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EVALUATION OF PUMPKIN SEED (*CUCURBITA PEPO*; KAKAI 35) AS A NEW SOURCE OF PROTEIN

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Four products were prepared from pumpkin seed (*Cucurbita pepo*, Kakai 35), defatted meal, protein concentrate, and two protein isolates (PPI-I and PPI-II). The functional properties of these products were studied.

Pumpkin seed products had high levels of crude protein content up to 72%, 77% and 96% in the case of meal protein concentrate and isolates, respectively. The yield of pumpkin seed protein isolate-I, pumpkin protein isolate-II and pumpkin concentrate was 62%, 59% and 87%, respectively.

The solubility of pumpkin seed meal and protein concentrate was minimal at pH 3, that of protein isolates at pH 4. The major protein fraction was globulin (60%). Pumpkin products showed excellent water and fat absorption, and emulsifying properties. The foaming capacity of pumpkin meal and protein isolate-I was much greater than that of protein isolate-II and protein concentrate. The foam of protein isolate-I was more stable than that of other pumpkin products. The neutralization of protein isolate (PPI-II) before drying seemed to have only a small effect on its functional properties except foaming and water absorption.

Keywords: pumpkin products, globulin fraction, protein solubility profile, functional properties

Pumpkin (*Cucurbita pepo*) seed could be utilized successfully as a source of edible protein and oil for human consumption as well as a feed. At the same time, it helps minimizing waste pollution problem, (LAZOS, 1986). In West Africa, especially in Nigeria cooked and dried pumpkin seeds are served as snacks. The seed might also be cooked, ground and fermented for use as a flavour enhancer in gravies and soups (NWOKOLO & SIM, 1987). In Egypt the pumpkin seed is eaten as a nut. In the European countries pumpkin seed is consumed as a snack. The extracted pumpkin seed oil is very popular in Europe.

To the best of our knowledge there are no reports on the preparation of isolated and concentrated protein from pumpkin seed meal. Therefore the aim of this study is to deal with the preparation and functional properties of protein isolate and concentrate from defatted pumpkin seed meal.

Akadémiai Kiadó, Budapest

1. Materials and methods

1.1. Meal

Peeled pumpkin seed was obtained from the Research Institute of Vegetable Oil and Detergent Industry, Budapest. Defatted meal was prepared by extraction of the ground seeds with hexane in a Soxhlet apparatus. Solvent was removed from the extracted flakes at room temperature. The meal was finely ground and passed through a 400 μm sieve.

1.2. Preparation of protein isolate (PPI-I)

The meal was extracted with 0.5% Na_2CO_3 (pH = 9.5) at room temperature. The solid to solvent ratio was 1:10 (w/v) and shaked for one hour. Insoluble materials were removed by centrifuging at 5000 r.p.m., for 15 min. The supernatant was acidified to pH = 3.0 with 1 mol l^{-1} HCl. The precipitate was washed twice with distilled water (after washing the pH value of the precipitate was 2.7) and acetone and dried in an oven under vacuum at 30 °C for 4 h.

1.3. Preparation of protein isolate (PPI-II)

Its preparation was similar to that of PPI-I except that it was neutralized to pH = 7.0 with HCl (2 mol l^{-1}) before drying. Both protein isolates were ground to pass through a 400 μm sieve.

1.4. Preparation of protein concentrate

Protein concentrate was prepared by the following five methods:

1.4.1. The meal was extracted with 75% ethanol for 1/2 h, the meal to solvent ratio was 1:10 (w/v), followed by an extraction at the isoelectric point (pH = 3.0) for 1/2 h and neutralized to pH = 6.6. Extraction was carried out at room temperature.

1.4.2. The meal was extracted with 75% ethanol to which 0.2% of sodium metabisulfit was added for 1/2 h followed by extraction at pH 3.0 for 1/2 h and neutralized to pH 6.6.

