Received: 9 June 1987; accepted: 21 January 1988)

Chemical composition, oxidative enzyme activities, color, strength and cooking quality of macaroni dried under high temperature (HT) and low temperature (LT) programs were compared. Drying of macaroni at HT compared to LT increased the losses of soluble protein and total lipids, decreased carotenoid pigment losses, inactivated lipoxygenase (LPO), peroxidase (PO) and polyphenoloxidase (PPO) in a greater extent, increased the yellow index (YI) and decreased the brown index (BI). Raw macaroni strength and cooking quality were less affected by the drying temperature. Drying of macaroni at HT offers definite advantages over the LT system. Beside the reduction of drying time, HT programme yields a macaroni with improved color without sacrifying cooking quality, strength or nutritional value.

Keywords: macaroni quality, drying temperature, oxidative enzymes, color indexes

The drying stage is a critical, time-consuming and difficult-to-control process in the production of pasta materials. The target of drying is to lower moisture content of the product from 31% to 12 or 13%. In the last decade, high-temperature drying, with air temperatures of 70 °C or more, has become widely accepted by European pasta processors for hygienic reasons, and because it permits shorter drying cycles in more compact drying lines of a given capacity (MANSER, 1978, 1980; PAVAN, 1980). It has been stated that the increased drying temperature improves pasta color and cooking quality (PAVAN, 1979a, 1979b; DEXTER et al., 1981; WYLAND & D'APPOLONIA, 1982). The improvement of color has been linked to the inactivation of oxidative enzymes (LAIGNELET, 1983), especially of lipoxygenase (DEXTER et al., 1981) during the high temperature processing.

This study was offered to compare the effect of HT and LT drying conditions on the quality properties, some chemical components and oxidative enzyme activities of the macaroni processed from the semolina of three Hungarian durum wheat varieties. To our best knowledge, such studies with Hungarian durum products have not been published yet.

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1. Materials and methods

1.1. Milling and purification

Seed samples of the Hungarian durum wheat varieties GK Minaret, SZ D 8 and GK Basa grown in the nursery of the Cereal Research Institute (Szeged, Hungary) from the 1984 harvest were used in this study. The grain samples were tempered (14.5% moisture), milled in a modified ÉLGÉP laboratory mill and purified with a laboratory purifier. Particle size of the purified semolina was 200–400 μm.

1.2. Macaroni processing

Macaroni (hollow long tubes of 3.5 mm diameter) was processed in the Institute of Milling and Baking Industry (Budapest, Hungary) with a laboratory scale PAVAN 1 type extruder (5 kg of semolina each) at 50 °C die and extrusion temperature, 31% absorption and at a vacuum of 55–60 Hg mm, according to the method of Szakács & Rosza-Kiss (1981). Each macaroni sample was dried in an experimental pasta dryer at LT and HT drying programs. In the LT procedure, macaroni was dried for a 30 h period with a controlled decrease in relative humidity from 97% to 67%, while temperature was maintained at 40 °C, followed by controlled decrease in temperature from 40 °C to 25 °C, during 3 h at constant 60% relative humidity. The HT cycle is featured by a high initial temperature (70 °C for 3 h, increased to 80 °C for 1 h), followed by a step-wise decrease of temperature over the next 4 h to 40 °C, at a parallel decrease of the relative humidity from 84% to 58%. The conditions were held at 40 °C for another 14 h followed by a step-wise decrease in temperature over the final 5 h to 25 °C, at a constant relative humidity of 58% (Fig. 1).

1.3. Mixing properties

Mixing properties of the semolina of each variety were assessed in duplicate by both farinograph (Irvine et al., 1961) and micro-mixograph techniques (Finney & Shogren, 1972).

1.4. Color indices

Semolina and macaroni color indices (YI and BI) were determined using the adaptation of the method of Alause and Feillet (1970) as described previously by Taha and Sági (1987).

1.5. Chemical analyses

Total protein content (N × 5.75) in semolina and macaroni was determined by the conventional Kjeldahl method (AACC, 1962).

Soluble protein content was measured spectrophotometrically according to SPECTOR (1980).

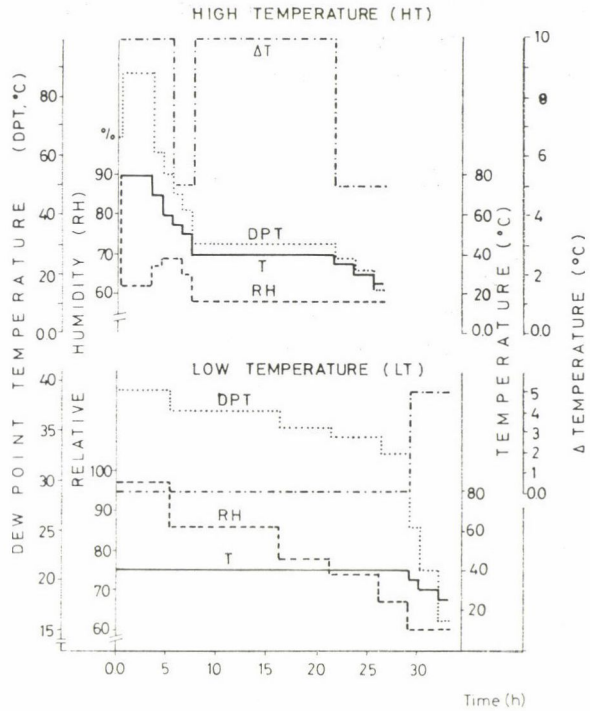

Fig. 1. Temperature and humidity conditions for low temperature (LT) and high temperature (HT) drying programs

Carotenoid pigments were determined as described in AACC (1968), except the preparation of the standard curve, which was made with potassium chromate instead of carotene according to BINNINGTON and GEDDES (1939).

Total lipids were extracted in a Soxhlet-apparatus for 20 h with chloroform–methanol (2 : 1 v/v) and weighed after evaporation of the solvent (KATES, 1972).

Oxidative enzyme activities were determined spectrophotometrically (lipoxygenase, LPO: MCDONALD 1979, SZÁNTHÓ et al., 1981; peroxidase, PO: HONOLD & STAHMANN 1968; KOBREHEL et al., 1974; polyphenoloxidase, PPO: HONOLD & STAHMANN, 1968; KRUGER, 1976; LAMKIN et al., 1981).

1.6. Macaroni quality

Macaroni cooking characteristics and strength of the dried products were determined as described in the HUNGARIAN STANDARD (1958).

1.7. Statistical analyses

The statistical analyses were made with a Commodore 64 personal computer according to SVÁB (1981).

The data presented here are averages of at least 3 parallel determinations.

2. Results and discussion

As indicated by the mixograms and farinograms (Fig. 2), the semolinae milled from GK Minaret had the highest mixing strength and that of GK Basa had the lowest. Semolina of SZ D 8 was intermediate in this respect.

As obvious from the data presented in Tables 1,2 and 3, the characteristics of macaroni seem to be affected partly by semolina characteristics and partly by the drying conditions.

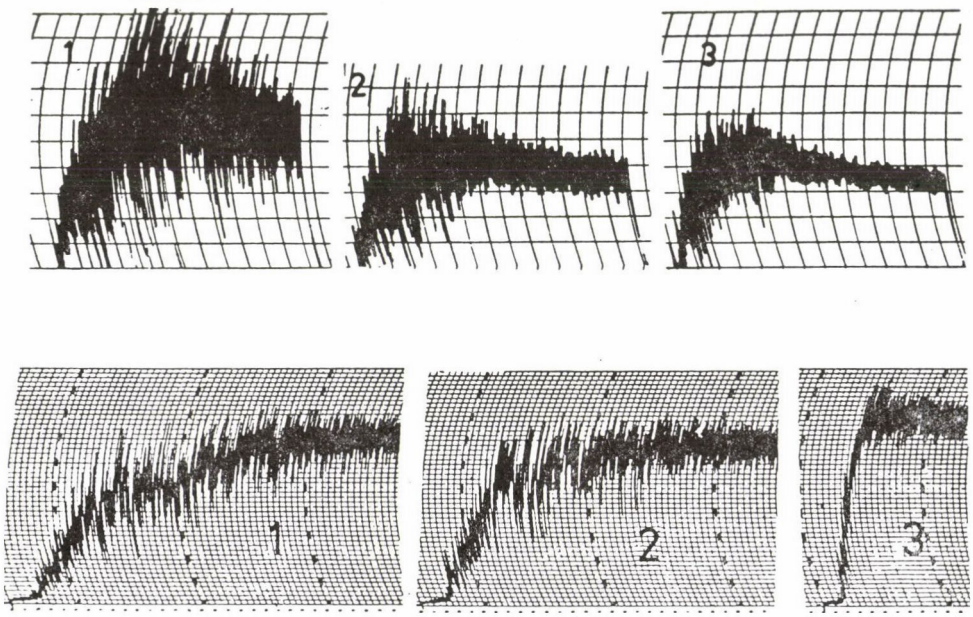

Fig. 2. Mixograms (2a) and farinograms (2b) showing mixing strength of semolina milled from durum wheat varieties. 1: GK Minaret, 2: SZ D 8 and 3: GK Basa (10 g of 14% moisture, 5.2 cm^3 water and mixing time 6 min were used for mixograph test, and 50 g of 14% moisture, 30 °C bowl temperature, and 33.5% water absorption for the farinograph test)

Table 1

Effect of drying conditions on macaroni chemical composition and color indices

Treatment and variety	Protein (%)[a] (N × 5.75)		Total lipid (%)[a]	Carotene (mg per kg)[a]	Enzyme activities			Color	
	total	soluble			PO (EU per 10 g)[a]	PPO (EU per 0.1 g)[a]	LPO (EU per 0.1 g)[a]	YI	BI
Semolina									
GK Minaret	15.9	1.61	2.51	3.22	10.5	90	40.3	29.2	33.5
SZ D 8	12.7	1.24	2.15	3.41	5.8	75	65.4	29.4	30.6
GK Basa	13.3	1.49	2.14	2.40	10.0	84	51.9	27.3	32.2
LT-macaroni									
GK Minaret	15.2	1.00	2.10	2.22	11.9	97	29.4	24.1	34.7
SZ D 8	13.2	0.73	1.73	2.71	6.5	67	53.3	27.4	32.4
GK Basa	14.7	0.93	1.64	1.99	12.0	82	33.5	23.8	40.0
HT-macaroni									
GK Minaret	14.6	0.37	1.36	2.75	6.4	37	28.2	25.8	26.6
SZD 8	13.5	0.29	0.99	3.38	3.4	53	12.0	28.3	28.7
GK Basa	14.3	0.49	0.94	2.26	10.5	96	11.8	26.2	33.4
SD 5% between varieties	1.59	0.18	0.06	0.39	2.91	46.36	27.18	11.42	5.48
SD 5% between treatments	1.59	0.18	0.06	0.39	2.91	46.36	27.18	11.42	5.48
F test for varieties	+	*	***	**	*	NS	+	NS	NS
F test for treatments	NS	***	***	*	+	NS	NS	NS	+

+ Significant at P $\geq$ 90 % probability level
* Significant at P $\geq$ 95 % probability level
** Highly significant at P $\geq$ 99 % probabilty level
*** Very highly significant at P $\geq$ 99,9 % probability level
[a]: on dry weight basis
NS: non significant

The values of YI and BI for semolina and macaroni (Table 1) revealed that the macaroni processed from SZ D 8 exhibited the best color values represented in the highest YI and lowest BI, compared to those processed from GK Minaret and GK Basa. The improved color of the SZ D 8 macaroni appears to be a result of its higher carotene content and lower PO and PPO activities. The YI is in a highly significant positive correlation with carotene content ($r = 0.88^{***}$) and negatively correlated with PO activity ($r = -0.58^{*}$), whereas BI is in a highly significant positive correlation with PO ($r = 0.79^{**}$) and PPO ($r = 0.71^{**}$) activities, but negatively correlated with carotene content ($r = -0.64^{*}$), (TAHA, 1987).

It is also clear from Table 1 that the macaroni dried at HT compared to that dried at LT conditions exhibited higher YI values and carotene content in all varieties. This result is in good agreement with that obtained by LAIGNELET (1977) and by DEXTER and co-workers (1981), and confirms the statement of PAVAN (1979b) that application of HT during initial drying is the most effective way for improving spaghetti color. HT also led to greater inactivation of all oxidative enzymes examined (except of PO and PPO in case of GK Basa) in coincidence with PAVAN (1979a, b) and LAIGNELET (1983).

It has been reported that HT may cause undesirable browning due to Maillard-type reactions (LAIGNELET, 1977; MANSER, 1978; PAVAN, 1979b). However, PAVAN (1979b) stated that if temperature was maintained below 80 °C, the browning effect could be greatly reduced. Beside the inactivation of oxidative enzymes, HT program used in this study prevents the product from browning due to Maillard-type reactions as obvious from the lower BI values of macaroni dried at HT compared to that dried at LT (Table 1), since the average of BI values in HT-macaroni did not exceed 82.9% the average LT-macaroni.

As it can be seen in Table 2, the amount of soluble protein, total lipids and LPO activity lost during macaroni processing under LT drying conditions were about half of the amounts lost under HT conditions. There were no losses in PO and PPO activities (except PPO in case of SZ D 8) under LT conditions, whereas 26.8% and 29.4% of semolina PO and PPO activities were lost during processing under HT conditions, respectively. In other words, HT drying cycle resulted in a highly significant decrease of protein solubility and, accordingly, the differences in the losses of oxidative enzyme activities were statistically not significant. An inverse relationship between the losses of carotenoids and LPO activity during processing is quite clear. As a consequence, the carotenoid

Table 2

Effect of drying conditions on the processing loss of some components, enzyme activities and color of macaroni

(%, based on the contents and activities in unprocessed semolina)

Macaroni samples	Soluble protein	Total lipid	Carotenoid pigments	Activity of			YI
				LPO	PO	PPO	
Low temperature							
GK Minaret	37.9	16.3	31.10	27.1	0.0	0.0	17.5
SZ D 8	41.1	19.5	20.50	18.5	0.0	10.7	6.8
GK Basa	37.6	33.5	17.10	35.5	0.0	0.0	12.8
Mean	38.9	23.1	22.9	27.0	0.0	3.6	12.4
High temperature							
GK Minaret	77.0	45.8	14.60	30.0	39.1	58.9	11.6
SZ D 8	76.6	54.0	0.88	81.7	41.4	29.3	3.7
GK Basa	67.1	56.1	5.80	77.3	0.0	0.0	4.0
Mean	73.6	52.0	7.1	63.0	26.8	29.4	6.4
SD 5% between varieties	14.76	18.02	12.79	93.02	70.79	91.61	8.67
SD 5% between drying cycles	12.05	14.71	10.44	75.95	57.80	74.80	7.08
F test for varieties	NS	NS	+	NS	NS	NS	+
F test for drying cycles	**	*	*	NS	NS	NS	+

\+ Significant at $P \geq 90$ % probality level
* Significant at $P \geq 95$ % probality level
** Highly significant at $P \geq 99$ % probality level

pigment loss was significantly lower during the HT cycle. Therefore, an increase in YI and decrease in BI of macaroni could be observed under HT compared to LT drying conditions (Table 1). These results emphasized that the color of pasta products can be improved by HT drying system, in agreement with the previous reports (MATSUO et al., 1970; MCDONALD, 1979; PAVAN, 1979a, b; DEXTER et al., 1981).

Although DEXTER and co-workers (1981) found that the spaghetti produced at HT drying cycles was significantly stronger than that dried at LT, the results of this study (Table 3) did not show significant effects of drying conditions on macaroni strength. However, LT drying cycle slightly increased raw macaroni strength. It appears that beside drying conditions, macaroni strength may be affected by other factors such as protein content and protein quality. Therefore, clarification of this point needs further research.

Although the averages of cooking time, weight increase, volume increase and cooking losses showed higher values under HT regime, surpassing those obtained under LT drying conditions (Table 3), the statistical analysis revealed that these differences are generally not significant, except cooking losses. The cooking quality parameters could not be changed considerably by the drying conditions. These results are partly in agreement with those reported by

Table 3

Effect of drying conditions on macaroni strength and cooking quality

Macaroni samples	Macaroni strength (N)	Cooking time (min)	Swelling		Cooking loss (%)[a]
			Weight (%)[a]	Volume (%)[a]	
Low temperature (LT)					
GK Minaret	409	6	133	183	7.52
SZ D 8	337	6	132	178	6.72
GK Basa	410	5	119	161	6.36
Mean	385	5.67	128	174	6.87
High temperature (HT)					
GK Minaret	396	9	154	211	7.72
SZ D 8	361	6.5	123	167	7.12
GK Basa	367	7	135	189	6.80
Mean	375	7.50	137	189	7.21
SD 5% between varieties	103.01	3.83	48.90	68.51	0.39
SD 5% between drying cycles	84.10	3.13	39.93	55.94	0.32
F test for varieties	NS	NS	NS	NS	*
F test for drying cycles	NS	NS	NS	NS	*

[a] on the uncooked macaroni weight basis
* Significant at P $\geq$ 95 % probability level

DEXTER and co-workers (1981), who found that neither spaghetti cooked weight nor cooked spaghetti diameter were affected by drying conditions, while cooking losses decreased in spaghetti dried by the HT cycle.

It can be concluded from the data presented in this study that applying HT (70–80 °C) during the initial stage of the drying programme offers advantages over the LT programme. Beside the reduction of drying time, HT yields a macaroni with significantly improved color without sacrifying cooking quality, strength or nutritional value.

*

The authors wish to thank Mr I. MAKÁRY and Ms M. ROSZA (Institute for Milling and Baking Industry, Budapest) for their kind help in macaroni processing.

Literature

AACC (1962): Crude protein — Micro-Kjeldahl method. American Association of Cereal Chemists, p. 46.

AACC (1968): Pigment content. American Association of Cereal Chemists, pp. 14–50.

ALAUSE, J. & FEILLET, P. (1970): Metode semplice obiettivo per la predizione del colore del pasta alimentare. *Tecnica Molitoria, 21,* 511–517.

BINNINGTON, D. S. & GEDDES, W. F. (1939): Rapid method for the determination of wheat and flour pigments. *Cereal Chem., 16,* 252–262.

DEXTER, J. E., MATSUO, R. R. & MORGAN, B. C. (1981): High temperature drying effect on spaghetti properties. *J. Fd Sci., 46,* 1741–1746.

FINNEY, K. F. & SHOGREN, M. D. (1972): A ten-gram mixograph—for determining and predicting functional properties of wheat flours. *Bakers Dig., 46,* (2), 32–35, 38–42, 77.

HONOLD, G. R. & STAHMANN, M. A. (1968): The oxidation-reduction enzymes of wheat. — Part IV. Qualitative and quantitative investigations of the oxidases. *Cereal Chem., 45,* 99–108.

HUNGARIAN STANDARD (1958): Tésztakészítmények vizsgálati módszerei. Fizikai tulajdonságok vizsgálata. (Methods for investigation of pasta products. Physical properties). MSZ 20500, 1–72.

IRVINE, G. N., BRADLEY, J. W. & MARTEN, G. C. (1961): A farinograph technique for macaroni doughs. *Cereal Chem., 38,* 153–164.

KATES, M. (1972): Isolation, analysis, and identification of lipids. — in: T. S. WORK (Ed.) *Laboratory techniques in biochemistry and molecular biology: Techniques of lipidology.* North-Holland Publishing Company, Amsterdam. pp. 428–444.

KOBREHEL, K., LAIGNELET, B. & FEILLET, P. (1974): Study of some factors of macaroni brownness. *Cereal Chem., 51,* 675–684.

KRUGER, J. E. (1976): Change in the polyphenol-oxidases of wheat kernel during growth and maturation. *Cereal Chem., 53,* 201–213.

LAIGNELET, B. (1977): Variazioni del colore e di altri fattori qualitativi delle paste durante l'essiccamento. *Tecnica Molitoria, 28,* 123.

LAIGNELET, B. (1983): Lipids in durum wheat and pasta products. — in: J. P. BARNES (Ed.) *Lipids in cereal technology.* Acad. Press, London, pp. 269–283.

LAMKIN, W. M., MILLER, B. S., NELSON, S. W., TRAYLOR, D. D. & LEE, M. S. (1981): Polyphenol-oxidase of hard red winter, soft red winter, hard red spring, white common club durum wheat cultivars. *Cereal Chem., 58,* 27–31.

MANSER, J. (1978): The influence of bacteria-count of pasta products during the drying process. *Buhler Diagram, 64,* 9.

MANSER, J. (1979): High-temperature drying of pasta products. *Buhler Diagram, 69,* 11.

MATSUO, R. R., BRADLEY, J. W. & IRVINE, G. N. (1970): Studies on pigment destruction during spaghetti processing. *Cereal Chem., 47,* 1–5.

MCDONALD, C. E. (1979): Lipoxygenase and lutein bleaching activity of durum wheat semolina. *Cereal Chem., 56,* 84–89.

PAVAN, G. (1979a): L'impiego dell'alta temperatura nel processo di essiccamento delle paste alimentari. *Tecnica Molitoria, 30,* 362.

PAVAN, G. (1979b): *High temperature drying process and pasta quality.* Report of Officine Meccaniche Pavan S. P. A., 35015 Galliera Veneta (Padova), Italy.

PAVAN, G. (1980): High temperature drying improves pasta quality. *Fd Engng int.,* (Feb.), 37.

SPECTOR, Th. (1978): Refinement of the Coomassie Blue method of protein quantitations. *Analyt. Biochem., 86,* 142–246.

SVÁB, J. (1981): *Biometriai módszerek a kutatásban.* Mezőgazdasági Kiadó. Budapest.

SZAKÁCS, L. & ROSZA-KISS, M. (1981): *Új típusú tésztaipari termékek gyártása és gyártmányfejlesztése.* (Production and development of pasta products of a new type.) MSKI report, Budapest. Manuscript.

SZÁNTHÓ, P. V., VIGYÁZÓ, V. L. & GYÁRFÁS, P. A. (1981): A búza lipoxigenáz aktivitásának meghatározása. (Determination of lipoxygenase activity in wheat.) *Élelmiszervizsg. Közl., 27,* 187–193.

TAHA, S. A. (1987): *Comparative biochemical-enzymological studies as related to the quality of durum wheats and their products.* Ph. D. Thesis, Hungarian Academy of Sciences.