1.4.3. The meal was extracted with 75% ethanol for 1/2 h followed by the extraction at pH 3.0 with the addition of 0.2% sodium metabisulfit for 1/2 h and neutralized to pH 6.6.

1.4.4. The meal was extracted twice with 75% ethanol for 1/2 h.

1.4.5. The meal was extracted with petroleum ether (1:4) for 1/2 h followed by the extraction with 75% ethanol for 1/2 h.

The products were dried with acetone and in a vacuum oven at 30 °C for 2 h, ground to pass through a 400 μm sieve.

1.5. Analytical methods

Moisture, ether extract, ash, crude fiber and protein solubility were determined according to the A.O.A.C. method (1980). Crude protein (N × 6.25) content was determined by the Kjeldahl-procedure in an automatic Kjel-Foss equipment (Model 16210, Denmark).

Non-protein nitrogen was determined by the method of BHATTY and FINLAYSON (1973) with the modification of NACZK and co-workers (1985). One g of meal was shaken for 1 h at room temperature with 40 cm^3 of 10% trichloro acetic acid (TCA) solution. The insoluble material was removed by filtration using Whatman No. 41 filter paper and rinsed three times with 15 cm^3 of 10% TCA solution. The filtrate was made up to 100 cm^3 with distilled water and an aliquot was taken for determination of soluble nitrogen by the Kjeldahl method in an automatic Kjel-Foss equipment.

Total carbohydrate was determined by Schoorl's method after hydrolysis with 2.5% HCl for 3 h.

Fractionation of pumpkin proteins was carried out by the OSBORN and CAMPBELL method (1897).

1.6. Functional properties

The pH values were determined using a 10% dispersion (w/v) of products in distilled water.

The nitrogen solubility index (NSI) was evaluated at pH = 7.0 according to A.A.C.C. (1969) methods as described by THOMPSON and co-workers (1982). It was also determined in water and NaCl (1 mol l^{-1}) at natural pH of the products.

Water absorption was measured by a centrifuge method of SOSULSKI (1962).

Fat absorption was measured by the method of LIN and co-workers (1974).

For the determination of the emulsifying capacity 0.5 g of the sample was dispersed in 23 cm^3 of distilled water and homogenized for 1/2 min at the highest speed (Ultra-Turrax homogenizer, Janke & Kunkel, TP 18/10 Staufen, Germany). Other steps were similar to those outlined by BEUCHAT and co-workers (1975). Emulsifying capacity is expressed as cm^3 sunflower oil emulsified by one gram of sample. Emulsifying activity and emulsion stability were studied by the method of YASUMATSU and co-workers (1972), but emulsions were prepared at a final sample concentration of 3.5% (w/v).

Foaming capacity was expressed as percent volume increase of 75 cm^3 of the sample dispersion (3%) whipped with homogenizer for 6 min (Ultra-Turrax homogenizer, Janke & Kunkel TP 18/10, Staufen, Germany) (approximately 10 000 r.p.m.). The mixture was poured into a 250 cm^3 graduated cylinder. Stability of the foam was expressed as volume of foam remaining after a standing period of 120 min (COFFMANN & GARCIA, 1977).

2. Results and discussion

Table 1 shows the proximate composition and non-protein nitrogen of the pumpkin seed products. Pumpkin meal has a high total protein content as compared to other oilseeds, such as rapeseed, peanut and sunflower (NACZK et al., 1985; IHEKORONYE, 1986; LIN et al., 1974). The percentage of crude protein was 72.1, 76.7 and 96.3% in meal, protein concentrate and isolates, resp. Non-protein nitrogen of pumpkin seed products ranged between 0.2–0.7%. LONGE and co-workers (1983) reported that the composition of defatted pumpkin seed were 69.7% of crude protein; 8.2% of crude fiber; 6.5% of oil; 9.3% of ash and 6.3% of carbohydrate (when dry).