TAHA, S. A. & SÁGI, F. (1987): Relationships between chemical composition of durum wheat semolina and macaroni quality. — Part II. Ash, carotenoid pigments and oxidative enzymes. *Cereal Res. Comm., 15,* 123–129.

WYLAND, A. R. & D'APPOLONIA, B. L. (1982): Influence of drying temperature and farina blending on spaghetti quality. *Cereal Chem., 59,* 199–201.

Acta Alimentaria, Vol. 17 (4), pp. 309–317 (1988)

PROPAGATION OF SOME FUSARIUM STRAINS AND STUDY OF THEIR ZEARALENONE PRODUCTION ON GROUND PAPRIKA

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(Received: 31 August 1987; accepted: 15 January 1988)

Moulds were isolated from ground paprika moulded under natural conditions. Three species dominated the samples: *Alternaria alternata*, *Fusarium equiseti* and *Penicillium cyclopium*. Potentially all the species isolated are toxigenic or facultative human pathogens.

Some of the strains isolated, and Fusaria obtained from a stock collection and known for their toxigenic character, their growth and zearalenone production were investigated. Investigations were carried out partly under laboratory conditions on ground paprika from Kalocsa, purchased on the retail market, and partly on stored samples of freshly picked sweet and hot paprika fruit.

In both cases propagation of moulds was very intense but zearalenone production by the different species differed substantially. Production of zearalenone was highly significant (10 000 $\mu g\ kg^{-1}$) when *Fusarium semitectum*, isolated from sweet and hot ground paprika was cultured under laboratory conditions. However, the same strain culture at an average temperature of 14 °C produced only 1000 $\mu g\ kg^{-1}$ zearalenone on both sweet and hot ground paprika.

When, on the other hand, ground paprika samples randomly bought on the retail market were tested for zearalenone none of the 20 samples contained the toxin.

The presence of zearalenone could not be detected in 4 paprika samples spontaneously moulded, in spite of the observation that ground paprika is a very good medium for zearalenone production in certain Fusarium strains. This can be explained partly by the concomitant microflora and partly by the storage temperature applied. To be able to draw final conclusions the number and variety of samples has to be increased.

Keywords: mycotoxin, seasonings, mould, zearalenone, ground paprika, Fusarium

Zearalenone is a mycotoxin which has oestrogenic effects. It is a secondary metabolite of certain Fusarium strains. It is thought to be carcinogenic because of its lactonic structure and because based on experiments carried out on animals, a correlation is assumed to exist between zearalenone and spontaneously formed tumors (THALMANN et al., 1985).

Literature data on fungal infection and mycotoxin contamination of seasonings are rather scanty although they are grown in countries where the conditions are favourable for the reproduction of moulds and for the biosynthesis of mycotoxin and they are used all over the world.

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Lately the interest in mycotoxin contamination and fungal infection of seasonings has increased. Thus, up-to-date and sensitive methods were developed for the determination of mycotoxins in seasonings (AWE & SCHRANZ, 1981).

Some of the authors report on the fungicidal activity of certain seasonings. HITOKOTO and co-workers (1980) found that mustard seed, garlic and cinnamon inhibit the proliferation of aflatoxin-producing moulds while black pepper, thyme and green tea leaves inhibit only the biosynthesis of aflatoxin (HITOKOTO et al., 1980).

In the course of these experiments answers were sought to the following questions:

- Is sweet and hot paprika from Kalocsa, collected directly after harvest, a suitable substrate for the multiplication during the storage period prior to processing, of some potentially toxin producing Fusarium strains, isolated from ground paprika becoming mouldy under natural conditions?
- Is toxin produced under laboratory conditions by some Fusarium strains obtained from the stock collection, known for their toxigenic capacity, as well as other Fusarium strains isolated from mouldy ground paprika purchased on the market?
- From a random investigation, what is the zearalenone content in ground paprika purchased on the market as well as in paprika samples spontaneously moulded under natural conditions?

1. Materials and methods

1.1. Materials

1.1.1. *Reagents.* The reagents were of analytical grade. Standard zearalenone solution: 100 μg of crystallized zearalenone (Makor Chemicals Ltd., Jerusalem, Israel) dissolved in 1 cm^3 methanol.

1.1.2. *Ground paprika.* For model experiments and the propagation of some Fusarium strains under laboratory conditions sweet ground paprika (Kalocsa), free from toxin, was purchased on the retail market.

Zearalenone production by some fungi was followed during the period between harvesting and processing of the paprika. The sweet and hot paprika was obtained from the Paprika Research Station, Research Institute for Vegetable Cultivation, Kalocsa, harvested in 1985.

In the course of the experiments with paprika from Kalocsa the zearalenone contents on the four batches of sweet and hot paprika, were determined. Further 20 samples (20 dkg bags) were randomly purchased on the market and analysed for their zearalenone content.

1.2. Microbiological methods

1.2.1. Isolation of fungi from mouldy ground paprika samples. The mould-producing fungi were isolated from the mouldy ground paprika samples, obtained from the Paprika Research Station, Research Institute for Vegetable Cultivation, Kalocsa. To isolate the fungi the surface of the samples was disinfected with the ten-fold dilution of commercial hydrogen peroxide then the bits of plant tissue were placed on wet, sterile filter paper and finally on peptone-PCNB medium.

Composition of the nutrient medium: 15 g peptone, 20 g agar-agar, 1.0 g potassium dihydrogenphosphate, 0.5 g magnesium phosphate, 0.1 g PCNB (pentachloronitrobenzene), 0.5 g sodium taurocholate, 100 mg streptomycin sulphate, 50 mg chlorocid, distilled water to 1 000 cm^3.

A medium containing sodium taurocholate and PCNB was necessary because the majority of the samples were highly contaminated with *Rhizopus* and thus rarely or not at all suitable for the isolation of true mould-producing fungi. The fungal colonies, when their purity was proven, were transferred to potato-saccharose or Czapek-Dox nutrient medium and the growth was used to identify the fungi according to BOOTH (1971).

1.2.2. Microorganisms used. A part of the microorganisms used in the experiments were kindly put at our disposal by Prof. L. Leistner (Bundesanstalt für Fleischforschung, Kulmbach, FRG) and were the following zearalenone producing strains:

Fusarium roseum (sp. 908)
Fusarium roseum (sp. 909)
Fusarium culmorum (sp. 976)
Fusarium graminearum (sp. 949)
Fusarium graminearum (sp. 977)

Another part of the zearalenone producing fungal strains was obtained from the stock collection of the Research Institute for Plant Protection, Hungarian Academy of Sciences, Budapest:

Fusarium graminearum (22–39)
Fusarium culmorum (22–234)

The rest of the strains originated from those isolated (as described in 1.2.1.) from moulded ground paprika and identified:

Fusarium semitectum (P_2)
Fusarium semitectum (P_3)
Fusarium oxysporum (P_4)
Fusarium equiseti (P_5)
Fusarium semitectum (P_6)

1.2.3. Propagation of isolated Fusaria on autoclaved ground paprika in the laboratory, and on freshly picked sweet and hot paprika from Kalocsa during the period between harvest and processing. Hundred g of ground paprika bought on the market and free of zearalenone was suspended in 200 cm^3 tap water, then autoclaved at 0.5 bar and finally inoculated with the isolated Fusarium strains listed in para. 1.2.2. The nutrient medium was incubated at room temperature for 2 weeks, then at 5 °C for 1 week and again at room temperature for another 2 weeks. Subsequent to the incubation period the samples were dried at 35–40 °C and pulverised in the mixer. The powder was used for zearalenone determination.

The sweet and hot paprika from Kalocsa was inoculated with Fusarium strains P_2, P_3, P_4, P_5, P_6, isolated from mouldy paprika and listed in para. 1.2.2. The samples were incubated at an average temperature of 14 °C for 8 weeks.

1.3. Analytical methods

1.3.1. Model experiments to determine zearalenone in ground paprika. Ten g ground paprika purchased on the retail market were injected with 50 $\mu g\ kg^{-1}$, 100 $\mu g\ kg^{-1}$, 200 $\mu g\ kg^{-1}$ zearalenone, respectively. The samples were allowed to stand for 3 h, then attempts were made to recover the zearelenone added in order to establish the percentage recovery.

1.3.1.1. Extraction — Twenty g quartz sand and 20 g of anhydrous Na_2SO_4 and 5 cm^3 absolute methanol were homogenized by grinding in a mortar for about 15 min. The mixture was shaken for 20 min with 50 cm^3 absolute methanol, then filtered through a folded filter paper. This extraction with methanol was twice repeated. The combined methanol solutions were evaporated under vacuum in a Rotadest apparatus (Labor MIM Budapest, Hungary) to about 50 cm^3.

1.3.1.2. Pre-purification by liquid-liquid extraction — The methanol containing zearalenone solution was defatted by adding 45 cm^3 distilled water and shaking in a separatory funnel three times with 40 cm^3 petroleum ether. Purification was continued by liquid-liquid extraction according to MIROCHA (1974): the pH of the aqueous methanol solution was set at pH = 13 with NaOH and extracted with 3×50 cm^3 chloroform in a separating funnel. After separating the phases the pH of the aqueous methanol solution was set at 9.5. The zearalenone was gained by shaking in a separatory funnel with 3×30 cm^3 chloroform. The extract was evaporated and using 0.5 cm^3 chloroform applied to the chromatography column.

1.3.1.3. Pre-purification on a Silica gel chromatography column — Purification was continued on a glass column of 200 mm length and 10 mm inner diameter. The column was provided with a tap and filled with Silica gel.

Contaminating substances were eluted first with 150 cm^3 petroleum ether, then with 150 cm^3 benzene. The zearalenone and the components eluted with it were eluted from the column with 90 cm^3 toluene–acetone (95 : 5) mixture. The toluene–acetone solvent was evaporated under vacuum and the residue applied to thin-layer chromatography.

1.3.1.4. Thin-layer chromatography — To determine the toxin Kieselgel 60_F–254 (Merck, FRG) layer was used.

Two-dimension running was applied the first solvent being petroleum ether–ether (1 : 1), the second chloroform–ethanol (95 : 5).

Zearalenone was identified by the following method, well known in related literature:

— In ultraviolet light zearalenone exhibits at 254 nm greenish-blue fluorescence, at 366 nm faint blue fluorescence (MIROCHA & CHRISTENSEN, 1974).

— The thin-layer, when sprayed with 2% 4-methoxybenzenediazonium fluoro borate solution, shows, after about 5 min, a reddish spot (SARUDI, 1974).

2. Results and discussion

2.1. Isolates from ground paprika

The isolates from mouldy ground paprika and identified are listed in Table 1.

Three species of mould-producing fungi dominated the samples: *Alternaria alternata*, *Fusarium equiseti* and *Penicillium cyclopium*. These three species formed about the half of the total mould fungi. The reason for breeding so many cultures was to gain the most complete possible list of species potentially present in the samples studied. The species isolated were all potentially toxigenic, some perhaps human pathogens.

2.2. Thin-layer-chromatographic method for the determination of F-2 toxin in ground paprika

Methods described in the literature for the determination of zearalenone in cereals or fodder (MIROCHA & CHRISTENSEN, 1974) could not be applied to ground paprika because at the height corresponding to the retention quotient of F-2 toxin (zearalenone) appeared on the thin-layer always an inpurity which showed a colour similar to that of zearalenone when sprayed with SARUDI (1974) reagent, thus zearalenone could not be evaluated. Only by the purification method as described in para. 1.3.1. could the chromatogram be made sufficiently pure and an evaluable spot obtained.

Table 1

Cultures isolated from mouldy ground paprika

	Number of isolates
Alternaria alternata	28
Aspergillus elegans	1
Aspergillus flavus	1
Aspergillus versicolor	1
Botrytis cinerea	1
Chaetomium sp.	1
Cladosporium cladosporioides	5
Cladosporium sp.	1
Fusarium dimerum	1
Fusarium equiseti	39
Fusarium hiterosporum	1
Fusarium oxysporum	11
Fusarium semitectum	6
Fusarium semitectum var. maius	1
Fusarium solani	2
Fusarium sporotrichioides	1
Penicillium brevi-compactum	13
Penicillium corylophilum	1
Penicillium cyclopium	51
Penicillium lanosum	2
Penicillium martensii	1
Penicillium piscarium	5
Phomopsis sp.	4
Scopulariopsis brevicaulis	3
Scopulariopsis candida	1
Scopulariopsis sp.	1
Stemphylium botryosum	2
Trichoderma koningii	1
Trichoderma pseudokoningii	1
Trichoderma roseum	1

Table 2 shows the sensitivity of the method (limit of detectability: μg per spot) in a standard toxin solution or upon adding the standard solution to 20 μl extract.

In Table 3 the percentages of recovery of the zearalenone quantities injected into the samples in the course of the model experiments are summarized.

Detection of zearalenone by thin-layer chromatography is shown in Fig. 1.

2.3. Toxin production by certain mould strains under laboratory conditions on ground paprika and on paprika during storage between harvest and processing

The growth and zearalenone production by mould-producing fungi described in para. 1.2.2. were studied under laboratory conditions on ground paprika and on paprika stored between harvest and processing. Ground paprika

Table 2

Sensitivity of the F-2 toxin determination method in a standard solution and upon adding the standard solution to 20 µl extract

Identification of toxin F-2	Standard solution (µg)	Quantity of standard toxin added to 20 µl paprika extract (µg)
254 nm UV	0.05	0.06
Using Sarudi reagent	0.02	0.03

(limit of detectability: µg per spot)

Table 3

Percentage recovery of zearalenone in model experiments with ground paprika

Added F-2 toxin ($\mu g\ kg^{-1}$)	Recovery (%) $\pm s$	n
50	60 ± 5.1	4
100	73 ± 4.3	4
200	81 ± 6.1	4

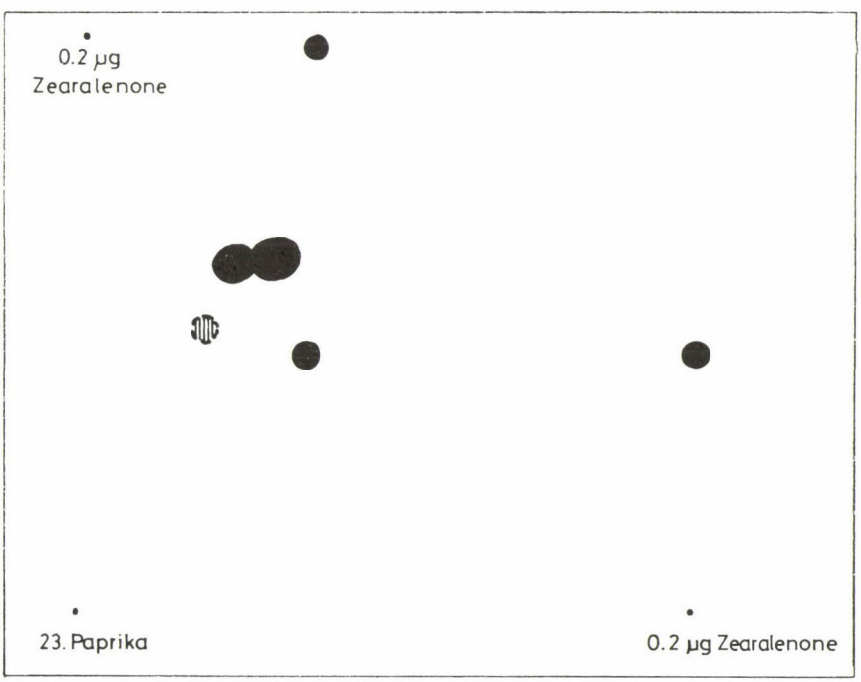

Fig. 1. Schematic diagram of the separation of zearalenone on a thin-layer chromatogram

proved to be an excellent substrate for the growth of all the strains examined and for zearalenone production by some of the strains. The growth of moulds was extremely intense both on ground paprika under laboratory conditions and on paprika stored between harvest and processing.

In contrast to the abundant mycelium production of all the fungal strains studied, however, there was a great difference in their zearalenone production. This observation is in accord with data in related literature and earlier experiences of the authors of toxin formation on different cereals (HALÁSZ et al., 1986) inasmuch as toxin production is a characteristic not only of the species but of the strain, too. Results are summarized in Table 4.

Table 4

F-2 toxin production of certain mould strains on ground paprika under laboratory conditions and on paprika freshly harvested and stored for 4–8 weeks

Mould strains		Zearalenone production ($\mu g kg^{-1}$)	
		Under laboratory conditions	Sweet and hot paprika in storage after harvest
Fusarium graminearum	(22–39)	0	0
Fusarium tricinctum	(22–234)	0	0
Fusarium roseum	(sp 908)	60	0
Fusarium culmorum	(sp 940)	0	0
Fusarium culmorum	(sp 976)	0	0
Fusarium graminearum	(sp 949)	0	0
Fusarium graminearum	(sp 977)	0	0
Fusarium semitectum	(P_2)	2 000	0
Fusarium semitectum	(P_3)	10 000	1000
Fusarium oxysporum	(P_4)	100	0
Fusarium equiseti	(P_5)	400	0
Fusarium semitectum	(P_6)	800	0

A highly significant (10 000 $\mu g\ kg^{-1}$) zearalenone production was obtained upon culturing *Fusarium semitectum* strain marked P_3, isolated from ground paprika under laboratory conditions. The same strain produced, at 14 °C average temperature both on sweet and hot paprika after 30 days storage, only 1 000 $\mu g\ kg^{-1}$ zearalenone.

The other fungal strains isolated from ground paprika produced zearalenone, too, under laboratory conditions, however, their production was lower than that of *Fusarium semitectum* P_3. The same strain when cultured at 14 °C temperature for 8 weeks did not produce toxin at all.

Of the strains obtained from the stock collection and known to produce toxin, only a single one, *Fusarium roseum* produced zearalenone when grown on ground paprika substrate under laboratory conditions.

Our results show that ground paprika is not so good as substrate for zearalenon production of fungi in comparison to rice or corn (BADEWEY et al., 1987). However, even in this case at longer storage time the development of moulds should be prevented.

2.4. Determination of the zearalenone content of ground paprika from Kalocsa, randomly purchased on the retail market and of spontaneously moulded ground paprika

The method as described in para. 2.2. was used to determine the zearalenone content of 20 ground paprika samples randomly purchased on the retail market and of 4 samples of spontaneously moulded ground paprika. Neither of the samples contained zearalenone.

Literature

AWE, M. J., & SCHRANZ, J. L. (1981): High pressure liquid chromatographic determination of aflatoxins in species. *J. Ass. off. agric. Chem.*, *64*, 1377–1382.

BADEWEY, A. HALÁSZ, A. SAWINSKY, J. & KOZMA, E. (1987): Influence of substrate on the F-2 and T-2 production of Fusarium species. – Part. I. Effect of corn and rice substrate. *Acta Alimentaria*, *16*, 29–43.

BOOTH, L. (1971): *The genus Fusarium*. Commonwealth Mycological Inst. Kew, Surrey, p. 237.

HALÁSZ, A., BADAWEY, A., SAWINSKY–ACSÁDI, J. & KOZMA–KOVÁCS E. (1986): F-2 toxin termelő penésztörzsek zearalenone képzése különböző szubsztrátumokon. (Zearalenone production on different substrates of F-2 toxin forming mould strains). *Élelm. Ipar*, *2*, 46–51.

HITOKOTO, H. S., MOROZUMI, T. & WAUKE, S. (1980): Inhibitory effects of spices on growth and toxin production of toxigenic fungi. *Appl. Environ. Microbiol.*, *39*, 818–822.

MIROCHA, C. J. & CHRISTENSEN G. M. (1974): Isolation, detection and quantitation of zearalenone in maize and barley. *J. Ass. off. agric. Chem.*, *57*, 1104–1110.

THALMANN, A., MATZENAUER, S. & GRUBER-SCHLEY, S. (1985): Untersuchungen über das Vorkommen von Fusarien Toxinen in Getreide. *Ber. Landw.*, *65*, 257–272.

SARUDI, J. (1974): Methode zum Nachweis des Zearalenon (F-2)-Toxins. *Z. Lebensmittelunters. u.–Forsch.*, *154*, 65–66.

Acta Alimentaria, Vol. 17 (4), pp. 319—332 (1988)

A HIGH PERFORMANCE SIZE EXCLUSION CHROMATOGRAPHIC METHOD FOR THE ANALYSIS OF THE MOLECULAR WEIGHT DISTRIBUTION OF STARCH

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(Received: 16 November 1987; accepted: 7 March 1988)

Three high performance size exclusion chromatographic (HPSEC) systems have been developed for investigating changes in the composition of wheat starch. The preparation of starch solutions was found to be an important factor in obtaining reproducible chromatograms of high molecular weight starches. In the best technique developed, dimethyl sulphoxide (DMSO) solutions of raw and processed starch extruded in a single screw extruder have been analyzed on styrene–divinylbenzene packed columns operated at 80 °C with DMSO as the eluant. Changes in the molecular size distributions of amylopectin were evident in the chromatograms of processed starch and the magnitude of the changes was related to the severity of the processing conditions. In the absence of sufficient high molecular weight reference materials, ratios of areas under the chromatographic peaks were used to express quantitative changes in the molecular size distributions.

Keywords: molecular weight distribution, HPSEC method, starch

In studies of the extrusion cooking of starches, attempts have been made to clarify the modifications on a molecular level which occurred during extrusion. By means of gel permeation chromatography and intrinsic viscosity, several researchers have demonstrated that starch polymers were degraded during extrusion processing (Mercier & Feillet, 1975; Colonna & Mercier, 1983; Colonna et al., 1984; Davidson et al., 1984a; Davidson et al., 1984b; Diosady et al., 1985). Although gel permeation chromatography is widely used in the determination of molecular weight distribution, it is a very tedious analysis. This method has limited accuracy, since there are no analytical standards available in the molecular weight range of the largest amylopectin molecules. The intrinsic viscosity is a relatively simple method correlating well with the average molecular weight of linear polymers. However, starch contains both linear molecules of amylose and branched molecules of amylopectin, and thus the average molecular weights obtained by this method contain

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large uncertainties. This method gives no indication of the molecular weight distribution within the sample. In order to develop an understanding of the mechanism of extrusion cooking of starches, a technique which quickly yields more detailed quantitative data on starch degradation is desirable.

High performance liquid chromatography (HPLC) has been recognized as a rapid, accurate technique for the analysis of simple and complex organic components. A number of researchers have been developing high performance size exclusion chromatography (HPSEC) for the analysis of starches and related polysaccharides (MEUSER et al., 1979; STONE & KRASOWSKI, 1981; KROGER & MARCHYLO, 1982; HIZURUKI & TAKAGI, 1984; SALEMIS & RINAUDO, 1984; KOBAYASHI et al., 1985; PRAZIK et al., 1986; CHUANG & SYDOR, 1987). However, its application to the analysis of starches has not been exploited fully, partly because HPLC column packings suitable for size exclusion of very large molecules have not been stable in the presence of the usual solvents for starches.

KOBAYASHI and co-workers (1985) published an HPSEC technique for the determination of amylose and amylopectin in starch samples. They used two Water's μ-Bondagel columns (E-linear and E-100) in series with dimethyl sulfoxide (DMSO) as the mobile phase. Although the manufacturer does not recommend the use of DMSO with these columns, no deterioration in column performance was reported. The same method was used by PATON (1985). The resolution of starch components was poor. MEUSER and co-workers (1979) used a combination of columns packed with porous glass beads, using DMSO as the mobile phase, to study the changes in molecular weight distributions in native and modified corn starch. However, the amylopectin fractions were eluted in the exclusion volume of the column combination, and the time required to obtain a chromatogram of the degraded product was about 270 min at an eluant flow rate of 0.15 $cm^3\ min^{-1}$. STONE and KRASOWSKI (1981) utilized HPSEC to compare molecular weight distributions of oxidized and hydroxyethylated corn starches using a three column set packed with Synchropak GPC with DMSO as mobile phase. HIZURUKI and TAKAGI (1984) used three sequentially linked columns of TSK-GEL with 50 mol l^{-1} sodium phosphate buffer (pH 6.1) as eluant to estimate the distribution of molecular weights for amylose. Recently, CHUANG and SYDOR (1987) reported a result of three types of columns (μ-Bondagel, Aquapore and μ-Styragel) with mobile phase of DMSO containing 0.03 mol l^{-1} sodium nitrate for the characterization of starch samples. However, there have been no reports of studies of changes in molecular weight distributions of wheat starch as a result of extrusion cooking when HPSEC has been used. This paper describes the development of a rapid, quantitative HPSEC method to characterize the structural changes in starch molecules.

1. Materials and methods

Fractionated potato starch amylose and amylopectin were obtained from BDH Chemicals (Toronto). Native wheat starch was donated by Industrial Grain Products Ltd. (Montreal). Three extruded wheat starch samples were prepared under different extrusion conditions (Table 1) in a 19 mm internal diameter, single screw extruder at the Food Research Centre of Agriculture Canada in Ottawa. High purity glass distilled DMSO was purchased from

Table 1

Extrusion conditions used in preparing wheat starch samples

Extruded samples number	Temperature (°C)	Moisture content	Screw speed (r.p.m.)
1	149	20	50
2	149	25	50
3	177	25	50

Caledon Laboratories Ltd. (Georgetown, Ontario). A phosphate buffer (pH = 7.2) was prepared by combining of 0.2 mol l^{-1} monobasic sodium phosphate (NaH_2PO_4) and 0.2 mol l^{-1} dibasic sodium phosphate (Na_2HPO_4), to which 0.02% Na_3N was added to prevent any microbial growth.

HPSEC analyses were carried out using a Hewlett-Packard Model 1090 instrument equipped with a Model 1037A refractive index (RI) detector. The column compartment and the detector were individually temperature controlled. The RI detector response was fed to an HP 3392 integrator and an HP 85B computer, which was equipped with HP 1090 GPC SYSTEM software for data handling. The whole system with the exception of the detector was controlled by the HP 85B computer. During analysis, the eluant was being continually degassed with helium.

Water soluble samples were prepared by using the method described by DAVIDSON and co-workers (1984a). The starch samples (100–150 mg) were first dissolved in DMSO and heated to 60–70 °C for approximately one-half hour. Ethanol (75%) was added to form an amorphous precipitate which was recovered by centrifugation. The dissolution and precipitation procedure was repeated at least three times. The final precipitate was washed with ethanol (75%) to remove any trace of DMSO; 30 cm^3 of distilled water were added and the dispersion was agitated for 5 min to form a solution. If any solid particles remained in suspension, the sample was discarded and the solubilization procedure was repeated.

The preparation of DMSO solutions of native and extruded wheat starch samples proved to be a very important part of the analysis. Starch

dissolved slowly in DMSO at room temperature. After 2 h at 70 °C, 0.02% w/v solutions appeared to be clear, however the analyses indicated that only a small fraction of the very high molecular weight components were actually dissolved. It was necessary to keep a sample solution at 80 °C for about 3 days before reproducible chromatograms were obtained.

Columns, eluant and operating conditions were varied for each case as described later in the results section.

2. Results and discussion

Several HPSEC column and eluant systems were tested that gave useful information on molecular weight distributions of starch polymers. Unfortunately, not all of these systems were suitable for reproducible quantitative analysis.

Two μ-Bondagel columns, E-high and E-500, 3.7 mm i. d. $\times$ 30 cm, purchased from Jordi Associates, Inc. Millis (Massachusetts), were connected in series. The columns were operated at 40 °C, using phosphate buffer (pH 7.2) as the eluant with a flow rate = 0.5 $cm^3\ min^{-1}$. The samples were dissolved in water as described above. The separation of the components of potato starch is shown in Fig. 1. The peaks of the amylopectin (*Ap* peak) and the amylose (*As* peak) were broad and overlapped. Despite the manufacturer's specifications, the columns proved to be unstable at pH values between 7.0 and 7.5.

Styrene–divinylbenzene packing is made by cross-linking styrene with divinylbenze to form small rigid gel particles with carefully controlled pore sizes. Unfortunately, the most widely used solvent for starches, DMSO, is thought to be incompatible with styrene–divinylbenzene according to the manufacturers. DMSO itself is a very viscous solvent that requires elevated temperatures to improve mass transfer and maintain low back pressure in the chromatograph. In the absence of a more suitable solvent, however, we decided to investigate the actual compatibility of DMSO with this packing.

Originally, a styrene–divinylbenzene column with a mixed porosity range of 10^3 to 10^6 Å, purchased from Jordi Associates, Inc. Millis, Massachusetts, was operated at 80 °C with an eluant (DMSO–H_2O, 75 : 25) flow rate of 3.0 $cm^3\ min^{-1}$. The RI detector temperature was kept at the recommended maximum temperature of 50 °C. Considerable baseline drift was observed even after one day's operation. To obtain a stable baseline, low detector sensitivity (attenuation 5) and high sample concentrations were used. Samples were dissolved in DMSO. Potato starch fractions injected as 200 μl of 0.5 (w/v) solutions were better separated (Fig. 2) than on the μ-Bondagel columns. Unfortunately, the column packing was also extremely short lived in this solvent.

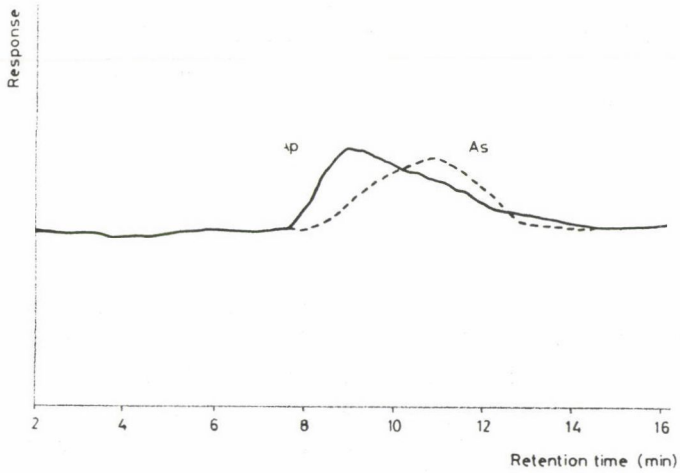

Fig. 1. HPSEC of potato starch fractions on μ-Bondagel columns. ————: Amylopectin fraction *(Ap)*; - - - - - - - : Amylose fraction *(As)*

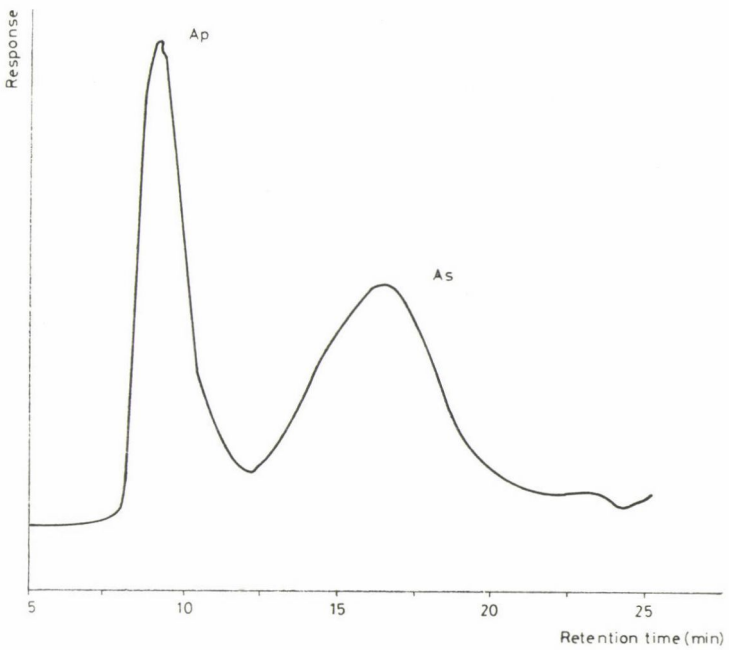

Fig. 2. HPSEC of potato starch fractions on styrene–divinylbenzene column (Jordi Assoc. Inc.). Ap = amylopectin; As = amylose

Other styrene–divinylbenzene columns, PL gel 10^6 Å, 10^5 Å and 10^4 Å were purchased from Polymer Laboratories, Amherst, Massachusetts. According to the manufacturer these were compatible with DMSO. The column specifications were as follows:

Length	30 cm
Diameter (i. d.)	0.75 cm
Frit size	5 μm
Particle size	10 μm

These columns were connected in series along with a PL gel guard pre-column. Pure DMSO was used as the sample solvent and eluant. The temperatures of the columns and the detector were 80 °C and 50 °C, respectively. Mobile phase flow rate, sample concentration and injection volume were optimized. A flow rate of 0.4 cm^3 min^{-1}, a sample concentration of 0.02% (w/v) and an injection volume of 100 μl gave the best separation.

The problem of base line stability was overcome by housing the RI detector in an insulated box and insulating the line connecting the columns to the detector to minimize heat losses. With this arrangement, a stable baseline could be obtained at the high detector sensitivity required. The scaling of the detector was 40 mV = 10^{-5} RIU. The detector was connected to the integrator, which had a full-scale deflection of 2mV. At this high sensitivity, a stable baseline could be obtained after one day of operation.

Figure 3 shows the chromatograms of potato starch fractions on a column set of 10^6 Å and 10^4 Å. As shown, the chromatographic profile of the amylose fraction contained three peaks: *Ap* (amylopectin), *As* (amylose) and *Ax* (an undefined component of intermediate molecular weight). The profile of the amylopectin fraction also contained a smaller *Ax* peak. As can be seen from Figs. 1–3, the PL gel columns had the best separation capacity for starch components. In addition, PL gel columns displayed excellent stability in DMSO even after 2 years of intermittent operation and storage, and therefore they seem to be well suited for HPSEC analysis of starches using DMSO as eluant.

For the analysis of starch degradation during extrusion, the columns selected should have a high separation efficiency in the high molecular weight range because amylopectin is degraded preferentially during starch processing (Davidson et al., 1984a). The combination of a 10^6 Å and a 10^5 Å column was therefore selected for this work.

As stated earlier in the Materials and methods section, the preparation of DMSO solutions of starch required about 3 days. The sample solution was too viscous to filter through a 0.5 μm-filter which is normally used in sample preparation. Clean sample solutions were injected without filtering, since the pre-column acted as a filter. In samples which had been heated for less than 3 days the chromatograms thus obtained did not correctly estimate the concen-

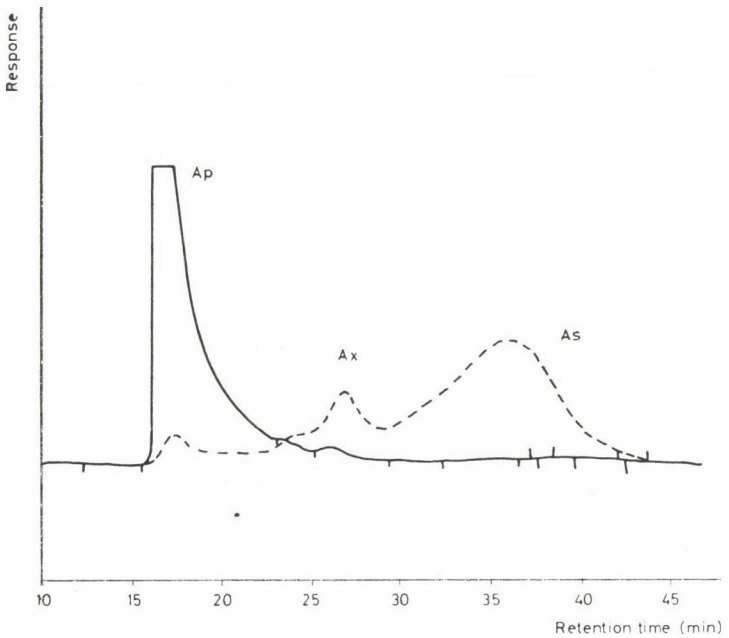

Fig. 3. HPSEC of potato starch fraction on PLgel 10^6 Å and 10^4 Å columns. ————: Amylopectin fraction; - - - - - - - -: Amylose fraction; *Ax:* undefined component of intermediate molecular weight

tration of high molecular weight components, of the original samples as these were removed by the pre-column. Figure 4 shows typical changes in the chromatographic profiles of an incompletely dissolved native wheat starch sample. This sample was heated at 70 °C for 2 h, and then it was injected after standing at room temperature for different times (T_r). After heating at 70 °C for 2 h, the sample continued to be dissolved at room temperature and the chromatographic profiles depended very strongly on the dissolution time. The amylopectin peak increased with T_r. In our experiments, it was found that after being heated at 80 °C for 72 h, samples seemed to be completely dissolved in DMSO, resulting in reproducible chromatograms. Higher temperatures degraded the starch polymers in DMSO, as shown in Fig. 5.

The combination of 10^6 Å and 10^5 Å columns yielded useful separations of the components of our processed samples. Figure 6 shows the chromatographic profiles of unprocessed and processed wheat starch samples. The *Ap* peaks of processed samples had different heights, shapes and retention times than the unprocessed samples. For all processed samples, the heights of the *Ap* peaks were lower than in the unprocessed samples, reflecting a decrease of the fraction of the largest molecular size component of the processed samples.

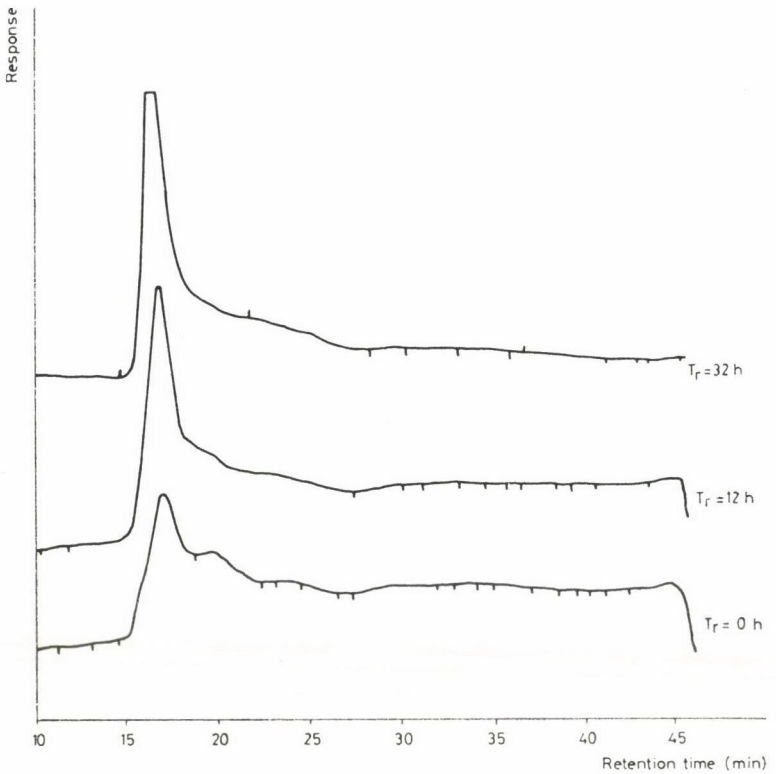

Fig. 4. Effect of dissolution time on HPSEC chromatograms. PL gel 10^6 Å and 10^4 Å columns. The unprocessed sample was heated at 70 °C for 2 hours in DMSO and then allowed to stand at room temperature for T_r hours.

This result was also in good agreement with that obtained from gel permeation chromatograms (Figs. 6, 7; ATHANASSOULIAS, 1986). Another feature not apparent in GPC chromatograms was the shift of the *Ap* peak of the processed sample when compared with raw starch. The *Ap* peaks of all processed samples were shifted toward the lower molecular weight range and were broadened, indicating a reduction in molecular size and changes in the molecular size distributions within the amylopectin component.

The HPSEC chromatograms contain much quantitative information, however the results would be more useful if standards were available for very high molecular weights. As it is, the changes of the molecular weight distribution could be described only in terms of peak area ratios. Peak areas were calculated for area slices in the chromatograms using the GPC software. Table 2 lists the area percentages of 3 minute area slices for triplicate injections. The result suggests that the analysis is reproducible. For the analysis of starch degradation, the peak area ratios should be measured in such a way as to

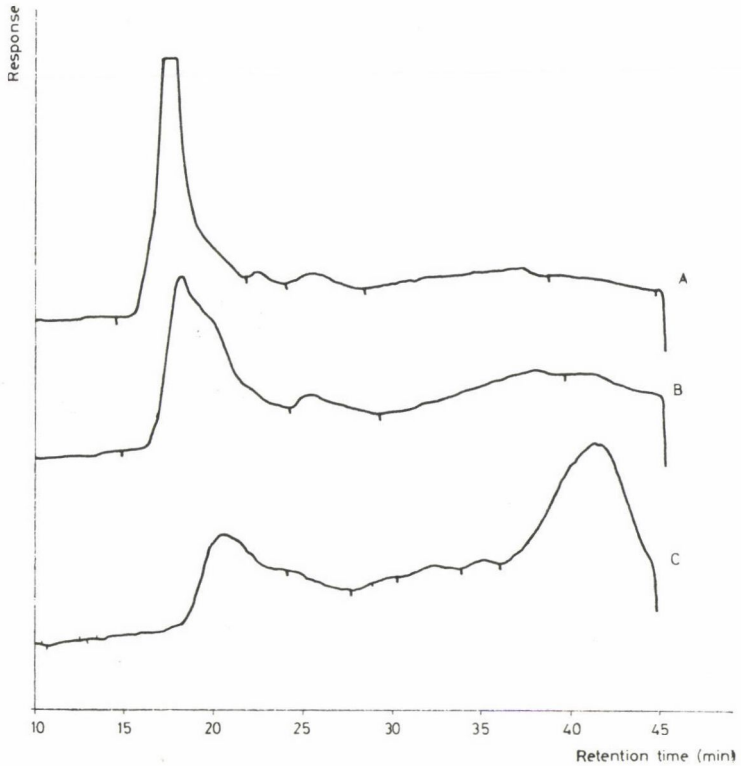

Fig. 5. Effect of high dissolution temperature on HPSEC chromatograms. PL gel 10^6 Å and 10^4 Å columns. The unprocessed sample in DMSO was heated: A: at 80 °C for 72 hours; B: at 150 °C for 2 hours; C: at 150 °C for 10 hours

reflect the two kinds of changes: the reduction of the fraction of the amylopectin in the processed sample, and the changes of molecular weight distribution within the amylopectin component. For example, as shown in Fig. 6, the total sample peak area, designated by S_t, was measured from retention time t=15 min (arrow A) to t = 45 min (arrow D). The area of the *Ap* peak, designated by S_{Ap}, was measured from t = 15 min to t = 24 min (arrow C), which lay between the *Ap* and the *Ax* peaks. Arrow B divided S_{Ap} into two parts: S_{ApI} and S_{ApII}, in order to describe the changes in molecular weight distribution within the amylopectin component. In this analysis, the position of B (t = 20 min) was chosen so that the S_{ApI} of the unprocessed sample could not contain the trailing part of the peak *Ap*. The relative changes in S_{ApI} and S_{ApII} correlate with the changes of size distributions within the amylopectin component. The peak area ratios S_{Ap}/S_t, S_{ApI}/S_t and S_{ApII}/S_t were measured and calculated directly by the GPC software. Table 3 lists the average data obtained from the triplicate injections of the unprocessed and the processed samples.