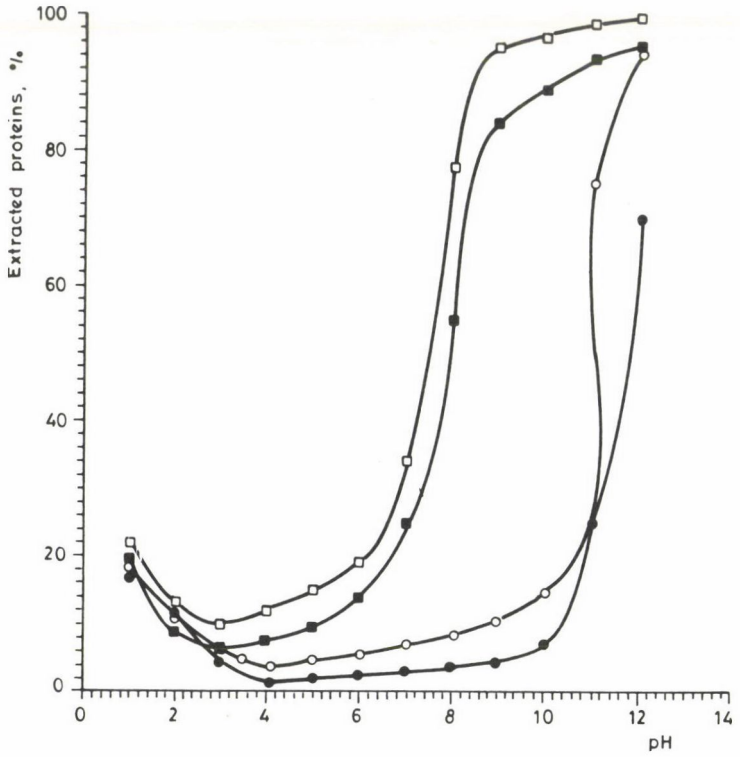

Fig. 1. Protein solubility profile of pumpkin seed products. □: Meal; ■: protein concentrate; o: PPI-I; ●: PPI-II

Table 1

Chemical composition of pumpkin seed products (on the dry weight basis)

Product	Crude protein (Nx6.25) (%)		Oil (%)		Crude fiber (%)		Ash (%)		Carbohydrate[a] (%)	Non-protein nitrogen (%)	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	$\bar{x}$	±s
Meal	72.1	0.1	4.3	0.2	2.6	0.2	9.4	0.3	9.1	0.7	0.02
Protein concentrate	76.7	0.2	2.2	0.2	4.0	0.3	9.9	0.1	6.7	0.6	0.01
PPI-I	96.3	0.1	1.7	0.1	-	-	2.7	0.1	0.3	0.2	
PPI-II	96.3	0.2	1.7	0.1	-	-	2.4	0.2	0.2	0.2	0.02

[a]: mean value of two determinations

$\bar{x}$: mean value of three determinations

±s: standard deviation

Figure 1 shows the protein solubility profile of pumpkin seed products. Both meal and protein concentrate have an iso-electric point at pH 3.0 and protein isolates at pH 4.1. The solubility of protein was low in the acidic region where it ranged between 17–22% at pH = 1.0, while the solubilities at pH = 11 were 96%; 93%; 75% and 25% in the case of meal protein concentrate, PPI-I and PPI-II, resp. The protein solubility profile of pumpkin meal was similar to that of other oilseed proteins, especially to sunflower seed (SAEED & CHERYAN, 1988). Both protein concentrate and isolates had a lower solubility than the meal, this is attributed to the removal of the more soluble nitrogen components of low molecular weight.