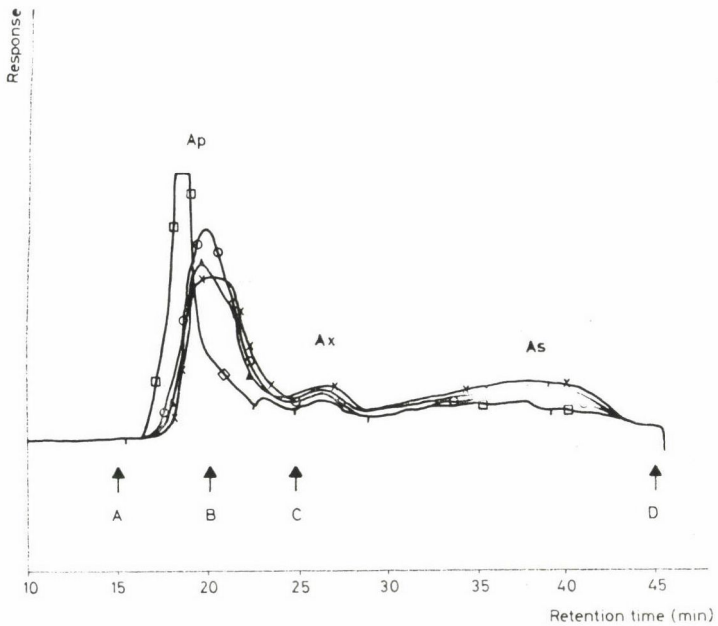

Fig. 6. HPSEC of wheat starch samples on PL gel 10^6 Å and 10^5 Å columns. □: unprocessed sample; ○: processed sample 1; △: processed sample 2; ×: processed sample 3. See Table 1 for processing conditions

Table 2

Values of peak area slices of triplicate analysis of sample 1

	Retention time (min)				
	15 – 18	18 – 21	21 – 24	24 – 27	27 – 30
Run 1	12.344	35.903	10.929	6.600	6.340
Run 2	12.792	38.066	10.616	6.334	5.502
Run 3	12.395	39.205	10.763	6.396	5.485
Average	12.510	37.724	10.769	6.443	5.776
Std. deviation	0.200	1.369	0.128	0.113	0.399
	Retention time (min)				
	30 – 33	33 – 36	36 – 39	39 – 42	42 – 45
Run 1	6.938	8.073	5.503	4.795	2.575
Run 2	6.282	7.584	5.328	4.966	2.530
Run 3	6.168	7.721	5.168	4.471	2.230
Average	6.463	7.793	5.333	4.744	2.445
Std. deviation	0.339	0.205	0.137	0.205	0.153

$$\text{Peak area (\%)} = \frac{\text{Area}}{\Sigma\ \text{Area}} \times 100\%$$

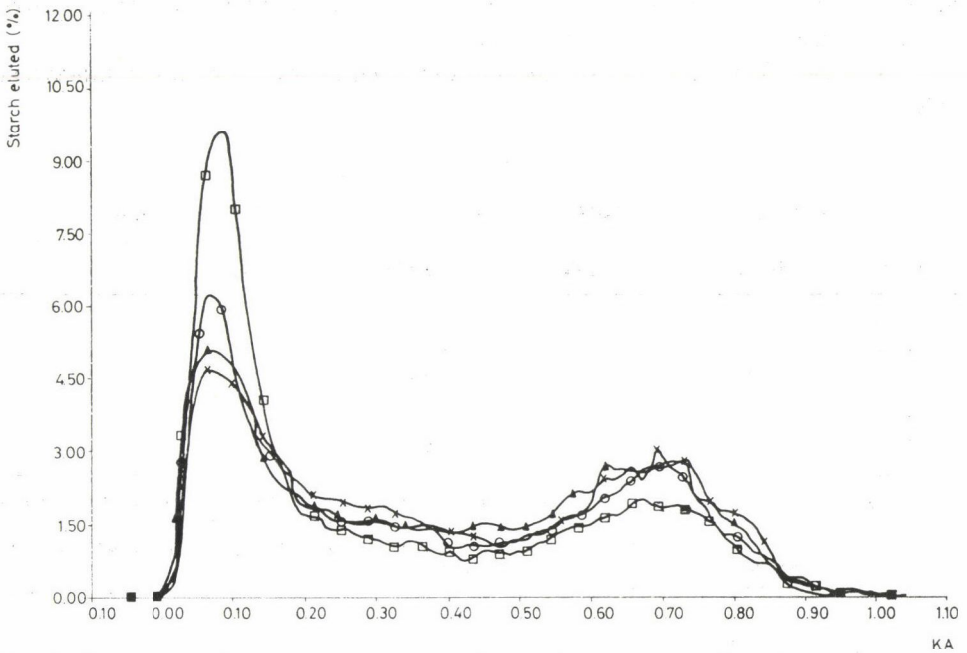

Fig. 7. Biogel — A 150 chromatograms of wheat starch samples (ATHANASSOULIAS, 1986). □: unprocessed sample; ○: processed sample 1; △: processed sample 2; ×: processed sample 3. See Table 1 for processed conditions

$$KA = \frac{V_e - V_o}{V_t - V_o},$$

where V_e = actual elutions volume; V_o = elution volume of an excluded substance (void volume); and V_t = total volume of gel bed

To characterize the extent of the degradation of the amylopectin, two parameters were introduced:

$$F_{Ap} = \frac{(S_{Ap}/S_t) \text{ of processed sample}}{(S_{Ap}/S_t) \text{ of unprocessed sample}}$$

$$D_{Ap} = \frac{(S_{ApII}/S_t) \text{ of processed sample}}{(S_{ApII}/S_t) \text{ of unprocessed sample}}$$

F_{Ap} denoted the fraction of large molecular weight material remaining in a processed sample, while D_{Ap} denoted the increase in the proportion of the smaller size molecules in the amylopectin component. Together, the two parameters described the change in the size distribution in the sample. From the results (Table 3), it can be seen that the decrease of the F_{Ap} and the increase of D_{Ap} depended on processing conditions, and D_{Ap} was more sensitive to processing conditions. By using the individual area slices more data may be

obtained that will be useful in developing a quantitative, molecular level model for the extrusion cooking of starch.

The key characteristics of the columns used in this work are summarized in Table 4.

Table 3

Values of peak area ratios of the unprocessed and the processed samples

Sample	S_{Ap}/S_t	S_{Ap}/S_t	S_{ApII}/S_t	F_{Ap}	D_{Ap}
Native starch	0.6966	0.5802	0.1164	—	—
1	0.6298	0.4406	0.1892	0.9041	1.625
2	0.5981	0.3904	0.2077	0.8596	1.784
3	0.5860	0.3484	0.2376	0.8412	2.041

S_{Ap}/S_t is the chromatographic area ratio of amylopectin peak (between arrow A and B in Fig. 6) to total sample (between arrows A and D in Fig. 6)

S_{ApI}/S_t is the chromatographic area ratio of the first part of amylopectin peak (between arrows A and B in Fig. 6) to total sample

S_{ApII}/S_t is chromatographic area ratio of the second part of amylopectin peak (between arrows B and C in Fig. 6) to total sample

$$F_{Ap} = \frac{(S_{Ap}/S_t) \text{ of processed sample}}{(S_{Ap}/S_t) \text{ of unprocessed sample}}$$

$$D_{Ap} = \frac{(S_{ApII}/S_t) \text{ of processed sample}}{(S_{ApII}/S_t) \text{ of unprocessed sample}}$$

Table 4

Column characteristics

Name of column	μ-Bondagel (Jordi Assoc.)	Styrene divinylbenzene (Jordi Assoc.)	PL gel (Polymer Lab.)
Packing material	silica-based	styrene divinylbenzene	styrene divinylbenzene
Type of solvent	phosphate buffer (pH 7.2)	DMSO/H_2O (72/25)	DMSO
Stability of packing in solvent	short term	short term	long term
Column arrangement	E-high + E-500	Single mixed (10^3 Å — 10^6 Å)	10^6 Å + 10^4 Å 10^6 Å + 10^5 Å
Analysis time (min)	17	25	45
Separation efficiency	*Ap* and *As* peaks overlapped	Good separation for *Ap* and *As* components	excellent separation for *Ap* and *As* components, with 10^6 + 10^5 Å providing more detail in the *Ap* component

Ap = amylopectin, *As* = amylose

3. Conclusions

A High Performance Size Exclusion chromatographic (HPSEC) technique has been developed for the determination of molecular size distribution of starches. The combination of PL gel 10^6 Å and 10^5 Å columns using DMSO as the mobile phase and as the solvent for the samples was found to be the most effective system for high resolution and reproducibility. The columns remained stable for at least one year.

Sample preparation was critical in obtaining reproducibility. Starch samples have to be maintained in DMSO at 80 °C for 3 days to ensure complete dissolution, without thermal breakdown of the higher molecular weight components.

The system was useful in the quantitative description of changes during extrusion-cooking of wheat starch. Although the lack of very high molecular weight standards prevented the calibration of the technique for accurate molecular weight determination, reproducible quantitative information was obtained, which may be correlated with molecular size.

The HPSEC technique was found to be much more rapid than the standard GPC analysis, and gave more detailed, reproducible results. It could be a useful technique in characterizing the extrusion cooking process.

*

The authors are grateful to Dr. D. PATON of the Food Research Centre, Agriculture Canada, and Dr. R. R. BAREFOOT for their advice and technical support. The research was supported by funds from the Natural Sciences and Engineering Research Council of Canada.

Literature

ATHANASSOULIAS, C. (1986): Degradation of wheat starch during extrusion processing. M.A.Sc. thesis, University of Toronto.

CHUANG, J-Y. & SYDOR, R. J. (1987): High performance size exclusion chromatography of starch with dimethyl sulfoxide as the mobile phase. *J. appl. Polym. Sci. 34*, 1739–1748.

COLONNA, P. & MERCIER, C. (1983): Macromolecular modification of manioc starch components by extrusion-cooking with and without liquids. *Carbohydr. Polym., 3*, 87.

COLONNA, P., DOUBLIER, J. L., MELCION, J. P., DE MONREDON, F. & MERCIER, C. (1984): Extrusion cooking and drum drying of wheat starch. — Part I. Physical and macromolecular modifications. *Cereal Chem., 61*, 538–543.

DAVIDSON, V. J., PATON, D., DIOSADY, L. L. & LAROCQUE, G. (1984a): Wheat starch in a single screw extruder: characteristics of extruded starch polymers. *J. Fd Sci., 49*, 453–458.

DAVIDSON, V. J., PATON, D., DIOSADY, L. L. & RUBIN, L. J. (1984b): A model for mechanical degradation of wheat starch in a single-screw extruder. *J. Fd Sci., 49*, 1154–1157.

DIOSADY, L. L., PATON, D., ROSEN, N., RUBIN, L. J. & ATHANASSOULIAS, C. (1985): Degradation of wheat starch in a single-screw extruder: mechano-kinetic breakdown of cooked starch. *J. Fd Sci., 50*, 1697–1699, 1706.

HIZURUKI, S. & TAKAGI, T. (1984): Estimation of the distribution of molecular weight for amylose by the low-angle Laser-light-scattering technique combined with high-performance gel chromatography. *Carbohydr. Res.*, *134*, 1–10.

KOBAYASHI, S., SCHWARTZ, S. J. & LINEBACK, D. R. (1985): Rapid analysis of starch, amylose and amylopectin by high-performance size-exclusion chromatography. *J. Chromat.*, *319*, 205–214.

KRUGER, J. E. & MARCHYLO, B. A. (1982): High-performance aqueous gel permeation chromatographic analysis of beta-limit dextrin hydrolysis by malted hard red spring wheat, malted durum wheat and fungal *(Aspergillus oxyzae)* alpha-amylases. *Cereal Chem.*, *59*, 488–492.

MERCIER, C. & FEILLET, P. (1975): Modification of carbohydrate components by extrusion-cooking of cereal products. *Cereal Chem.*, *52*, 283.

MEUSER, F., KLINGER, R. W. & NIEDIEK, E. A. (1979): Trennung von Stärkendekülen durch Hochdruckflüssigkeitschromatogtaphie. *Getreide Mehl Brot*, *33*, 295–299.

PATON, D. (1985): Personal communication.

PRAZNIK, W., BURDICEK, G. & BECK, R. H. F. (1986): Molecular weight analysis of starch polysaccharides using cross-linked allyl-dextran gels. *J. Chromat.*, *357*, 216–220.

SALEMIS, P. & RINAUDO, M. (1984): Gel permeation chromatography of starch and other unchanged polysaccharides. *Polym. Bull.*, (Berlin) *11*, 397–400.

STONE, R. G. & KRASOWSKI, J. A. (1981): Determination of molecular size distributions of modified corn starch by size exclusion chromatography. *Analyt. Chem.*, *53*, 736–737.

Acta Alimentaria, Vol. 17 (4), pp. 333—344 (1988)

PARTIAL PURIFICATION AND PROPERTIES OF LIPOXYGENASE FROM GERMINATED TOMATO SEEDS

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(Received: 10 December 1987; accepted: 8 February 1988)

Lipoxygenase was extracted from germinated tomato seeds with several media and was partially purified using two Sephadex gel filtration columns. Addition of Triton X-100 at 0.5% to the extracting buffer solution caused an increase of 892% in the total enzyme yield. Fractionation of seed extract proteins by Sephadex gel filtration resulted in 14.1fold purification. The purified enzyme showed an optimum pH of 6.5 and a K_m value of 0.6×10^{-5} mol l^{-1} when linoleic acid was used as the substrate. Chlorophyll and carotenoid bleaching activity of lipoxygenase was measured by a high-performance liquid-chromatographic method which revealed that different pigments differ in their sensitivity to lipoxygenase-catalyzed linoleic acid oxidation.

Keywords: enzymes, lipoxygenase, tomato

The seeds of tomato fruits are important by-products of tomato processing. Their oil and protein content makes them useful and serviceable for human and animal nutrition. Unsaturated fatty acids, such as linoleic and linolenic acids were found to be abundant in the oil of tomato seeds (SZÁNTÓ-NÉMETH, 1980). The same author reported considerable amounts of γ-tocopherol in the extracted oil of these seeds. Therefore they had been added to some fatty foods to reduce autooxidation of unsaturated fatty acids which is considered as the major contributor to off-flavor in many foods.

Lipoxygenase (EC 1.13.11.12) which catalyzes the oxidation of unsaturated fatty acids containing 1,4-pentadiene systems, has been demonstrated to play an important role in the physiology of plants and to contribute to the breakdown of many plant products during processing and storage (ESKIN et al., 1977; VICK & ZIMMERMAN, 1987; KERMASHA & METCHE, 1987). In spite of the great number of papers on lipoxygenase of plant seeds, the activity of this enzyme in some vegetable and fruit seeds has not been estimated because of some serious difficulties such as insolubility of enzyme protein, its membrane-bound nature, presence of effective antioxidants in the extracts and insufficient sensitivity of the method of assay.

Germination of plant seeds always activates some enzyme to a high level so that the determination of enzyme activity becomes possible even though disturbing factors are present in the enzyme preparations. The purpose of this work was to isolate and purify lipoxygenase from germinated tomato seeds and to study some of its enzymatic properties.

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1. Materials and methods

1.1. Materials

The seeds of *Lycopersicon esculentum* var. *Ventura* were obtained from the University of Horticulture and Food Industry (Budapest), Sephadex G-50 and G-150 were from Pharmacia Fine Chemical Co. (Uppsala, Sweden). Linoleic acid and Tween-20 were obtained from Sigma. Other chemicals used in this work were from Reanal, Budapest. Redistilled water was used in the preparation of the different solutions.

1.2. Methods

1.2.1. Seed germination. Two grams of tomato seeds were spread on a moist filter paper placed in a Petri-dish and allowed to germinate at 30 °C for 48 h. The germinated seeds were kept at −20 °C when not in use.

1.2.2. Enzyme extraction. Three grams of germinated seeds were disintegrated in a mortar with quartz sand and homogenized with 30 cm^3 of the extracting medium. The initial homogenate was strained through a double layer cheese cloth and centrifuged at 15 000 × G for 20 min at 0 °C in a Beckman (USA) type JA-20 refrigerated centrifuge. The supernatant was carefully removed with a syringe to avoid remixing of the lipid pad. This preparation was the crude extract of seed lipoxygenase.

1.2.3. Partial purification. Fractionation of the crude extracted proteins was tried by using two different methods:

— The enzyme protein was salted out by adding ammonium sulphate, at increasing concentrations, to the crude extract at 0 °C with continuous stirring of the precipitate obtained after centrifugation. After each step of fractionation the precipitated protein was redissolved in a minimum volume of 0.1 mol l^{-1} Tris-HCl buffer, pH = 7, containing 0.25% Triton X-100. The different fractions were examined for protein concentration and enzyme activity.

— Sephadex G-50 and G-150 were also applied to purify the enzyme. The crude extract was first fractionated on a Sephadex G-50 column (2 × 40 cm). The active fractions were pooled and further purified on a Sephadex G-150 column of similar dimensions. The fractions containing the highest activity were collected and stored at −20°C in small vials when not in use. The parameters of chromatographic separation are given in Table 1. Preparation and packing of the columns were carried out according to the instructions of Pharmacia Fine Chemical Co. (Uppsala, Sweden).

1.2.4. Enzyme assay. Linoleic acid substrate was prepared, by the procedure previously described (Daood & Biacs, 1988).

The spectrophotometric assay was carried out in the spectrophotometer cell: 0.05 cm^3 of linoleic acid substrate was mixed with 2.9 cm^3 of 0.1 mol l^{-1} phosphate buffer, pH 6.5, in the cuvette and the mixture was then vigorously shaken. The reaction was initiated by adding 0.05 cm^3 of the enzyme preparation and the increase in absorbance at 234 nm was measured on a Specord M-40 (Carl Zeiss, Jena) spectrophotometer. One unit is defined as the amount of enzyme which causes an increase of 0.01 in absorbance per minute at 234 nm.

Table 1

Chromatographic parameters of gel filtration on Sephadex G-50 and G-150 columns used in partial purification of tomato seed lipoxygenase

Parameters	Sephadex G-50	Sephadex G-150
Eluent	0.05 mol l^{-1} TRIS–HCl buffer pH 7	0.05 mol l^{-1} TRIS–HCl buffer pH 7
Flow rate	1.0 cm^3 min^{-1}	0.5 cm^3 min^{-1}
Hydrostatic pressure	35 cm	30 cm
Temperature	4 °C	4 °C
Injected volume	4–5 cm^3	4–5 cm^3
Volume of collected fraction	5 cm^3	3 cm^3

Blank was prepared in the same way, but with heat-inactivated enzyme preparation.

1.2.5. Protein determination. A spectrophotometric method was used for purification studies (WHITAKER, 1972), applying the following formula:

$$\text{mg protein per cm}^3 = 1.55\ A_{280\ nm} - 0.75\ A_{260\ nm}$$

Absorbance was read against a blank. This consisted of 0.1 mol l^{-1} TRIS–HCl buffer pH 6.5 containing 0.5% Triton X-100. Protein content of the individual fractions obtained by gel filtration was estimated at $A_{280\ nm}$ only, whereas that of crude extract and pooled fractions of gel filtration were calculated by the formula.

1.2.6. Determination of pigment bleaching activity. Pigments of green and red tomato fruits, respectively, were extracted and chromatographically separated and identified as described previously (DAOOD et al., 1987).

HPLC was used to determine the pigment bleaching activity of lipoxygenase according to BIACS and co-workers (1987). The method included solubilization of the extracted pigments in 50 μl linoleic acid and 50 μl Tween-20 with 3 cm^3 of chloroform which then was evaporated under vacuum. The residues were resuspended in 5 cm^3 of 0.1 mol l^{-1} phosphate buffer, pH 6.5. Bleaching was initiated by adding 1 cm^3 of the partially purified enzyme extract. Twenty μl of the mixture was withdrawn at 15 min time intervals and

injected on a Chromsil-C_{18} column eluted with acetone–water (9 : 1) as the mobile phase. The decrease in the peak areas of the individual components was considered as an index of bleaching activity.

2. Results and discussion

2.1. Extraction and partial purification

The effects of various extracting solutions on the extractability of lipoxygenase from tomato seeds are shown in Table 2. The result showed that water and buffer solutions were not sufficient to solubilize and extract the

Table 2

Effect of different extracting media on the extractability of tomato seed lipoxygenase

Extracting media	Lipoxygenase activity (unit cm^{-3})	Yield (%)
Redistilled water	56±3.5	100
0.1 mol l^{-1} TRIS–HCl buffer	80±6.8	142
TRIS–HCl buffer + 0.5% Triton X-100	500±30	892
TRIS–HCl buffer + Triton + 1% EDTA	305±15.2	544
TRIS–HCl buffer + Triton + 1% ascorbic acid	325±16	580

The values represent the mean of 3–4 replicates ± standard deviation

enzyme. TRIS–HCl buffer containing 0.5% Triton X-100 was the best extracting medium. These results revealed the membrane-bound nature of this enzyme and its association with some organelles or macromolecules of the seed. This nature was demonstrated for lipoxygenases from different plant tissues (Kim & Grosch, 1978; Feys et al., 1982; Kar & Feierabend, 1984). When ascorbic acid was added to the extracting media, alone or with EDTA as reported by Bonnet and Crouzet (1977) and Cayrel and co-workers (1983) to protect the enzyme protein against oxidizing factors, partial inhibition was observed.

Ammonium sulphate fractionation was applied as a primary step to concentrate the enzyme in the purification procedure. Uneven and unreproducible precipitation of the enzyme protein was obtained with a high loss in overall activity. This problem may be the consequence of the precipitation of the detergent and/or of its interference with the concentration technique used (Feys et al., 1982). The decrease in enzyme activity was accompanied by

marked browning appearing during the fractionation process. This indicates that the seeds contain a very active phenolase enzyme which oxidizes the phenols present in the extracts of the seeds producing more effective inhibitors.

Gel filtration was tried as a rapid technique to purify the enzyme and to remove polyphenols from the active protein. Figure 1 shows the elution pattern of tomato seed proteins fractionated on a Sephadex G-50 column.

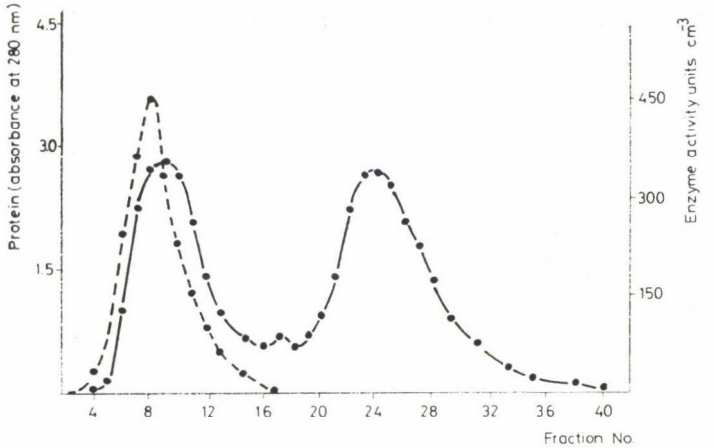

Fig. 1. Elution pattern of crude extract on Sephadex G-50 column. o——o protein concentration; o- - - -o enzyme activity. Sample: 92 mg protein, for conditions see Table 1

It can be seen that the active protein was eluted with the proteins of high molecular weight. The active fractions from Sephadex G-50 were pooled and further purified on Sephadex G-150; the elution pattern of this fraction can be seen in Fig. 2. The enzyme protein eluted with the first fractions indicates that the enzyme has a high molecular weight or associates with some macromolecules of protein nature.

Typical results of purification by gel filtration are presented in Table 3. It was found that the total activity of the pooled fractions from Sephadex G-50 fractionation was higher than that of the crude extract. This increase is probably due to the removal of phenolic inhibitors which eluted later from the Sephadex columns because of their low molecular weight. A 14.1fold purification was achieved using this technique. A 12.5fold purification was reported for lipoxygenase from niebe flour purified by a conventional procedure including ion-exchange chromatography (N'Doye et al., 1985), while Cayrel and co-workers (1983) reported 2.9fold purification using two columns of Sephadex to purify grape lipoxygenase. The one-step purification of the crude enzyme on Sephadex G-150 resulted in a 7.5fold purification only but the yield was

relatively higher than that obtained in the two-step Sephadex gel filtration procedure. Because of their high stability, the active fractions obtained from two-step gel filtration were pooled and used in the subsequent studies.

It is important to mention that further purification by advanced methods such as DEAE-Cellulose, CM-Cellulose and DEAE-Sephadex G-50 with linear gradients of NaCl or $(NH_4)_2SO_4$ was not successful; troubles occurred during removal of the detergent by dialysis and aggregation of proteins on the column material. The latter could not be eluted even with a 7 mol l^{-1} ammonium sulphate solution.

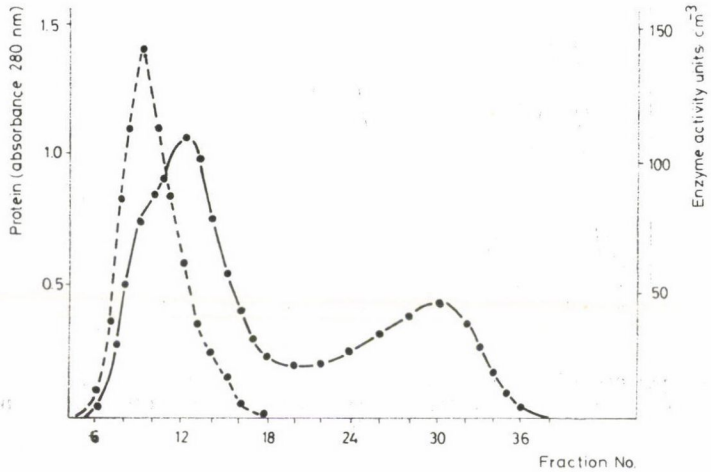

Fig. 2. Elution pattern of active G-50 eluates on Sephadex G-150 column. o——o protein concentration; o- - - -o enzyme activity. Sample: 31 mg protein. For conditions see Table 1

Table 3

Partial purification of lipoxygenase from tomato seeds by Sephadex gel filtration

Fractions	Protein (mg cm^{-3})	Activity (unit cm^{-3})	Specific activity (unit mg^{-1} protein)	Total activity (unit)	Yield (%)	Purification (×)
Two-step crude	18.4	1500	82	6000	100	1
Sephadex G-50	6.15	1200	195	7200	120	2.4
Sephadex G-150	1.72	370	212	3330	36	14.1
One-step crude	24	1549	64	7745	100	1
Sephadex G-150	0.9	435	483	4830	62.4	7.5

2.2. Enzyme concentration and reaction rate

At the substrate concentration used the increase in absorbance at 234 nm was proportional to time in the first minutes of the reaction. Then a slight decrease was observed (Fig. 3). The decrease in the absorbance at the maximum wavelength of linoleic acid hydroperoxide (234 nm) might be due also to substrate depletion product inhibition or hydroperoxide decomposing factors present beside lipoxygenase in the partially purified preparation. Such factors were found in many plant tissues and identified as hydroperoxide lyase or

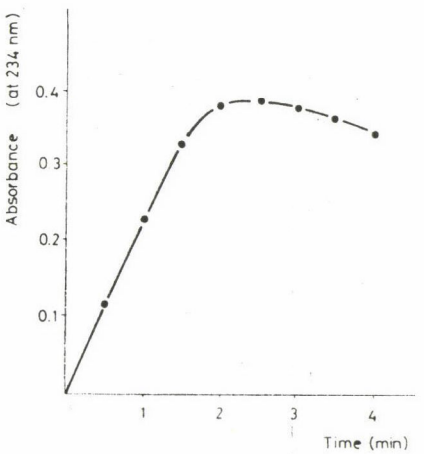

Fig. 3. Progress curve for the formation of hydroperoxy-linoleic acid by tomato seed lipoxygenase. Substrate concentration: 0.05×10^{-4} mol l^{-1}

isomerase (Vick & Zimmerman, 1976; Schwarz & Pyler, 1984; Kermasha & Metche, 1987).

A linear relationship between the initial rate of the reaction and the enzyme concentration was observed (Fig. 4). A short lag period was noticed with the crude extract at the lowest concentration of the enzyme. This disappeared when the enzyme was purified by gel filtration indicating that some inhibitors were present in the crude extract.

2.3. Effect of pH on enzyme activity

Figure 5 shows the pH-response curve of tomato seed lipoxygenase to be similar to those of the enzymes from broad bean (Al-Obaidy & Siddiqi, 1981), apple (Kim & Grosch, 1979), sunflower seeds (Leoni et al., 1985) and niebe flour (N'Doye et al., 1985). Tomato seed lipoxygenase exhibited a pH optimum between 6–6.5 with marked sensitivity to the alkaline values. At pH 8.5 no activity was observed ruling out the possibility of hem-catalized oxidation.

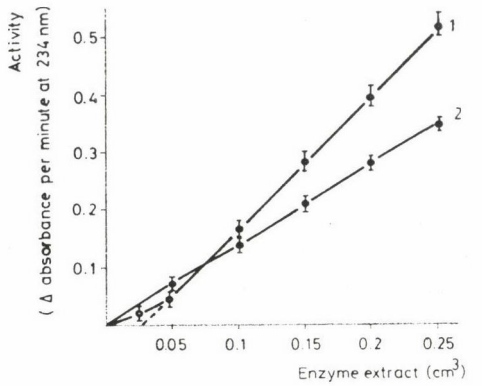

Fig. 4. Effect of enzyme concentration on lipoxygenase-catalyzed linoelic acid oxidation. Substrate concentration: 0.1×10^{-4} mol l^{-1}; 1: crude, $y = 2.3x - 0.058x$, $r = 0.99$; 2: purified, $y = 1.4x + 0.001$, $r = 0.99$

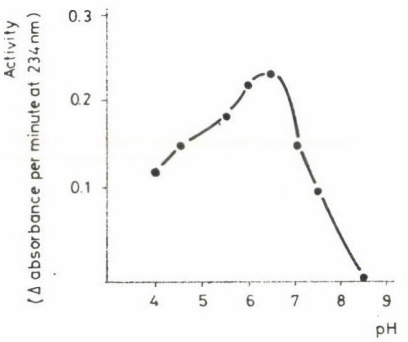

Fig. 5. Effect of pH on the activity of lipoxygenase from tomato seeds. Substrate concentration: 0.05×10^{-4} mol l^{-1} enzyme concentration: 0.1 cm^3 (0.17 mg protein)

2.4. Effect of substrate concentration

The activity of the partially purified lipoxygenase was studied at pH 6.5 with varying concentrations of linoleic acid. The results indicated that tomato seed lipoxygenase followed the Michaelis-Menten equation when the reaction rate was plotted against linoleic acid concentration (Fig. 6). The apparent K_m value calculated from the Lineweaver-Burk plot (Fig. 7) was 0.6×10^{-5} mmol l^{-1}. This value is higher than that previously reported for tomato pulp lipoxygenase (DAOOD & BIACS, 1988) but much lower than the values 0.20 mmol l^{-1}, 0.35 mmol l^{-1} and 2.8 mmol l^{-1} reported for lipoxygenase from pea seed, rice bran, some legume seeds, and broad bean, respectively (REYNOLDS & KLEIN, 1982; SHASTRY & RAO, 1975; KLEIN, 1976; AL-OBAIDY & SIDDIQI, 1981). However, this value is very close to the 0.67×10^{-5} mol l^{-1} reported for sunflower seed lipoxygenase under similar conditions (LEONI, 1985).

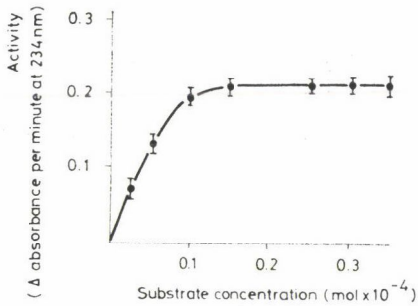

Fig. 6. Effect of linoelic acid concentration on the activity of tomato seed lipoxygenase. Enzyme concentration: 0.1 cm³ (0.17 mg protein)

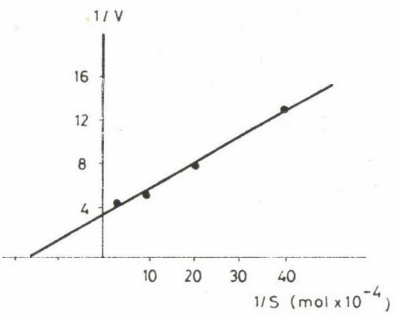

Fig. 7. Lineweaver-Burk plot for tomato seed lipoxygenase. $y = 0.24x + 3.29$, $r = 0.99$

2.5. Stability of the enzyme preparations

The crude extract was found to be unstable even at refrigeration temperature. The loss in enzyme activity could be noticed after few hours when the extract was stored at 4 °C. The loss in lipoxygenase activity was accompanied by marked browning indicating the presence of a very active polyphenol oxidase and its substrates in the crude extract. The inhibitory effects of some phenols on lipoxygenase reaction were examined by YASUMOTO and coworkers (1970). The author found that phenolic antioxidants inhibited soybean lipoxygenase either as competitive inhibitors or as irreversible inactivators. The stability of the enzyme has substantially increased after purification by the gel filtration technique. The enzyme could be stored at refrigeration temperature for 3 days without loss in its original activity. The stability of the enzyme was found to be similar to that of LOX-1, LOX-2 and LOX-3 purified from wheat germ by gel filtration (NICOLAS et al., 1982).

The partially purified enzyme lost 13.5% of its original activity when kept at 60 °C for 5 min, whereas no activity was observed in the preparation

heated at 80 °C for 2 min. The enzymes from broad bean and tomato fruit ACE 55 VF showed similar heat stability (AL-OBAIDY & SIDDIQI, 1981; BONNET & CROUZET, 1977). Furthermore, the enzyme purified from the pulp of the fruit of the same variety exhibited very similar thermal stability (DAOOD & BIACS, 1988).

2.6. Pigment bleaching activity

The HPLC method used for the determination of chlorophyll and carotenoid bleaching activity of lipoxygenase had been previously confirmed to be more suitable and specific than the spectrophotometric methods (BIACS et al., 1987). The sensitivy of different pigments to enzymatic degradation could simultaneously be estimated. Figure 8 shows HPLC profiles of green tomato extract incubated with heat-inactivated and active tomato seed lipoxygenase in the presence of linoleic acid as the substrate. It was found that in the presence of lipoxygenase and its substrate the polar pigments such as chlorophyll B, pheophytin B and lutein were more susceptible to the bleaching action than the less polar ones (Fig. 9). Moreover, the chlorophylls of B structure were more sensitive than those of A structure to enzymatic oxidation. These findings suggest that lipoxygenase takes part in chlorophyll degradation throughout its action in cooxidation of these components to unstable intermediates. These decompose rapidly to colorless compounds of small molecular weight at the

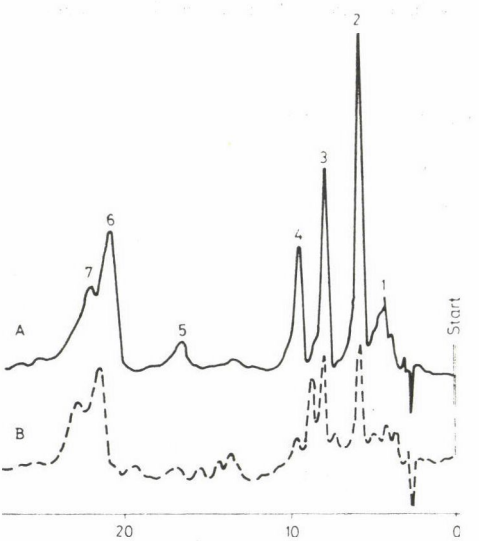

Fig. 8. HPLC profile of green tomato extract on Chromsil C-18 column eluted with acetone–water (90 : 10) as the mobile phase. ———— incubated with heat-inactivated enzyme, - - - - - incubated with active enzyme 1: Zeaxanthine, 2: Lutein, 3: Chlorophyll B, 4: Pheophytin B, 5: cis-Neurosporene, 6: Pyropheophytin, A. 7: β-Carotene

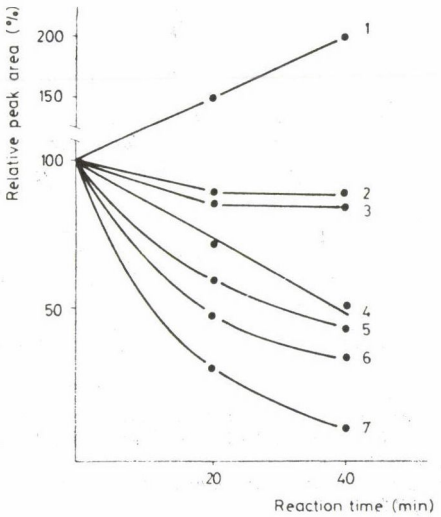

Fig. 9. Changes in the relative concentrations of the major chlorophylls and carotenoids of green tomato fruit in the presence of seed lipoxygenase and linoleic acid. 600 units of enzyme were used. For conditions see text. 1: Oxidation product, 2: β-Carotene, 3: Pyropheophytin, 4: cis-Neurosporene, 5: Chlorophyll B, 6: Lutein, 7: Pheophytin B

onset of ripening and senescence of some fruits and vegetable. The role of lipoxygenase in chlorophyll decolorization has been reported (HSIEH & MCDONALD, 1984) but the actual mechanism of this process was questionable yet.

It is worth mentioning that the bleaching capability of lipoxygenase depends not only on the type of the enzyme but also on the type of pigment used and/or the conditions under which the method of assay is being carried out. E. g. tomato seed lipoxygenase could not oxidize β-carotene in the presence of some chlorophylls of green tomato, but β-carotene was highly susceptible when the extracted pigments of ripe red tomato fruit were incubated with the same enzyme in the presence of linoleic acid as previously reported (BIACS et al., 1987).

Literature

AL-OBAIDY, H. M. & SIDDIQI, A. M. (1981): Properties of broad bean lipoxygenase. *J. Fd Sci., 46*, 597–600, 605.

BIACS, P. A., DAOOD, H. G., HAJDU, F. & CZINKOTAI, B. (1987): *Investigation of chlorophyll and carotenoid degradation by HPLC.* Annual American-East European Simposium on Liquid Chromatography, Budapest.

BONNET, J. L. & CROUZET, J. (1977): Lipoxygenase from tomato fruit: Partial purification and study of some properties. *J. Fd Sci., 42*, 625–628.

CAYREL, A., CROUZET, J., CHAN, W. S. & PRICE, K. R. (1983): Evidence for the occurrence of lipoxygenase activity in grapes (variety Carignane). *Am. J. Enol. Vitic., 34*, 77–82.

DAOOD, H., BIACS, P., HOSCHKE, Á., HARKAY-VINKLER, M. & HAJDU, F. (1987): Separation and identification of tomato fruit pigments by TLC and HPLC. *Acta Alimentaria, 16*, 339–350.

DAOOD, H. G. & BIACS, P. A. (1988): Some properties of tomato fruit lipoxygenase. *Acta Alimentaria, 17*, 53–65.

ESKIN, N. A. M., GROSSMAN, S. & PINSKY, A. (1977): Biochemistry of lipoxygenase in relation to food quality. *CRC Crit. Rev. Fd Sci. Nutr. 9*, 1–40.

FEYS, M., DE MOT, R., NAESENS, W. & TOBBACK, P. (1982): Solubilization, partial purification and properties of lipoxygenase from apple. *Z. Lebensmittelunters. u. -Forsch., 174*, 360–365.

HSIEH, C. C. & MCDONALD, C. E. (1984): Isolation of lipoxygenase isoenzymes from flour or durum wheat endosperm. *Cereal Chem., 61*, 392–398.

KAR, M. & FEIRABEND, J. (1984): Metabolism of activated oxygen in detached wheat and rye leaves and its relevance to the initiation of senescence. *Planta, 160*, 385–391.

KERMASHA, S. & METCHE, M. (1987): Change in lipoxygenase and hydroperoxide isomerase activities during the development and storage of french bean seed. *J. Sci. Fd Agric., 40*, 1–10.

KIM, I. S. & GROSCH, W. (1978): Solubilization, partial purification and properties of lipoxygenase from apple. *Z. Lebensmittelunters. u. -Forsch., 167*, 324–427.

KIM, I. S. & GROSCH, W. (1979): Partial purification of lipoxygenase from apple. *J. agric. Fd Chem., 27*, 243–246.

KLEIN, B. P. (1976): Isolation of lipoxygenase from split pea seeds, snap bean and peas. *J. agric. Fd Chem., 24*, 938–942.

LEONI, O., IORI, R. & PALMIERI, S. (1985): Purification and properties of lipoxygenase in germinating sunflower seeds. *J. Fd Sci., 50*, 88–92.

N'DOYE, A. S., DRAPRON, R. & NICOLAS, J. (1985): Purification of lipoxygenase from niebe (*Vigna unguiculata* L.) flour. *Sci. Aliments, 5*, 465–482.

NICOLAS, J., AUTRAN, M. & DRAPRON, R. (1982): Purification and some properties of wheat germ lipoxygenase. *J. Sci. Fd Agric., 33*, 365–372.

REYNOLDS, P. A. & KLEIN, B. P. (1982): Purification and characterization of a type-1 lipoxygenase from pea seeds. *J. agric. Fd Chem., 30*, 1157–1163.

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

SCHWARZ, P. B. & PYLER, R. W. (1984): Lipoxygenase and hydroperoxide isomerase activity of malting barley. *Am. Soc. Brewing Chem. J., 42*, 47–53.

SHASTRY, D. S. & RAO, M. R. R. (1975): Studies on lipoxygenase from rice bran. *Cereal Chem., 52*, 597–603.

VICK, B. A. & ZIMMERMAN, D. C. (1976): Lipoxygenase and hydroperoxide lyase in germinating watermelon seedlings. *Pl. Physiol., 57*, 780–788.

VICK, B. A. & ZIMMERMAN, D. C. (1987): The lipoxygenase pathway — in: STUMPF, MUDD & NES (Eds) *The metabolism, structure and function of plant lipids*. Plenum Press, New York, pp. 383–399.

WHITAKER, J. R. (1972): *Principles of enzymology for the food sciences*. Marcel Dekker, New York, p. 25.

YASUMOTO, K., YAMAMOTO, A. & MITSUDA, H. (1970): Effect of phenolic antioxidants on lipoxygenase reaction. *Agr. biol. Chem. J., 34*, 1162–1168.

Acta Alimentaria, Vol. 17 (4), pp. 345–381 (1988)

ABSTRACTS

of papers presented at the

INTERNATIONAL BIOCHEMICAL SYMPOSIUM ON FOOD PROTEINS

STRUCTURE, MODIFICATION AND FUNCTIONAL PROPERTIES OF PROTEINS FOR HUMAN FOOD

Organized by

HUNGARIAN BIOCHEMICAL SOCIETY

6–10 October 1987

Balatonszemes, Hungary

TECHNOFUNCTIONAL PROPERTIES OF FOOD PROTEINS

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When the functional properties of proteins are studied the biochemist is always thinking of the biological function of proteins and the function–structure relations. In the eyes of the food scientist protein is the most important nutrient but also a compound influencing on the one side the physical, organoleptic properties of food products and on the other the methods of processing technology. Thus in food production the term "functional properties" of proteins is strongly connected to the operations included in food processing and also to the physical (eventually other) properties of the end product. To avoid misunderstanding in this field I always proposed the use of the term "technofunctional" properties of "functionality" instead of functional properties. There are two approaches in the definition of the term functional properties. The more general definition includes any property of food or food ingredients except its nutritional value which affected its utilization. The second approach is connected with the growing use of plant, resp., animal protein preparations in the manufacture of food products. A great variety of protein preparations and the properties they possess necessitate to determine precise technological procedures that are to be applied in the particular products in order to utilize their potential functionality. This is an indispensable condition for obtaining the final products with appropriate desirable properties

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to be accepted by consumers. Such approach requires deep knowledge of properties of the particular protein preparations and their impact on the properties and quality of the product in which they are incorporated. All these properties of protein preparations are celled technofunctional properties. In other words technofunctional properties are those properties of the protein preparation which affect the behaviour of the product to which it is added during processing, storage and consumption.

In this paper only the technofunctional properties of protein preparations used as ingredients in the manufacture of foods will be treated.

Properties of the protein preparations which affect their functionality and thereby their utilization comprise first all their physico-chemical properties and in second line their sensory properties.

In the frame of this paper only the physico-chemical properties, being the dominant attribute that affects functionality of proteins in food, will be treated. Physico-chemical basis of protein functionality originates first of all, in the properties of the proteins themselves, and is also affected by other components (carbohydrates, lipids, etc.) as well as by conditions of processing (temperature, pH, ionic strength, etc.) and others.

The physical behavior of protein is determined by its amino acid composition and amino acid sequence coded in its genes, its molecular size, the conformation of the protein molecule, the charge of the protein and its distribution, the quality, quantity and distribution of the secondary intra and intermolecular bonds including quaternary structure. Conformation of protein appears to be critical in governing their functionality in food systems. As it is known, there are two basic types of proteins from the point of view of conformation:

— globular proteins,
— asymmetric fibrillar (lamellar) proteins.