Table 2

Effect of treatment on the yield and colour of pumpkin seed products

Product	Treatment	Colour	Yield (%)
Meal	–	white-greenish	–
PPI-I	–	yellow-greenish	62.1
PPI-II	–	green-yellowish	59.3
Protein concentrate	1.4.1: ethanol and isoelectric point	green	89.1
	1.4.2: ethanol + 0.2% SMBS[a] and isoelectric point	dark green	87.8
	1.4.3: ethanol and isoelectric point + 0.2% SMBS	dark green	87.1
	1.4.4: ethanol (twice)	green	90.2
	1.4.5: petroleum ether and ethanol	light green	88.6

[a]: sodium metabisulfite

The effect of different treatments on the yield and colour of pumpkin seed products are given in Table 2. The yield of PPI-I was 62.1% and the colour was yellow-greenish. The loss in the yield and change in colour of PPI-II is attributed to the neutralization process.

Addition of 0.2% sodium metabisulfite during the preparation of the protein concentrate caused a dark green colour and the yield was slightly lower. Extraction of the meal twice with ethanol or with ethanol followed by the extraction with water at the isoelectric point produced a green colour. The best colour (light green) was observed by extraction with petroleum ether followed by extraction with ethanol.

Table 3

Classification of pumpkin seed proteins by the Osborn method

Pumpkin product	Protein fraction soluble in (in % w/w unit)								Protein in residue (%)	
	H_2O		1 mol l^{-1} NaCl		70% ethanol		0.2% KOH			
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Meal	15.7	0.8	60.0	1.1	2.5	0.6	12.3	0.6	9.6	3.1
Protein concentrate	11.2	0.7	56.7	1.3	1.3	0.2	12.3	0.3	18.5	1.0
PPI-I	6.6	0.2	1.7	0.1	1.0	0.1	0.6	0.1	90.1	0.3
PPI-II	0.9	0.2	2.6	0.4	0.8	0.1	1.1	0.1	94.6	0.2

$\bar{x}$: mean of three determinations
±s: standard deviation

Data presented in Table 3 show the protein fractions of pumpkin products. The major protein fraction in pumpkin seed protein is globulin which represent 60% of the meal protein. Protein concentrate had a high ratio of residual protein, attributed to the method used for its preparation, whereas the extraction of meal with ethanol reduced the solubility of proteins, probably due to the denaturation of the proteins. Both protein isolates had a low solubility in different solvents. This might be due to the denaturation of the proteins by acetone treatment during drying.

Functional properties of pumpkin seed products are given in Table 4. The NSI in water of meal was 23% as compared to 15.7, 8.3 and 2.1% for the protein concentrate; PPI-I and PPI-II, resp. LIN and co-workers (1974) reported that the protein solubility index of soybean and sunflower flours in water were 21.4 and 16.1%, resp. The NSI in NaCl was greater than in water and at pH = 7.0. Of the meal and protein concentrate about 92 and 57% of nitrogen was dissolved in the NaCl solution, respectively.

Absorption of water is very important in certain foods such as doughs and processed meat products. The pumpkin meal absorved 127.3% of water and other products even more. Of all pumpkin products PPI-I absorbed the greatest amount of water. This results are similar to those reported by LIN and co-workers (1974). They found that the water absorption of soy flour was 130% less than of soy protein concentrate and isolate, resp. PPI-II absorbed less water than PPI-I. We know that the water absorption capacity reflects interactions between the hydrophilic groups of the protein molecule, therefore the neutralization of protein before drying (PPI-II) might reduce the interaction with water.

Table 4

Functional properties of pumpkin seed products

Product	pH	NSI in water (%)		NSI at pH 7.0 (%)		NSI in NaCl (%)		Water[a] absorbtion (%)		Fat[a] absorbtion (%)		Emulsifying activity (%)		Emulsifying stability (%)		Emulsifying capacity cm^3oil g^{-1}	
		$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Meal	6.6	23.0	0.8	25.8	0.6	91.9	0.6	127.3	6.3	353.3	1.8	53.7	1.2	64.8	0.3	183	8.3
Protein concentrate	6.7	15.7	0.3	17.5	0.7	57.1	0.6	185.6	6.1	316.2	4.4	49.6	0.5	63.7	0.8	97	4.7
PPI-I	2.5	8.3	0.4	8.2	0.5	9.5	0.2	221.7	4.1	66.2	4.5	47.2	1.4	49.1	0.4	95	4.7
PPI-II	7.0	2.1	0.5	2.8	0.0	3.8	0.3	182.4	4.8	75.3	4.4	46.5	1.5	47.4	0.5	94	8.1