The molecular weight of globular proteins is low, the structure is compact with relatively high amount of helical structure. More polar groups are oriented towards the surface. This favours solubility and hydration. The unfolded elongated conformation is desirable for gel formation, foam stability, formation of fiber like structures, etc.

After this general survey let us drop the specific technofunctional terms.

Protein preparations used in food production contain always numerous polar side chains. Dry protein, depending on the relative humidity of air, interacts with water molecules and the polar groups will be hydrated and form a monomolecular layer.

At higher relative humidity values multilayer adsorption may occur and depending on the colloidal structure of the protein preparation liquid water condensation can occur in the capillars. Immersing the protein preparation

in liquid phase water additional water absorption occurs due to swelling (osmotic water absorption). This type of water absorption could be much higher than the first type of hydration. Depending on the structure of protein molecules and their colloidal properties the swelling could be limited and a cohesive swollen mass will be formed, or unlimited and we obtain a protein solution. Which of the two types of hydration process occurs depends on the properties of the end product. It should be mentioned that polysaccharides that may constitute a part of the protein preparations also participate in the extent of water absorption. Thus the ability to absorb water by the given preparation depends not only on the origin and concentration of protein ingredient but also, to a large extent, on the content and nature of other non protein compounds, method of processing, including pH, heat treatment and others.

It is assumed that the *solubility* of proteins is the property which affects their functionality and application. This results from the fact that high solubility of a protein preparation is usually linked to its good emulsifying and foaming properties. Good solubility is needed also in dryed instant products serving for the preparation of beverages. In another group of products solubility is not so important in the evaluation of the usefulness of the protein preparation. In meat processing favourable technological effects can also be obtained by the use of protein preparations with low solubility. The solubility of proteins is also dependent on different factors such as pH, temperature, presence of other compounds, etc.

The effect of protein preparation on the *viscosity* is depending on a number of factors. One of the most important factors is the structure (conformation) of the proteins. The viscosity of the solutions of globular proteins increases slowly with the increase of concentration. In the case of fibrillar protein solutions the increase of viscosity is rapid especially if the degree of polymerization is high. The viscosity needed depends always on the product to be prepared.

The prerequisit of the good formation of emulsions is first of all a decreased surface tension and the formation of an intermediate layer between the hydrophylic and hydrophobic (oil) phase.

In both processes the proteins may play an important role if these are sufficiently soluble, surface active, if they have polar or charged and hydrophobic groups and finally if they are able to form a layer around the fat (oil) particles. From point of view of stabilization the mechanical strength of the interphase layer and the increased viscosity of the continuous phase are the most important. Special importance has the formation and stabilization in the production of different products from chopped and comminuted meats such as frankfurters, sausages, etc. Meat emulsions of this type are prepared as initial mixtures serving for the manufacture of such products. The meat emulsions

contain minimally four components: meat protein, added protein preparation, fat and water. For the stabilization of this system the uniform distribution of fat particles (globules) is needed and the formation of mechanically stable stabilizing layer around the fat droplets.

There are two typical ways of formation of gels:

— from solution by interaction of protein molecules resulting in a three-dimensional network,
— from dry protein mass (xerogel) by hydration and swelling.

Although it is possible to form gels from concentrated solutions of globular proteins, the unfolded linear protein molecules form stronger gels and at a lower protein concentration. It depends on the product to be manufactured which type of protein will be used. The ability of protein isolates (concentrates) to form gels and retain in such structure water and other components is extremely useful in producing sausages. Although the protein concentration of these meat emulsion preparations (e. g. soy isolate, sodium-caseinate) is lower than that required in gelation processes (these are globular proteins), the proteins of the preparation may further meat proteins in the formation of gel. Thus, water and fat molecules are immobilized and thereby stabilize meat emulsion. This effect is especially well observed when the used meat is of lower quality or when mechanically deboned meat is used. The ability of protein preparations to bind and retain fats is an important property especially in the manufacture of meat products (minced meats, hamburgers, luncheon meats, etc.). Although the mechanism of fat absorption has not been fully explained, absorption of fat is attributed mainly to the physical entrapment of oil.

The ability of proteins to form textured macrostructures (fibrils, films, sheets, expanded threedimensional networks, etc.) is of particular importance in the production of a lot of new protein foods, including simulated or "synthetic" food products. The special processes resulting in textured structure of an "amorphous" protein mass or a mixture containing proteins, is called *texturization*. The most known processes are the following ones:

— thermal coagulation and film formation,
— fiber formation (meat analogues),
— thermoplastic extrusion,
— freeze texturization.

Since the first three methods are widely known we will discuss here only freeze texturization. In recent years there has been increased practical interest in using freezing as a method of creating aligned fibrous structure in an amorphous protein slurry. The current aim of processes inducing fiber formation by freezing is to fabricate a product which simulates some of the textural characteristics of meat, especially the fibrous nature of its proteins myosin

and actin arranged in a parallel direction. The general freeze texturization process entails freezing of an aequeous protein solution in such a way as to control and align the ice crystal growth. Until an eutectic point is reached, only the water freezes, forming distinct and separate ice crystals. Cooling only one surface of the protein system will generally cause the crystals grow aligned and perpendicular to that surface. As the ice crystals grow from the surface as "spears" into the slurry, they force the proteinaceous material out of the space occupied by the advancing crystals and draw water molecules out of the slurry and bind them onto the ice crystal surface. This action concentrates the proteinaceous material in the interstitial spaces between the ice crystals and the branches of each crystal.

Upon melting the ice, the proteinaceous material remains compacted in the former interstitial spaces, and long, thin, parallel gaps are formed where the crystals had been. In extracted meat or poultry proteins the freezing and frozen storage will partially set the fibrous structure. In most cases the fiber set must be stabilized by heat. The characteristics of the fibrous protein structure may be influenced by rate of cooling, resp., by the ratio of the rate of nucleation and rate of crystal growth.

As it is known different food industries need different protein preparations with specific technofunctional properties. Thus for an optimal supply of food production with protein preparations there is a need of "tailor made" proteins. Due to the fact that natural sources of possible protein preparations (cereals, legumes, oil seeds, etc.) contain proteins which are not fully satisfactory from point of view of technofunctional properties, there exists a need for modification of these properties. As regards the methods which may be used the main possibilities are summarized as follows:

- improving of the proteins of raw materials (cereals, legumes, oil seeds, etc.) by breeding (classical method or applying genetic engineering),
- chemical modification of protein isolates (e. g. acetylation of *Vicia faba* proteins, etc.),
- enzymatic modification of proteins (e. g. plastein reaction),
- optimization of protein mixtures from the point of view of technofunctional properties.

Probably in the future the methods mentioned above will play a significant role also in industrial practice. Generally methods of evaluation of functional properties of protein preparations fall into the following three groups:

- measurement of general technofunctional properties (ingredient test),
- examination on model systems (model test),
- pilot processing of the product required (utility test).

The first type tests (measuring water absorption, fat absorption, viscosity, emulsifying activity, etc.) are the simplest. The main disadvantage of these methods is the lack of the knowledge of the exact correlations between characteristics of end product and the measured data. The most complex information could be obtained by utility test, but this is the most expensive and time consuming. The model test seems at this time to be the most suitable. Compared to utility test, the model test is inexpensive, uses simple equipment, has high resolution and tests for properties of multiple utility.

CRYOSTRUCTURIZATION OF MYOFIBRILLAR PROTEINS

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The use of proteins form new, non conventional sources as food requires their processing into certain food forms of which textured forms are of particular interest. New approaches in this respect are offered by the thermo-irreversible anisotropic gel formation phenomenon observed on freezing-thawing of polymer solutions or suspensions. Such gels are termed cryogels and their formation cryostructurization. In the framework of our research project cryostructurization of myofibrillar proteins from marine resources was investigated.

One of the most promising sources of myofibrillar proteins could be certain species of mesopelagic fishes and chiefly Myctophidae. The estimated quantity of Mictophidae which may be fished out only in explored regions is over one million tons per annum. Since efficient methods are presently available for isolation of miofibrillar proteins from fish and other marine animals, the solving of texturization of these proteins becomes rather urgent.

The mechanism of cryostructurization of fish protein isolates was studied using electronmicroscopy and the factors influencing the properties of the cryostructurized protein were controlled. Analysing a typical temperature – –time curve it can be stated that in the first cooling period no marked changes of the system could be observed. In the second period solvent crystallization begins causing intensive dehydration and resulting in protein–protein interaction and gel formation. For the last period of freezing an almost complete solidification is characteristic.

The character of protein – protein interaction is depending on the nature of the protein source. In native actomyosin suspensions the hydrogen bonds and hydrophobic interactions are the most important. In the case of denatured actomyosin the interaction of sulfohydryl groups has also a significant influence.

Additional possibilities of controlling cryogel properties are offered by their dependence on pH. The mechanically strongest gels are formed in the pH range close to the actomyosin isoelectric point.

Although the results mentioned above concern the cryostructurization of fish myofibrillar proteins, we suppose the generalities pointed out would apply to other protein systems as well.

DETERMINATION OF FOAMING PROPERTIES OF PROTEINS

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In connection with the estimation of foaming properties of proteins a new laboratory method has been developed. The equipment consists of a direct current motor-driven whisk/stirrer. An electronic-control assembly makes the rotation speed constant independent of the rheological properties of whipping agents and the energy which is necessary to maintain a constant rotation speed is measured.

With the new laboratory whipping principle two new characteristic features are obtained: the necessary mechanical work for foam production and the volume-specific mechanical work for foaming (that means the energy necessary to produce 1 cm^3 foam).

The volume-specific mechanical work for foaming is independent of the equipment used for producing and/or characterizing protein foams and is therefore a generally acceptable value for the energy expense in foaming processes.

In comparing examples for performing the whipping operation egg white, fresh and dried were differently aerated, and the foaming properties were determined. Whipping was performed on industrial scale as well as with devices widely used in laboratory. Foaming properties measured suggest that only the new, laboratory whipping principle reflects the actual performance of protein based whipping agents and that it is far better adapted to large-scale processing than the laboratory equipment most commonly used.

STEPWISE REGRESSION ANALYSIS OF RELATIONSHIPS BETWEEN WHEAT QUALITY AND CHEMICAL COMPONENTS

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The quality of flour and that of bread and pastry products prepared from flour is determined in the first place by the quantity and quality of the protein components of flour and interaction with other wheat components.

Stepwise regression analysis was applied to investigate the correlation between the quality characteristics (measured in the loaf and with Valorigraph) and quantity of proteins and lipids in wheat and flour. From the calculated equations the quality characteristics may be estimated.

The first variable step in the multivariate regression equation was the total protein and the determination coefficient higher than 50% ($100 \times R \times R$). The R value between the loaf volume and further 4 variables [ground free neutral lipid/ground free phospholipid, Atl. 66 — Pedigree, albumin and globulin, flour free total lipid/flour w. s. buthanol soluble total lipid] is $R = 0.88$.

The R value between the valorigraph value and further 5 variables (fraction of soluble protein in acetic acid by HPLC, fraction of insoluble protein in acetic acid by HPLC, Ran, Bez 2, Prod — Pedigree) is $R = 0.90$.

On the basis of these results the protein content plays an important part in these relations.

In the next stage of our investigation the loaf volume and Valorigraph value were divided by the protein content and these values were chosen as independent variable. The lipid data were divided in the case of flour by the flour protein content and in case of ground by the ground protein content, too.

The loaf volume/protein value can be calculated from lipid data by $R = 0.95$ value but the Valorigraph value only by $R = 0.54$.

To improve the equation draw into the equation the other variables, too.

The loaf volume/protein value can be estimated from flour free neutral lipid, flour buthanol soluble polar lipid, glutenin/gliadin, soluble protein in acetic acid by $R = 0.96$.

The valorigraph value/protein can be estimated from flour buthanol soluble glycolipid, ground free phospholipid, flour free total lipid/flour w. s. buthanol soluble total lipid, Mir, Ran, Bez 2, Pedigree, gliadin, protein soluble in acetic acid, fraction of protein soluble in acetic acid by HPLC, ground free total lipid by $R = 0.87$.

EMULSIFYING AND FOAMING FABA BEAN PROTEINS

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Faba bean is one of the most important leguminous seeds and can be used as raw material for plant protein products. The dehulled seeds contain about 30% protein. About 70 to 80% of the faba bean proteins are globulins, e. g. vicilin (7S) and legumin (11S). Ten to 15% of the faba bean proteins are albumins according to the Osborne classification.

Globulins have some good functional properties, depending on their preparation. Native globulins for instance are not best suited as emulsifying agents because of their compact globular shape. Therefore the globulins are modified to yield a more unfolded, flexible structure with enhanced hydrophobicity and higher negative charge. It is shown that acetylation of faba bean globulins is an effective and economic way to get products particularly suitable for stabilizing food oil-in-water emulsions. Examples of preparation and application of acetylated faba bean globulins are given.

It is also possible to produce good foaming agents from faba bean proteins. A special process is presented to get preparations with high foaming activity and good foaming stability. Such factors as defatting of raw material and protein composition play an important role. The foaming faba bean protein preparations are applicable for instance in icecream and sweets.

FUNCTIONAL PROPERTIES OF SQUID–FISH–MEAT HOMOGENATES

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Some fish minces deteriorate rapidly during frozen storage. Squid flesh, being resistant to freezing changes, could improve the functional properties of these minces.

The materials were characterized by water holding capacity (WHC) and cooked minces by free drip, separation of fat, product yield, and rheological properties. The WHC vrs pH curve of squid: water homogenate was similar to that of meat and fish, with pH_{min} 4.5–5.0. Heating the squid homogenate to 80 °C shifted the pH_{min} to 5.5–6.0 and decreased WHC. Adding 2% NaCl increased the WHC by 20% in the raw homogenate and by 50% after cooking. The WHC of frozen stored squid was higher than that of fresh cod and frozen blue whiting, while after heating it was much lower than that of cod. The WHC of mixtures of squid and whiting flesh was significantly lower than expected from the properties of the components. A squid–fat–salt–water

mince formed after cooking a firm gel just like cod mince, of higher yield limit than corresponding blue whiting mince. Adding squid to whiting minces and to sausage formulations containing lowest grade beef improved the rheological properties of the products.

FUNCTIONAL PROPERTIES OF PEA PROTEIN FRACTIONS

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The effects of chemical composition (protein, moisture, oil and carbohydrate content) and functional properties of six pea protein fractions (made by air fractionation with different protein content) were investigated.

The following functional properties were determined: emulsifying activity, emulsion stability and capacity, foaming capacity and stability and gelation.

The connection between protein content and emulsification, foam characteristics and gelation of the fractions were studied. The effect of the concentration of NaCl and pH on the foaming characteristics of the fractions were also determined. The gelation at different temperatures and pH was investigated as well.

It was concluded that the protein fractions of pea protein influence their functional properties.

CHEMICAL AND FUNCTIONAL PROPERTIES OF RYE PROTEINS

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In some rye producing countries the proteins of this cereal may play a role in the protein supply. Knowledge about these proteins and in particular on albumin and globulin fractions, however, are not sufficient. In our laboratory the proteins of Pluto, an important rye cultivar grown in GDR were investigated.

The availability of lysine, the number of thiol and disulfide groups, the electrophoretical behaviour as well as the solubility and foaming characteristics of some protein fractions were investigated.

Sixty to 90% of rye proteins may be extracted from flour with distilled water in the presence of sodium palmitate according to Kobrehel. The protein yield presumably depends among others on the grain size of the flour. Emulsification properties of the material are remarkable.

EFFECT OF LOW TEMPERATURE IRRADIATION ON THE PROTEINS OF CUT UP CHICKEN MUSCLES AND THEIR FUNCTIONAL PROPERTIES

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Chicken breast and leg samples were irradiated at different dose levels of 0.20, 0.70 Mrad, respectively. In all cases the temperature of irradiated samples was approximately –20 °C. The treated samples were stored at –15 °C for 180 days.

Chicken muscles were analyzed for the changes in moisture, total nitrogen, total amino nitrogen, fat, free fatty acid, thiobarbituric acid values, pH value and drip loss before and after irradiation and during frozen storage.

Results showed that moisture, total nitrogen, fat and pH value of all samples were decreased with increasing the dose of irradiation and the storage period. The TBA values, total amino nitrogen, free fatty acids and drip loss increased with increasing the dose level of irradiation and after frozen storage at –15 °C for 180 days.

The water holding capacity and consistency of sausages produced from different chopped samples were also investigated. A decrease of water holding capacity and consistency was observed.

CHARACTERISTICS OF THE NON-COAGULABLE NITROGEN COMPOUNDS OF THREE GENETICALLY DIFFERENT RAPE VARIETIES

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The meals from three genetically different rape varieties (traditional, low-erucic and double-zero variety) were extracted by HCl and NaOH solutions within the range of pH values from 1.2 to 10.8. Minimum points of nitrogen solubility occurred within the limits of pH values 3.6–4.2 for all varieties tested. The solubility of nitrogen containing substances at pH 10.5–10.8 amounted to 78–81% of total N.

Curves of precipitation of proteins as the function of pH values suggested the presence of the main protein fraction with the isoelectric point at pH 4.5. The yield of precipitation of proteins from improved varieties was at the above pH values higher as compared to the traditional variety.

The non-coagulable nitrogen compounds (NCN) as a residue after precipitation of alkaline extracts were characterized by gel electrophoresis on polyacrylamide and gel chromatography on Sephadex G-75. The molecular weight determined for NCN fractions from the rape varieties of higher level of glucosinolates amounted to 25 000 and 1 000 but for NCN from the double zero variety amounted to 56 000, 25 000 and 1000.

The analysis of the amino-acid composition of NCN fractions has proved considerable differences in this composition.

THE EFFECT OF MECHANICAL TREATMENT AND SPECIAL ADDITIVES ON THE STRUCTURE OF WHEAT PROTEINS

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The effect of different mixing times, as a mechanical treatment, on the development of dough made of wheat flour and the composition of wheat protein was studied. The changes in rheological properties and the amounts of wet gluten and salt soluble proteins caused by different quantities of oxidants, as well as reducers together with mechanical treatment were also investigated.

It was found by means of penetrometer studies that the increase of mixing time, in the presence of increasing amounts of oxidant (ascorbic acid) resulted in stronger dough structure and in the presence of reducer (cystein) softening was observed.

The increase of mixing time gave rise to a slight gradual growth in wet gluten, i. e. it promoted the formation of gluten network. The formation of intermolecular bonds was also induced by increasing amounts of ascorbic acid, but was weakened by adding cystein.

The salt-soluble proteins extracted from doughs mixed for different periods, i. e. which do not take part in the formation of the gluten network, became more available under the influence of ascorbic acid, while the added cystein resulted in decreasing amounts of salt-soluble proteins.

Examining the different factors exerting an influence of the structure of proteins in the construction of dough, gives characteristic data about the processing possibilities of flours.

POSSIBILITY OF THE PRODUCTION OF CASEIN HYDROLYSATE FROM CHEESE FOR DIETETIC PURPOSES

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Casein hydrolysates are mostly obtained as a result of enzymatic digestion of casein by means of such proteolytic enzymes as trypsin, papain, pepsin, etc. In the present work, an attempt was made to explore possibilities of using ripening cheeses in casein hydrolysate production. Three types of cheeses were used in the preparation of hydrolysates: Swiss-Ementhal, Dutch-Edam and Swiss-Dutch-Tilsit. Hydrolysates were obtained as cheese water extract, these were then freeze dried and subjected to organoleptic and chemical composition assessment.

On the basis of the results of preliminary tests, Tilsit cheese was eliminated as a raw material for obtaining hydrolysates due to its unfavourable organoleptic properties. Edam and Ementhal cheeses were characterized by broth flavour, saline with a touch of flavour typical of a given cheese. More favourable taste had freeze-dried extracts of Ementhal cheese. Hydrolysates obtained, as a result of additional digestion by means of trypsine, were characterized by slightly bitter flavour the intensity of which was incomparably lower than that of trypsine casein hydrolysate.

CHARACTERISTICS OF LIPOPROTEIN COMPLEXES IN INFANT FORMULA MILK AND MILK POWDER

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The formation of lipoprotein complexes in milk concentrates is the result of protein and polar lipid interaction.

Lipoprotein complexes are not toxic, nevertheless they considerably lower the nutritional value of protein so the investigation of these compounds in infant formula milk is of particular interest.

Lipoprotein complexes were extracted from formulas and milk powder using a mixture of chloroform and methanol (2 : 1) and petroleum ether.

In tests of fresh formulas of Laktowit I, Bebiko I and vitamin enriched milk powder, slight differences were noted in the number of lipoprotein complexes (1.04–1.24 g per 100 g) and slightly higher differences in the amounts of protein in them (18.8–21.1%). But during storage at 18 °C for 6 months the increase of lipoprotein complexes, diversification of their amount (2.1–3.1 g per

100 g) and increase of protein in them (22.6–27.8%) was observed. From the analysis of amino acid composition of lipoprotein complexes in fresh samples the following components appeared: proline 10.8–12.0 g per 16 g N, glutamic acid 9.6–10.9 g per 16 g N and leucine 7.9–9.6 g per 16 g N. These results show essential differences in the amino acids of these compounds compared to milk powder.

During storage changes in amino acids of lipoprotein complexes were found, especially in reference to the amount of glutamic acid, phenylalanine, alanine and valine. These changes result from progressive process of interaction between proteins and lipids and from the increase of protein in them.

FUNCTIONAL PROPERTIES OF CEREAL PROTEINS AND THEIR MIXTURES

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It is well known that the biological value of cereal proteins can be improved if they are complemented with one another. An other important property of these proteins is their functionality. We investigated the water absorption, the fat absorption, the emulsifying activity and emulsion stability of rice, wheat, corn and their mixtures with peas and lentils.

It was found that, in some cases, the addition of peas and lentils improved the emulsifying activity and emulsion stability. The addition of lentils effected a stronger increase of the emulsion stability than the addition of peas. Water absorption and fat absorption were not improved by the addition of peas or lentils in most of the investigated cases.

STRUCTURAL STUDIES ON NATIVE AND CHEMICALLY MODIFIED RAPESEED PROTEINS

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Structural changes in two main rapeseed storage protein fractions, the oligomeric high-molecular mass (300 kD) 12 S globulin and the low-molecular mass (14 kD) basic albumin ("napin"), after succinylation and acetylation have been studied using chemical analysis, CD and fluorescence spectroscopy, viscometry and ultracentrifugation.