x: mean value of three determinations

±s: standard deviation

[a]: mean value of four determinations

The fat absorption of pumpkin meal was 353%, of protein concentrate 316% and of protein isolates between 66 and 75%. Contrary to water absorption both protein concentrate and isolates absorbed more water than meal, thus there is a negative relationship between the fat absorption and crude protein content and the other components of the meal absorb more fat than the protein. The fat absorption of PPI-II was higher than that of PPI-I probably due to the pH value of the product.

On the other hand, the pumpkin meal, protein concentrate and PPI-I formed an emulsion of high viscosity and white colour, while the emulsion from PPI-II had a low viscosity and a white-yellowish colour. The pumpkin products meal had the highest emulsification properties among the pumpkin products. These results are similar to those reported for sunflower protein preparations (LIN et al., 1974). The authors found that both protein concentrate and isolate had a lower emulsifying capacity than the meal. Pumpkin emulsions had a great resistance to heat treatment and their emulsion stability was increased by heating. Pumpkin products had a higher emulsifying capacity than emulsifying activity and emulsion stability. Emulsification characteristics increased as a rule with the increase of NSI values. Similar results were obtained by DEV and MUKHERJEE (1986), they have reported that emulsifying activity of protein products are related to their soluble protein content. The neutralization of protein isolate had a very small effect on the emulsification properties.

Table 5

Foaming capacity and foam stability of pumpkin seed products

Product	Volume increase on whipping (%)		Time after whipping (min) 15		30		60		120	
			Volume of foam (cm^3)							
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Meal	247	13.5	36	7.6	10	5.3	5	1.2	3	1.2
Protein concentrate	24	7.5	12	5.3	8	0.6	5	1.2	5	1.2
PPI-I	191	14.0	142	9.7	133	11.0	119	6.4	100	17.2
PPI-II	57	17.2	30	17.4	22	11.2	13	6.4	8	3.5

$\bar{x}$: mean value of three determinations
±s: standard deviation

Foaming capacity and foam stability of pumpkin products are presented in Table 5. Pumpkin meal had a high foaming capacity compared to protein concentrate and isolates. This is attributed to the denaturation of protein during preparation.

YASUMATSU and co-workers (1972) reported that heat processing diminished the protein solubility of soy proteins and reduced their foam capacity. The other components of the meal might aid in the formation of whipped foam (LIN et al., 1974). Foam from PPI-I was more stable as compared to other pumpkin products. This might be due to the pH value of the PPI-I.

SATHE and co-workers (1982), studied the functional properties of the proteins of Lupin seeds. They found that their maximum foaming capacity was at pH 2.0, whereas their highest foam stability was measured at pH 4.0.

3. Conclusions

Pumpkin seed has a high crude protein and ash content as compared to other oilseeds such as peanut, sunflower and rapeseed meal (IHEKORONYE, 1986; LIN et al., 1974; NACZK et al., 1985).

The yield of pumpkin protein isolate was (62%) much higher than of other oilseeds especially rapeseed and peanut (MANSOUR et al., 1992; MOSTAFA et al., 1988).

The high water and fat absorption and the emulsification properties suggest that pumpkin seed products could be used successfully in processed meat products and doughs (IHEKORONYE, 1986).

Emulsification plays an important role in several foods, especially in icecream, meat products, cakes and baby foods, thus pumpkin products might be used as a substitute for synthetic emulsifiers. Functional characteristics of foods containing pumpkin products should be tested in the future.

Generally, pumpkin products have a very poor foam stability, they are not suitable for sponge products like sponge cakes.

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PROTEIN SOLUBILITY, MINERAL CONTENT, AMINO ACID COMPOSITION AND ELECTROPHORETIC PATTERNS OF SOME GOURD SEEDS

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This investigation was carried out on cantaloupe, sweet melon and water melon kernels. All varieties contained uniformly high crude protein, with values of 54.29%, 53.90% and 43.41% in cantaloupe, sweet melon and water melon, respectively. Fat content was highest in water melon kernels (46.16%), in sweet melon it was 37.67% and in cantaloupe kernels 36.01%. All studied samples are valuable source of the major elements and of iron, zinc, manganese and copper. The solubility of protein was higher when the concentration of NaCl was higher.

Gourd kernels meal supplies high amounts of total nitrogen required for the synthesis of the dietary dispensable amino acids. It also supplies high amounts of threonine, leucine, phenylalanine and tryosine. PAGE showed four bands in cantaloupe kernels protein. Moreover, sweet melon and water melon kernel protein had typical banding patterns with respect to the number of bands (seven bands) and migration distances of both high and low molecular weight protein fractions.

Keywords: gourd seeds, fractionation of total protein, polyacrylamine gel electrophoresis, amino acid analyzer, atomic absorption

Cantaloupe, sweet melon and water melon are grown in many areas of Egypt. They are very popular and consumed fresh in large quantities in summer. There is no definite use for the remaining by-products.

Gourd seeds are available for direct consumption after salting and roasting by the majority of the population. The seeds have a relatively thick husk and edible kernel. The kernel contains about 53% of oil and 5.8% of nitrogen. The amino acid composition of the kernel compares favourably with that of other important oily seeds such as soybean (EZEIKE, 1988).

The gross chemical composition of some gourd seeds i.e., vegetable marrow and water melon, have been determined by several authors (KARMANDE, 1971; VIGO et al., 1973 and BADAWY, 1979). The data showed that the seeds contained 40.6 to 53.0% of crude protein; 28.4 to 45.1% of oil; 2.5 to 2.9% or 1.9 to 2.3% of ash.

OYENUGA and FETUGA (1975) have reported that water melon seeds contain high amounts of potassium, calcium and magnesium. Previous data were focussed on the chemical composition and amino acid content of some gourd seed proteins

(AHMED et al., 1966). This study was therefore conducted to supplement the existing data on the gross chemical composition, in order to assess the potential nutritional quality of these seeds.

1. Materials and methods

1.1. Materials

Cantaloupe (*Cucumis melo* L.), sweet melon (*Cucumis melo*) and water melon (*Citrullus lanatus*, var. Giza 1) were obtained from the local market in Assiut city during the summer of 1991. The seeds were removed from the tissues by hand, washed with tap water and air dried at room temperature (25 to 30 °C) for about one week.

The hull of the dried seeds were manually removed, the kernels were ground, then kept in tightly closed brown glass jars and stored at 5 °C till their analysis.

1.2. Analytical methods

1.2.1. Moisture, proteins, lipids and ash were determined by A.O.A.C. methods (A.O.A.C., 1980). Total carbohydrates were calculated from the differences.

1.2.2. Non-protein nitrogen (NPN). The NPN content was determined in the supernatants after precipitation of protein using 10% trichloroacetic acid (TCA) as described by SINGH and JAMBUNATHAN (1981). Nitrogen content of the supernatants was determined by means of the microkjeldahl procedure (EGAN et al., 1981). The true protein content was obtained from the following equation:

$$\text{True protein (\%)} = (\text{\% total nitrogen} - \text{\% NPN}) \times \text{factor}$$

1.2.3. Fractionation of total protein on the basis of solubility. The classification of protein was done according to ABD EL-AAL et al. (1986) using distilled water, 0.5 mol l^{-1} NaCl and 1 mol l^{-1} NaCl.

1.2.4. Polyacrylamide gel electrophoresis (PAGE)

1.2.4.1. Preparation of defatted seed meals – The meals were defatted by stirring three times with cold acetone (1:1, w/v) for 30 min each time air dried at room temperature and kept frozen till used.