The 12 S globulin undergoes significant changes at all levels of structural hierarchy (quaternary, tertiary, secondary structure) depending on the degree of succinylation. These alterations are analogous to those in other 12 S plant proteins.

The albumin fraction shows, however, a high structural stability and only small alterations after succinylation or acetylation. Its secondary structure, characterized by a high content of α-helix conformation (40%), does not change after the exhaustive modification. Only small changes in the CD and fluorescence spectra point to some alterations in the tertiary structure which is stabilized by inter- and intrachain disulfide bridges.

FUNCTIONAL PROPERTIES OF ACETYLATED FISH PROTEIN ISOLATES

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Chemical modification of food protein isolates, though permits to improve their functional properties, but, at the same time, often deteriorates the nutritional value. That is why it is important to find the optimal extent of modification of the protein in isolate for each item of its use in various food.

Present work demonstrates changes in solubility, emulsifying capacity and foaming capacity of the protein isolates from whiting *(Theragra chalcogramma)* with the extent of its changing upon acetylation. Experimental results illustrate, that the solubility of isolates in the neutral pH range (7–8) greatly improved as the extent of acylation reached and exceeded 67%. The trinitrobenzenesulfonic acid of Kakade was used to determine the extent of acetylation of the protein isolates. Maximum foaming capacity was observed for derivatives with amino groups modification of 67%. Acetylation of 43% and 67% of the ε-amino groups of lysine increased emulsifying activity. Maximum emulsifying capacity was measured in proteins, acetylated to 89% and 97% in the mixtures with high contents of oil.

Thus, optimal degrees of acetylation of whiting protein isolates were found to lie in different ranges for achieving maximum solubility, foaming capacity and emulsifying capacity of the product.

PROTEIN TRANSFORMATIONS UNDER DIFFERENT CHEMICAL CONDITIONS

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The modern automated spectrophotometric methods of protein determination need a rapid and total solubilization of the protein content of foods. The different physical, chemical or combined methods of solubilization may cause changes in the solubilized proteins influencing probably also the analytical results. Since our knowledge about the possible changes is relatively poor, there is a need of additional investigations.

In our laboratory different methods of solubilization applying organic acids (formic acid, citric acid) and other additional chemicals (alcohols, methylene chloride, sodium hydroxide, hydrochloric acid) and heat treatment were studied. Wheat flour, egg white and pork meat were the raw materials investigated. Free amino acid content, structural changes of proteins using HPLC-method, the effect of the splitting of disulfide bonds were measured. The formic acid digestion under heat treatment (100 °C, 60 minutes) was found to be the most suitable. Formic acid digestion caused only a moderate decomposition of the structure of different proteins. Amino acids are produced in low ratio. No harmful degradation of amino acids could be detected. Formic acid digestion may be used for different types of foods and solubilization of their proteins. The reaction mixture is well suitable for the spectrophotometric determination of protein content.

DESIGNED PROTEIN MODIFICATION BY ENZYMATIC PEPTIDE MODIFICATION

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The biological value of proteins is mainly determined by their essential amino acid content. Besides the knowledge of the demand for quantity and composition of amino acids another question also remains to be answered: whether these amino acids can be optimally utilized as free amino acids, in form of peptides or as proteins.

According to the current understanding of the science of nutrition, the biological utilization of mixtures of free amino acids is much less effective and energy-consuming than that of foods consisting of peptides and proteins of the same composition.

RELATIONSHIP BETWEEN PHYSICO-CHEMICAL PROPERTIES OF FISH PROTEIN ISOLATES AND CONDITIONS OF THEIR PREPARATION

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It is well known that alkaline extraction of myofibrillar proteins from fish muscle tissue followed by isoelectric precipitation is the general method for the isolation of proteins. In this connection the effect of pH of the medium during the extraction on the composition and physico-chemical properties of carp protein isolates has been studied. Using the method of exclusion chromatography and electrophoresis in PAAG it was found that actomyosin and corresponding aggregates have been extracted from muscle tissue at pH 11–11.5 ($T = 23$ °C, $t = 40$ min) while at pH 12–12.5 the actomyosin complex dissociates with depolymerization of G-actin and elimination of light chains of myosin. It was shown by the circular dichroism method that the alkaline dissociation of actomyosin resulted in the conformation transition of protein macromolecules. The full transition of α-helix $\rightarrow$ β-sheet occurred at pH 12.5.

The morphology and sedimentation stability of the suspension particles of protein isolates formed as the result of isoelectric precipitation were influenced by the velocity of adding acid to the alkaline extract of proteins. Fast isoelectric precipitation resulted in the formation of suspension particles characterized by asymmetrical shape which tended to coagulation contacts and the formation of a structural network. The suspensions of this type had high sedimentation stability. Slow isoelectric precipitation of the isolated proteins resulted in the formation of suspension particles of spherical form which aggregated to a lesser extent and were characterized by low sedimentation stability. The conditions of isoelectric precipitation essentially influenced the rheological properties of the concentrated suspensions of protein isolates and their syneresis. The samples prepared by fast isoelectric precipitation of myofibrillar proteins (1 min) had higher rigidity and syneresis than the samples prepared by slow precipitation provided the pH was the same during extraction.

Enzymatic peptide modification (EPM) proved to be suitable to obtain protein products of designed amino acid composition. In this study methionine-enriched protein was produced from casein hydrolysate by enzymatic peptide modification. The structural changes in the products were investigated. The results revealed that methionine was incorporated by covalent bonds and transpeptidation took place during the EPM treatment.

PROTEIN–PHYTIC ACID INTERACTIONS

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The ability of phytic acid to complex proteins has been a subject of investigation for several reasons but predominantly from chemical and nutritional viewpoints. Recently the effect of phytates on the functional properties of protein concentrates and isolates was also studied.

The phytate content of different legumes and cereals varies in a wide range (0.8–5.6%). Depending on the conditions of processing the phytate content of protein concentrates and isolates may be also very different (e. g. in soy protein products 0.1–1.6%).

Having dissociable hydrogens (protons), depending on the pH phytic acid has always more or less charged groups. So in all cases if protein is also charged (pH below or over isoelectric point) a possibility of ionic interactions exists. This is evident from several studies indicating involvement of lysil, histidyl, arginyl and amino terminal groups in the formation of protein–phytic acid complexes. Formation of ternary complexes of phytic acid, bi- or multivalent cations and proteins is also possible. Finally an esterification of primary alcoholic groups (e. g. serine side chain) may not be excluded.

Although the functional properties especially of soy protein preparations were intensively studied, few data have been collected concerning possible effects of phytate content on the technofunctional properties. The main purpose of investigations carried out in our laboratory was to extend our kowledge in this field.

Four types of protein preparations (soy concentrates and isolates, vital gluten, lupine seed protein concentrates) were investigated. Phytic acid (Sigma) was added to some samples to increase the concentration level of this compound in the protein preparations.

Nitrogen solubility, water absorption, oil absorption, emulsifying activity, resp., emulsion stability and least gelation concentration was determined using methods described by Dev and Mukherjee.

On the basis of experiments it can be stated generally that the effect of phytic acid content on the functional properties is relatively low. A slight decrease in the solubility of soy and lupine protein preparations was observed. It was also stated that low phytate protein preparations have better emulsifying properties.

The facts mentioned above also suggest that the lowering of phytate content in protein preparations may be needed in first line from the nutritional point of view.

EFFECTS OF HEATING ON BIOLOGICAL VALUE OF SOYBEAN

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Soybean, because of its high protein content and relatively well-balanced amino acid pattern is a potentially valuable protein source both for humans and animals. To increase the nutritional value of raw soybean some antinutritional factors, mainly trypsin inhibitors must be inactivated. Different heat treatments are known to eliminate the adverse effects of antinutritional factors and produce soybean products with good protein nutritional quality.

The aim of this study was to investigate the effect of dielectric heating on the biological value of protein in soybean.

Whole soybean (*Glycine max*, var.: Ewans) was heated at three different temperatures by a pilot plant dielectric equipment. Protein nutritional values of raw and treated samples were estimated by rat assay and characterized by Net Protein Ratio (NPR), Net Protein Utilization (NPU), True Digestibility (TD) and Biological Value (BV). Trypsin inhibitor activity of the samples was measured.

It was found that the in vivo nutritional values of treated samples were significantly higher (NPR = 23.3–25.3, NPU = 58.4–61.9, BV = 65.7–77.8) than the respective values of raw soybean (NPR = 14.9–17.1, NPU = 42.1–42.5, BV = 52.7–54.9). Residual trypsin inhibitor activities of treated samples were 5–10% of the original.

THE ROLE OF PROTEIN–LIPID INTERACTIONS OF GLUTEN IN THE FUNCTIONAL PROPERTIES

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Although the lipid content of wheat flour is usually close to 2% this minor constituent nowadays is in the focus of scientific interest. Lipids, forming protein–lipid complexes during the dough-making process mediate the surface nature of certain gluten proteins, effect their capability to form protein–protein interactions, which interactions are the key to determine baking properties of the flour.

The study of lipid content and composition of wheat and wheat flour as well as their complexes with proteins helps to understand the relationship between chemical composition, structure and technofunctional properties of wheat as well as the change of the latter one during storage or sprouting. Using lipid data, easily determined from even some wheat kernels, baking properties can be predicted which are extremely important in the early stages of breeding programs.

The review summarized the stage of knowledge in this area of cereal chemistry in the light of results of the last 2–3 years based on which the confusing earlier data can be re-evaluated.

STUDIES ON RAPESEED PROTEIN–PHYTIC ACID INTERACTION

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The phytic acid binding to the main proteins from rapeseed (*B. napus* L.) — the 12 S globulin and the lowmolecular basic albumin fraction — in insoluble complexes has been studied using turbidimetric titration and chemical analysis after coprecipitation. It has been found that the amount of phytic acid bound to the proteins increased with decreasing pH. A 1 : 1 stoichiometry of phytic acid binding to the globulin has been found at pH 3.0, whereas only 0.8 mol phytic acid phosphate per mol basic group were bound to the albumin at the precipitation point. The precipitation yields of the globulin and the albumin by interaction with phytic acid were 100% and 80% at pH 3.0.

The formation on soluble complexes has been studied in the albumin – phytic acid system at pH 5.0 by means of scattering gel chromatography and

quasi-elastic light. A dimerization and oligomerization of the protein has been observed. This interaction did not change the secondary structure of the protein. It causes, however, an inhibition of heat-induced aggregation.

INTERACTIONS BETWEEN PROTEINS AND REDUCING CARBOHYDRATES

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The desirable and undesirable physiological and also psychological effects of the Maillard-reaction were and continue to be a reason also for us to investigate intensively the interactions between proteins, peptides and/or amino acids with saccharides. The promotion or the more difficult inhibition of the Maillard-reaction require for their mechanism to be segregated as precisely as possible, the latter being coined by the active pairing between internal and external parameters. The internal parameters include the molecular structures of the reactants; external factors are e. g. the temperature, the pH-value, and the salt content of the system. In the initial phase, the N-compounds are glycosidized, the number and sequence of the sugar are retained in the colourless glycosides formed.

In the advanced phase of the Maillard-reaction, particularly the volatile aromatic substances and the low-molecular soluble compounds are formed, which react in the 3rd phase with the N-components to the slightly soluble, mainly brown pigments.

Our concern is especially attached to the starting phase of the Maillard-reaction, and like the group around Örsi, we study the reaction between amino acids and glucose.

In doing this we found the β-D-glucose to react faster with the natural amino acids, such as phenylalanine, than the α-form, which is at the same time a pointer to the ionic nature of the initial reactions. Next, the saccharide reacts on the amino acid, as a rule acid-catalyzed and/or synchronously, forming the glycoside. The unstable, not isolable glycoside transforms into the Amadori-compounds in accordance with the model of the Lobry-De-Eruyn-Van-Eackenstrin-rearrangement. As an intermediate stage, and in conformity with Obretenov, the not safely identified enaminole must be formulated.

We are of the opinion that dry amino-acid/glucose-mixtures or glycosyl-amines also yield Amadori-compounds in the derivatography, the object of the joint work with Örsi. The relatively stable fructose-compounds can continue to react in several ways. With another saccharide molecule, bisketosyl-amino acids are formed, with an amino acid, glycosyl-bis-amino acids are formed. From the N-carbohydrate components of the composition 1 : 1, 2 : 1 thus

formed and with the elimination of N-compounds, 1-, 3-, and 4-desoxyosones will emerge; the catalytic function of the amino acids and thus the first phase of the Maillard-reaction have come to an end. The highly reactive dicarbonyl compounds enter into the Strecker degradation, the source for many aromatic substances. The synchronous mechanism of this degradative reaction might be linked up with our experimental findings that D-amino-acids react faster with glucose than L-amino-acids. Finally we should like to state that carbohydrates with α-amino-acids yield preferably aromatic substances and with ε-amino-acid preferably melanoidines.

THE EFFECTS OF SURFACE-ACTIVE AGENTS ON THE PROTEINS OF MACARONI DOUGH

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In our experiments with macaroni dough made from flour of aestivum wheat cultivars MV-8 and Jubilejnaja 50, glycerine-monostearat and DP-40 have been used as surface-active materials at concentrations of 0.2, 0.4, 0.6 and 0.8%.

We have determined the characteristic features of dough protein content, dry matter content, cooking characteristics, the quantity of absorbed water, the increase of volume, the cooking loss, the quantity of dissolved protein. The quantity of sodium chloride solubility, alcohol-solubility and glutenin fraction has been measured by fractional process. The penetration and shear force values have been measured so as to characterize the rheological properties.

Dough structures have been examined by microscopic method and the organoleptic evaluation of the samples has also been carried out.

Calculating the F and t tests we have established the characteristic features which cause sigfinicant changes. The optimal concentration of the applied materials has been determined too.

The surface-active materials applied improve the quality of dough. They help the formation of structure from gluten proteins, change the solubility of the individual fractions and doing so they influence the quality of dough.

AMINO ACID ANALYSIS, PRESENT PRACTICE AND FUTURE TRENDS

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The need for determination of amino acid composition of different proteins, resp. foods is rapidly growing in many countries including also Hungary. Among the factors causing this increase the following ones may be mentioned:

- determination of the structure of different proteins in biochemical and food science research,
- evaluation of the foods from point of view of nutrition,
- optimation of protein mixtures,
- evaluation of new protein sources,
- investigation of the effect of processing on the proteins and amino acids.

The mainly applied automatic amino acid analysis based on ion exchange chromatography is relatively expensive and also time consuming. Over the world big efforts are made to develop more effective (productive) and cheaper methods. On the basis of current experience it seems that the following four methods are the most promising:

- capillary GLC of the derivatives of amino acids,
- HPLC analysis of the amino acid derivates,
- OPTLC methods,
- NIR methods.

Experiments made in our laboratory with OPTLC showed that until now we were not able to find such combination of solvents which is equally good for all the 18 amino acids to be determined.

A disadvantage of the methods is the non adequate quantitative determination and the difficulties in automation.

The HPLC method gives good results but under Hungarian conditions is too expensive.

The best results were achieved by capillary GLC. The efficacy is very good and the time of analysis may be reduced to 10 minutes. For derivation n-butanol and trifluoroacetic acid anhydride or 1,1-dichloro-tetrafluoroacetone was used. A glass capillary of 15 m length wetted with SE-54 stationary phase gave the best results.

The main disadvantage of the method is the relatively complicated preparation of the samples to be analysed.

PHOTOMETRIC DETERMINATION OF SOME ESSENTIAL AMINO ACIDS IN FOODSTUFFS AND FORAGES

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Lately there has been a quick development in the ion exchange column chromatography of amino acids, but the determination of all amino acids forming protein still requires 1–1.5 hours. The advantage of the photometric methods is, that there is no need for expensive apparatus and determinations can be carried out fast and in several repetitions. Another advantage of the method is that only the essential amino acids can be determined. Because of the above mentioned these methods were first used to determine two essential sulphur containing amino acids under laboratory conditions. The results were compared to those of amino acid analyser and in the cause of authenticity having oxidized two sulphur containing amino acids they were determined in the form of cysteic acid and methionine sulphone.

For photometrical determination of methionine we thought the best Toennis' and Kolb's platinum-iodine method. Since during the preliminary experiments results could be compared best with those of ion exchange column chromatography, later this method was used.

It is well known that metals of the platinum group (Pt, Pd) form coloured complex iodines, which in the presence of organic sulphids or mercapto compounds like cysteine and methionine loose their colour because of the sulphureous compounds. Cysteine has much less effect on platinum iodine complex, than methionine.

With the method of orange platinum iodine based on fading having determined methionine content of milk powder, meat flour, soybean, maize and wheat, no significant difference could be proved in the methionine content resulting from photometrical and ion exchange methods.

Following Ellman's method for photometrical determination of cystine and cysteine we used the reaction between 5,5'-ditiobis-(2-nitrobenzoic acid) and cysteine. According to Cleland cystine was reduced to cysteine with erythro-1,4-dimercapto-2,3-butanediol (DTE) and threo-1,4-dimercapto-2,3-butanediol, and the unnecessary reducing agent as suggested by Zahler and Cleland, was fixed by sodium meta arsenite. The orange 3-mercapto-6-nitrobenzoic acid resulting from the reaction of cysteine and Ellman reagent was determined by photometry at 412 nm.

In the course of comparative analysis — similarly to methionine — no significant difference between the ion exchange and photometrical determination, was found.

PROBLEMS IN THE DETERMINATION OF PROTEOLYTIC ENZYMES IN RENNET

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The determination of proteolytic activities depends on the kind of substrate, the separation method of reaction products and the methods for quantification of hydrolysis products. The bovine rennet contains the acid proteases chymosin and pepsin in changing relations, depending on the age of the animal being the source of the enzyme preparation.

The proteolytic activity of chymosin and pepsin depends on pH. Therefore the chymosin–pepsin activity-relation also changes with pH. The maximum efficiency of chymosin in rennets is observed near the enzyme's isoelectric point at pH 4.6 but it is sufficiently high at the pH value of milk, too.

The correlation between the chymosin–pepsin activity-relation, determined as proteolytic activity and milk coagulating activity was found to be not linear. This fact influences the calculation of rennet efficiency during the cheese ripening process.

IMMUNOELECTROPHORETIC CHARACTERIZATION OF PROTEINS

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The immunoelectrophoretic methods have recently been applied to the identification and characterization of minute quantities of proteins, e.g. enzymes, in clinical and biological laboratories. Five of the most commonly usd techniques, immunoelectrophoresis (IE), rocket immunoelectrophoresis (RIE), fused-rocket immunoelectrophoresis (FRIE), crossed-immunoelectrophoresis (CIE) and crossed-immunoelectrofocusing (CIEF) were applied in our experiments to characterize the Glycocladium cellulolytic enzyme system obtained in our laboratory. Nineteen different antigenic components, most of them glycoproteins, could be detected.

However, classical immunoanalytical techniques are not very appropriate for the investigation of processed denatured mixtures e.g. soy protein in meat products. For sterilized products the enzyme-linked immunosorbent assay (ELISA) has been developed to quantitate soya protein. Unlike the classical immunochemical methods, ELISA does not rely on the precipitation of the antigen-antibody complex; the presence of the complex is quantitatively

monitored by colorimetric measurement of the activity of the enzyme linked to it. The technique has been applied successfully to the quantitative determination of soya protein even in severely heat-processed meat products.

BIOLOGICAL VALUES OF NEW PROTEIN SOURCES

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The protein management of nations and the health preserving nutrition strategies require that higher priority should be given to the qualification of proteins. In the possession of adequate methods the protein value gained from unit area can economically be increased and this can provide the basis for the development of an up-to-date nutrition pattern.

In our laboratories protein concentrates with balanced amino acid pattern were prepared by enzymatic peptide modification. By enzymatic technique first the methionine was built into the protein bonds. Methionine plays an important role as an essential amino acid and as an exclusively methylizing agent in the living organism.

The purpose of the present investigation was to study the biological value and digestibility of protein concentrates based on casein partially enzyme-hydrolysed, prepared from α-chymotrypsin (Cas) and soyprotein isolate (SI) for utilization as formula food. The Cas and SI were balanced (Sulphuric Amino Acids = 3.5 g per 16 gN) with free L-methionine (Cas-Met, SI-Met) and with enzymic peptide modified Cas (EPM) prepared by building in the peptide bonds of methionine-methyl-ester (Met = 6.8 g per 16 gN) with NOVO Alcalase enzyme-catalyzed reactions (Cas-EPM, SI-EPM).

The in vitro biological value was characterized by the CS-value (Chemical Score) calculated on the basis of the essential amino acid content (FAO, WHO, 1973). The in vivo biological value was determined by animal feeding experiments on growing male rats. Changes in Net Protein Ratio (NPR), Net Protein Utilization (NPU), Biological Value (BV) and True Digestibility (TD) were determined by direct measurement of the body-mass, the body- and fecal-nitrogen (UNU, 1980).

The results indicate that the biological value of the balanced protein concentrates (Cas-Met, SI-Met and Cas-EPM, SI-EPM) is more favourable. The supplementation with EPM on the basis of SI resulted in a significant improvement of the biological value.

INVESTIGATION OF DIGESTIBILITY OF ENZYMATICALLY TREATED YEAST

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Cell-walls of microorganisms are hardly available to digestive enzymes of human being and laboratory animals. In case of yeasts, disregarding some strains which have cell-walls of high permeability (e.g. brewer's yeast), the cell-wall decreases the digestibility of the protein. In order to increase the digestibility the authors carried out experiments regarding the digestibility of different kinds of yeast strains *(Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Rhodotorula glutinis, Candida guilliermondii)*.

Experiments were extended to the investigation of the effect of the yeast protease inhibitor. They carried out it by pancreatine enzyme in pH-stat system and they compared degrees of hydrolysis (DH) when the time of hydrolysis was 60 minutes.

The yeast suspensions were treated with enzymes, heat, physical effects and combinations of them. Comparing the effects of the treatments in the digestibility of each strain, it was found that in case of *Saccharomyces cerevisiae* the pretreatment with its intracellular protease yielded significantly better digestibility.

In case of *Rhodotorula glutinis* every treatment changed its digestibility significantly, but only the pretreatment by its intracellular protease was more successful compared to the control sample. In case of the other strains none of the treatments could change the digestibility significantly.

IMMUNOCHEMICAL CHARACTERIZATION OF SOYPROTEIN PRODUCTS

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Immunoprecipitation techniques and enzyme-linked immunosorbent assays (ELISA) for the measurement of soyprotein levels in foods have already been published. The application of immunoassays for the determination of soyproteins depend on the composite nature of soyprotein ingredient and the biological variability of its composition and on the processing involved in the manufacture of the ingredient (e.g. soy flour, concentrate, isolate or extrudate).

The polyclonal rabbit antiserums to soya isolate and 115 soyprotein subunit prepared by Thanh and Shibashaki (1976) have been developed in the Central Food Research Institute. The purpose of the present investigation

was to study the possibility of using these serums for the determination of various soyprotein products in a model mixture.

The soybean varieties (McCall, Eszter, S 1345, ISZ 15) and processed soy-products (texturate, extrudate, concentrate, isolate) were checked with immunoelectroforesis test by Laurell (1967) and with ELISA by Notermans and Henvelman (1985). The affinity purified (Erdei et al., 1986) immunoglobulin was labelled with horse radish peroxidase by Avrameas (1969).

It was found that these assays are applicable to raw and processed soy-products and are not dependent on soya variety and type of soyprotein product composition and processing conditions.

BIOLOGICAL VALUE AND CHANGE OF MILK PROTEIN IN CATTLE, GOATS AND SHEEP DURING LACTATION

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During the past 20 years in the interest of economy and increase of milk production far-reaching changes have taken place in cattle breeding. Nowadays there are several genotypes, and these different breed constructions give considerably dissimilar milk. Changes having taken place in sheep and goat breeding lagged far behind cattle breeding, but because of the increasing demand for sheep's and goat's milk attention has been focused on these breeds.

Biological value of milk protein in the following breeds and breed constructions were determined:

Cattle: Hungarian red spotted (Hrs), Holstein-Friesian (HF), Hungarian red spotted × Holstein-Friesian F_1 (F_1), Hungaro-Friesian B (HFB), Holstein-Friesian fatherhood (HFf).

Goat: Saanen (S), Alpine (A), Hungarian home breed (H), improved Hungarian white (iH).

Sheep: Hungarian combing merino (Hcm).

From the animals belonging to the above mentioned groups milk samples were taken after parturition on 3–5 occasions and on 3–10 occasions during lactation. The amino acid pattern of the milk samples followed by 6 mole hydrochloric acid hydrolysis was determined by an LKB-4101 automatic amino acid analyser, then using Morup's and Olesen's method the biological value was counted.

The biological value of the colostrum in cattle after calving is practically unchanged (105) until the 48th hour then it gradually decreases till the 30th day of lactation (74–75).

The biological value of milk increased till the second month of lactation then it decreased until the fifth month, while towards the end of lactation it increased again. The results for goat and sheep were similar with the difference, that while the biological value for cattle and sheep colostrum coincide that for goat was 20% higher.

The biological value of goat's milk milked during lactation was 15–25% higher that of cattle corresponding to a higher protein ratio in goat's milk

No significant difference was found between the biological value of the milk of animals being under the same conditions of lactation neither between certain genotypes of cattle.

ESTIMATION OF THE DISTRIBUTION OF LOW MOLECULAR WEIGHT PEPTIDES IN PROTEIN HYDROLYSATES

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To estimate molecular weight distributions of proteins and larger peptides gel chromatography with Sephadex or SDS gel electrophoresis using molecular weight standards are most common. With highly hydrolyzed proteins, consisting of small peptides and amino acids, disturbances occur as a result of partial content of aromatic amino acids with retarded running properties.

The proposed method eliminates these disturbances and consists of the following steps:

— Gel chromatography of 50 mg of a peptide mixture in 0.5 cm^3 0.1 mol l^{-1} acetic acid on a Sephadex G-25 column collecting 3 cm^3 fractions.

— Determination of NH_2-groups with trinitrobenzenesulfonic acid before and after total hydrolysis (TH) with 6 *N* HCl in each fraction,

— Calculation of the NH_2-groups before and after TH and of the amino acids per peptide (Pn = NH_2 after TH/NH_2 before TH), ranking of the Pn-values by size and summing up in steps of the respective NH_2-values after TH by a computer program,

— Plotting the stepwise summarized NH_2-values (Σ mol%) against the ranked Pn-values and presenting the molecular weight distribution by taking the differential NH_2-values for the integers of Pn from the above plot.

Some molecular weight distribution patterns of different protein hydrolysates are given as examples.

ENZYMATIC HYDROLYSIS OF MAIZE PROTEIN

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An enzymatic modification or hydrolysis as it is performed e.g. on soy, wheat, meat, fish or milk proteins by a direct proteolytic attack is not possible to a similar degree with maize gluten. Maize proteins are hydrolyzed by proteolytic enzymes to a much lesser extent. An industrial use of this process has not become known yet.

The present investigations with technical maize gluten deal with the following problems:

- comparison of the enzymatic hydrolysis of maize and wheat protein and investigation of some functional properties,
- comparison of different proteinases,
- influence of different kinds of pretreatment (HCl, α-, and β-amylase, heating) on the enzymatic proteolysis,
- estimation of molecular weight distribution with maize protein hydrolysates.

From the results it is obvious that the solubilities of maize gluten are significantly lower than those of wheat gluten at comparable degrees of hydrolysis. Special investigations of solubilities and the distribution of molecular weights reveal that preferably small peptides besides not or only little attacked proteins are found as hydrolysis products. Despite of using selected pretreatment methods the extent of hydrolysis can be increased only partly.

YEAST AS A HUMAN PROTEIN SOURCE

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Yeasts raise great interest as SCP for food purposes not only for their good fermentation characteristics but the high biological value of their protein and technofunctional properties. For direct human consumption following yeast species can be used: *Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Kluyveromyces fragilis, Candida lypolitica, Candida tropicalis, Candida utilis.*

An unlimited utilization of yeast as main protein source is only possible after the reducing of RNA content below 2%. Activation of intracellular RN-ases seems to be very convenient especially in case of yeasts with high cell

wall permeability. Our experimental works shows that good growth rate, high protein content and biological value are equally important aspects for strain selection. The composition of fermentation medium and aeration level also influence the SCP quality.

In the amino acid composition of yeast protein methionine is a limiting essential amino acid. Methionine content can be increased by mutation and protoplast fusion technique, too. Among fermentation parameters aeration intensity (mmol $O_2\,l^{-1}h^{-1}$) has a significant effect on methionine content. Higher aeration levels cause a decrease in methionine synthesis.

Yeast protein digestibility is dependent on the cell wall, partial destruction of it resulted in an increase of digestibility.

INVESTIGATION OF THE PROTEINS OF LUPIN SEED

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Protein of two cultivars (Giza-1 and Giza-2) of lupin species *Lupinus termis* and two cultivars (Luplance and Lucky) of species *Lupinus doux* grown in Egypt, were investigated.

Total protein content of different cultivars ranged from 28,1% to 43,1%. Amino acid composition showed high levels of glutamic acid, leucine and arginine and low level of tryptophan being the first limiting amino acid. Digestibility of lupin seeds and kernels ranged from 61.7% to 71.1%.

Globulin is the major protein fraction in all cultivars followed by albumin. The quantity of all other (residual) proteins is about 10%. Using HPLC technique 5–8 subfractions of globulins may be separated. Changes in the molecular weight distribution of thermally treated protein fractions may be well demonstrated using HPLC. Boiling in water for 1 h changed significantly the solubility of globulin fractions.

Protein isolates of lupin seeds have a relatively good solubility and satisfactory emulsifying properties.

CHARACTERIZATION OF SOY- AND PEA PROTEIN FRACTIONS

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Different fractions of soy- and pea proteins were characterized to get more information about their applicability in food industry. The fractionation of soy- and pea proteins from soybean and pea flour were made according to methods of Osborne, and Than and Shibasaki, respectively. Their molecular weight distribution was determined by SDS-PAGE, quantities of SH and SS groups in proteins were determined by the method of Beveridge. The next functional properties were investigated: water binding capacity, fat binding capacity, emulsifying activity, emulsion stability, foaming activity and foam stability.

The results showed that soy- and pea proteins could be well fractionated into different fractions. They have specific molecular weight distributions, different quantities of SH and SS groups and different functional properties. Investigating the same fractions of soy- and pea proteins some similarities also could be noticed. It is suggested that composition and structure of food proteins could influence their technofunctional properties.

THE PRODUCTION OF SINGLE CELL PROTEIN FROM AGRICULTURAL WASTES BY FUNGI

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Results throughout this study revealed that the chemical composition of corn and cotton stems are considered basic and suitable nutritional sources for microbial growth, because of their high fibre constituents, 46.62% and 48.35% for corn and cotton stems, respectively.

The optimum concentration of the cellulosic waste in the fermented media for SCP production and the maximum yield of mycelium which was the aim of this study, depends greatly on both fungal strains and the type of waste. Two strains of fungi were used for SCP in this work, *Sporotrichum* and *Aspergillus* as cellulase enzyme producing microorganisms.

In the growth media 1.8% of powder corn stems was an optimum concentration for SCP production and maximum yield of mycelium for the *S. pulverulentum* strain, as the fungi produced 21.5 g mycelium per 100 g of waste; 1.5% of powdered cotton stems was optimum concentration for SCP for the same fungal strain, as it produced 20 g of mycelium per 100 g waste.

For *A. niger* strain 2.1% of powdered corn and cotton stems, respectively, were optimum concentrations for SCP production as the fungi produced 24 and 21 g of mycelium per 100 g of the mentioned agricultural cellulosic wastes, respectively. The protein content of fungal strains SCP was 28 and 30.6% for *S. pulverulentium* and *A. niger*, respectively, which is considered a high protein content.

ACIDIFIED FABA BEAN PROTEIN GELS

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A protein fraction is prepared from faba bean (*Vicia faba* L. minor) protein isolate at pH 2 which forms gels in the acidic pH range. The aim of this study was to investigate the influence of pH value, protein concentration, heat treatment, calcium ions, sucrose and dextrose syrup on the gelation of this faba bean protein fraction.

For preparing gels the components are mixed and the pH is adjusted by adding citric acid or HCl. The mixture is heated and then cooled to form gel. Gel strength is measured with the penetrometer AP 4/II. The gel strength rises with increasing pH up to pH 3.5. Above pH 3.5 the protein suspension does not form a gel but it becomes curdlike. The gel strength also depends on the protein concentration, it decreases with decreasing protein concentration. At pH 2 protein suspensions with less than 11% protein do not form gels, at pH 2.8 with less than 10% do not form gels. The extension of heating time and temperature increases the gel strength, the minima for gelation are 15 min at 100 °C or 30 min at 85 °C (13% protein, pH 2.8). Addition of calcium ions to the protein suspension (13% protein, pH 2.8) up to 15 mmol increases the gel strength. With more than 15 mmol calcium gelation does not occur, the protein suspension becomes curdlike. Addition of sucrose to the protein suspension (14% protein, pH 2.8) leads to a decrease of the gel strength. Protein suspensions with more than 120 g sucrose to 100 g do not form a gel, they have after heat treatment and cooling a syrup-like consistency. Addition of dextrose syrup to the gel up to 25 g to 100 g gel (13% protein, pH 2.8, 80 g sucrose to 100 g gel) increases the gel strength. Addition of more than 25 g dextrose syrup is detrimental because the gel becomes cloudy.

YEAST STRAIN SELECTION FOR FOOD PROTEIN SOURCE

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Yeast strains were chosen according to the FDA permission. Growth rate, protein content, cell concentration, RNA content, RN-ase activity and efficiency of RNA reduction by intracellular RN-ase had been determined for *S. cerevisiae*, *Candida guilliermondii* and *Rhodotorula glutinis*. Cell propagation was done in batch fermentation on date syrup medium. Growth rate was significantly influenced by C-source concentration but the sensitivity of the three strains is different.

Highest limiting substrate concentration was found for *R. glutinis*. Protein content of yeast biomass was highest for *S. cerevisiae* at each substrate concentrations above the limiting value. RNA content is influenced by the strain and fermentation conditions as well. At date syrup concentrations between 0.5–1.0% however there is no significant difference among RNA values except for *S. cerevisiae*, produced at 1% substrate concentration.

On comparing protein production values *C. guilliermondii* showed poorest and *R. glutinis* was slightly significantly better than *S. cerevisiae*. RN-ase activity of the yeast strains is significantly different and efficiency of RNA reduction by heat-shock treatment also.

As RNA content of SCP for food protein purposes should not be higher than 2%, *R. glutinis* was found to be the best strain.

COMPLETE UTILIZATION OF THE PROTEIN RICH FABA BEAN IN HUMAN NUTRITION

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Faba bean is one of the four most important legume crops in the world. The recent annual production exceeds 4 million tons. Faba beans (*Vicia faba* L. minor) contain after dehulling about 28% of proteins, 45% of starch and 1–5% of lipids beside some other components.

A complete processing scheme is presented for faba beans, to yield special roasted kernels, hulls, special flours, protein isolates (native and acetylated), starch and lipids. Roasted kernels are applicable as a partial nut substitute in sweets or as a snack. Hulls are used as a component with a high dietary fiber content. The faba bean flours show water and fat binding capacity and form gels by heating. Different pretreatments reduce the beany taste and the content of undesirable components. Faba bean flours can be used for

example in sausages, bread and other bakery products, ice cream and pasta products. Faba bean protein isolates are gel forming, emulsifying, emulsion stabilizing and foaming products. Acetylation enhances gel strength and emulsion forming and stabilizing properties. Faba bean protein isolates are applied as high functional additives to processed foods in small amounts (mostly less than 5%). Typical application fields are: sweet jellies and fillings, mayonnaise, salad cream, dressings, margarine, sauce for canned meat or fish. Faba bean starch has white color, is tasteless and provides a high amylose content of about 30–35%. Therefore faba bean starches form strong gels, but tend to produce syneresis. Starch is applicable as gel forming or thickening agent, for instance in cooked puddings. The lipids of the faba bean consist of more than 40% phosphatides and are applicable similarly to soya lecithin, for instance as emulsifier in chocolate. The complete processing and utilization of faba bean is an example for the exhaustive exploitation of a protein containing raw material in human nutrition.

OFF-FLAVOUR REDUCTION IN FABA BEAN PRODUCTS

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The application of modified protein isolate from faba beans as substitute for traditional gelling agents in the acid range can be difficult from the sensory aspect. In fruit gel systems an unpleasant off-flavour can occur.

The categorical judgement of small intensities enables the determination of the off-flavour amount of acid modified faba bean protein isolate in the application system "Fruit gel". This sensory method was used to evaluate the effect of various processing methods on the reduction of undesirable components, which cause the off-flavour in the acid range.

Variance analysis of the results shows that defatting or isoelectric washing, respectively, reduces the off-flavour significantly. A further weakening of the off-flavour is possible by addition of a strawberry aroma concentrate. A combination of defatting and isoelectric washing of faba bean flour gives the highest removing rate of off-flavour components.

EFFECT OF PROTEIN QUALITY AND DIETARY LEVELS OF IRON, ZINC AND COPPER ON THE INDICES OF OVERALL DEVELOPMENT OF RATS

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The bioavailability and utilization of trace elements depend on many dietary factors such as content of protein, fibre, oxalates, tannins, etc. This investigation was only a part of wider studies designed to examine the effect of protein quality and dietary levels of iron, zinc and copper on mineral metabolism.

The study was performed in male Wistar rats with a mean initial weight of 100 g. Casein, wheat gluten and a caseingluten mixture (1 : 1) were used as the sources of protein. The protein content of the semisynthetic diets was 20%.

Three levels of examined minerals were used:

- moderately low level of Fe, Zn and Cu simultaneously (60% of recommended level),
- adequate level (recommended for rats),
- high level (three times higher than recommended in rats diet).

It was found that under conditions of this experiment

- protein quality and level of Fe, Zn and Cu in the diet affect weight gain of the rats, diet intake and food efficiency ratio independently from one another. Casein and recommended levels of minerals were optimal,
- minerals had no effect on the relative weights of liver, spleen and kidney, however, casein resulted in slightly higher weights than wheat gluten,
- high levels of minerals resulted in the highest hemoglobin concentrations (16.1 g 100 cm^{-3} at high levels and 12.7 g 100 cm^{-3} at low levels).

This study was supported by CPB-R Grants No. 10–16/4.2.8/67.

EFFECT OF PROTEIN QUALITY AND DIETARY LEVELS OF IRON, ZINC AND COPPER ON MINERAL METABOLISM IN THE RATS

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The purpose of this study was to compare the concentration of iron, zinc and copper in tissues of rats fed experimental diets containing 20% protein as casein, wheat gluten or mixture of both (1 : 1) and three different levels of the examined minerals (approximately 60%, 100% and 300% of the recommended amount simultaneously for Fe, Zn and Cu). The experiment was carried out using male growing Wistar rats.

The level of Fe, Zn and Cu was measured as well as total iron binding capacity (TIBC) and transferring saturation in liver, kidney and plasma. The iron and copper content and TBIC of plasma were highest using casein and high level of minerals in the diet. Transferring saturation and level of the zinc was not affected by mineral level in the diet.

The higher mineral level of diet increased the mineral content of kidney and liver, but the quality of protein affected only the iron concentration of liver. Gluten protein in diet resulted in the highest iron content.

This study was supported by CPB-R Grants no 10–16/4.2.8/67.

CHANGES IN THE ACTIVITY OF POLYPHENOLOXYDASES DURING STORAGE OF APPLES

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Four apple varieties (Jonathan, Starkimson, Golden delicious, Idared) were investigated harvested in 1982, 1983 and 1984. The apples were stored in normal and controlled atmosphere (14% O_2, 3.5% CO_2). Some of the samples were treated with irradiation (0.5–1.5 kGy, Co^{60}) and calcium chloride (2% solution).

It was found that generally small (10–20%, relative) change occurs in the polyphenoloxydase (PPO) activity during 6 month storage. Nevertheless significant differences were observed between varieties (e.g. the initial PPO activity of Idared variety harvested in 1986 was 28.6 units and that of Starkimson only 13.5 units). Treatment with calcium chloride decreased the initial PPO activity (−20 −30%). Irradiation and changes of the atmosphere during storage had no significant effects on PPO activity levels.

BOOK REVIEW

Microorganisms in foods

4. Application of the hazard analysis critical control point (HACCP) system to ensure microbiological safety and quality

J. H. Silliker, A. C. Baird-Parker, F. L. Bryan, J. H. B. Christian, T. A. Roberts and R. B. Tompkin (Eds.)

Blackwell Scientific Publications, Oxford, 1988; 357 pages

Book 4 in the series of the International Commission on Microbiological Specifications for Foods (ICMSF) may be regarded as original work due to its thematics. The Hazard Analysis Critical Control Point (HACCP) system, adopted in this book aims to identify the microbiological hazards existing in a process or practice, to identify the critical control points (CCPs) at which these hazards can be controlled, and to establish systems based predominantly on chemical and physical testing, and on visual observation, by which the effectiveness of control can be monitored.

The book contains 357 Arabic and XIV Roman numbered pages. The publication is divided into two parts. In Part 1: Principles are discussed in 8 chapters in 136 pages. Here, the new considerations and methods concerning the HACCP system and its application are introduced. Part 2 is the Applications in 5 chapters in 176 pages. It deals with the problems of the: Production and harvesting of plant foods; Production of animal products; Food processing; Marketing and retail stores; Food service and Homes.

The very useful book is completed by 6 Appendices introducing the organization and the activities of ICMSF. 40 tables contain the data of the book and 46 figures contribute to the better understanding.

Abundant literature is available for experts dealing with these questions. Therefore, this new edition will be an essential reading in post-gradual education, in the hygienic design of food operating areas and in research.

I. Varsányi

Biotechnology and food industry

J. Holló and D. Törley (Eds.)

Akadémiai Kiadó, Budapest, 1988; 707 pages

The book contains the Proceedings of the International Symposium held in Budapest, Hungary between October 5—9, 1987.

The volume comprises the subject matter of the Symposium, including papers delivered by experts from 20 countries. Some papers deal with general problems of the biotechnology and the general situation of biotechnology in some countries, other are engaged in the most recent results of genetic engineering and physiology. A large number of contributions are aimed at the production and application of enzymes and microorganisms. Many papers review technological applications in the production of alcohol, citric acid, beer wort, bread, feeds, the utilization of waste and other waste substances, the application of new reactors and technologies, etc.

The Symposium covered 4 main topics through 65 presentations.

The first chapter is the General Topics with 8 lectures. The importance of biotechnology is discussed with special regard to its role in the food industry. The food industry applies the methods of "positive" microbiology in a more wider range. Several lectures are examples for this.

The second chapter contains 10 lectures and introduces the newest results of researches in the fields of genetic engineering and physiology. Special emphasis is given to the role of modifying some yeast and mould strains in the production of biologically

Akadémiai Kiadó, Budapest
Kluwer Academic Publishers, Dordrecht

active materials. Lectures on the newest application of protoplast fusion; on the further study of the characteristics of brewer's yeast and on the development of microbiological wine improvement convincingly prove that the Hungarian biotechnology is a dinamic part of not only the researches but of the industrial practice.

The third chapter discusses in 23 lectures the biotechnological application of enzymes and microorganisms. Lectures account on the preparation, purification and on the usage of enzymes produced mainly by fungi. The newest results in enzyme immobilization, in the determination of enzyme activity and on certain industrial applications are shown.

The fourth chapter presents the technological applications in 24 lectures. Biotechnological methods may be utilized on various fields of food industry. Lectures were not only given from the meat-, dairy-, brewing industries and from viticulture but also on the possibility of applying single-cell protein; on waste utilization and also on the methods of traditional biotechnology.

Both the opening addresses and the closing conclusions emphasize the necessity of utilizing the biotechnological methods in a wide range and also the positive role of exchanging the research results at international symposia. The new possibilities offered by technology contribute to the development of the food industry.

This volume gives information on the present state, developmental trends and results achieved in biotechnology in the food industries. Researchers as well as industrial specialists, graduate students in food chemistry, biochemistry and biotechnology will find in this book many ideas that may be useful in the solution of their own problems.

L. Somogyi & I. Varsányi

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Continued from back cover

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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.

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