1.2.4.2. Extraction procedure – Aliquots of 250 mg of the defatted meals were mixed in a test-tube with 5 cm^3 of a solution consisting of 4% sodium dodecyl sulfate (SDS) and extracted by mechanical shaking in an electric shaker (Voss Intrument LTD, type WF 5, Maldon, Essex, England).

1.2.4.3. Electrophoresis – The electrophoresis of sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) was performed by the method of DAVIS (1964) in 0.01 mol l^{-1} sodium phosphate buffer of ph 7.8 and 7.5% gel. Amido black was used to stain the protein bands and the unbound dye was removed with 7.5% acetic acid.

1.2.5. Analysis of amino acids. The amino acids were determined in the acid hydrolysate according to MOORE and co-workers (1958), using a Beckman amino acid analyzer (Model 121 M) as described by YOUSSEF and co-workers (1986). Fifty mg of the ground sample were placed in a 250 cm^3 conical flask fitted with a ground glass neck and 50 cm^3 of 6 *N* hydrochloric acid containing 0.1% mercaptoethanol was added. A reflux condenser was connected and the samples were refluxed for 24 h on a hot bath at 100 °C. The hydrolyzed samples were cooled to room temperature and filtered through the Whatman No. 1 filter paper. The flask and the precipitate on the filter paper were washed with distilled water and diluted to 100 cm^3 in a volumetric flask. As aliquot (10 cm^3) of the filtrate was placed in a 50 cm^3 beaker and kept in a desiccator to dry under vacuum over potassium hydroxide in the presence of concentrated sulfuric acid. The residue was dissolved in sodium citrate buffer (pH 2.2), diluted to the volume required suitable for amino acid analysis.

1.2.6. Minerals. Manganese (Mn), copper (Cu), iron (Fe) and zinc (Zn) were determined by using the Perkin Elmer Atomic Absorption spectrophotometer (Model 2380) according to A.O.A.C. (1980). Sodium (Na) and potassium (K) were estimated by a Carl-Zeiss Jena flame photometer.

2. Results and discussion

2.1. Chemical composition

Table 1

The approximate composition and energy value of some gourd kernels (% dry matter)

Constituents	Gourd kernels					
	Cantaloupe		Sweet melon		Water melon	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Fat	36.01	0.28	37.67	0.95	46.16	0.23
Crude protein	54.29	0.41	53.90	1.27	43.41	0.58
Total ash	4.43	0.61	4.94	1.33	4.26	0.37
Total carbohydrates[a]	5.27	0.38	3.49	0.69	6.17	0.24
Energy (cal per 100 g)	562.33	0.47	568.59	0.83	613.76	1.07

[a]: calculated difference

$\bar{x}$: averages of three determinations

±s: standard deviation

In order to evaluate the possibility of using cantaloupe, sweet melon and water melon kernels as sources of protein for human consumption, it is of great importance to carry out comparative studies of the chemical composition of the investigated kernels. The gross chemical composition of gourd kernels are given in Table 1.

All varieties contained uniformly high crude protein, with values of 54.29%, 53.90% and 43.41% in cantaloupe, sweet melon and water melon, respectively. Fat content was the highest in water melon kernels (46.16%), that of sweet melon was 37.67% and of cantaloupe kernels 36.01%.

Ash contents showed no significant differences: the values obtained were 4.43%, 4.94% and 4.26% in cantaloupe, sweet melon and water melon kernels, respectively. The protein levels of the studied kernels were higher than reported for groundnut and soybean (EL-DENGAWY, 1990).

The obtained data were found to be in agreement with those given by KARMANDE and co-workers (1971); VIGO and co-workers (1973) and OYENUGA and FETUGA (1975).

2.2. Mineral composition

The mineral constituents of cantaloupe, sweet melon and water melon kernels are presented in Table 2.

Table 2

The mineral constituents of some gourd kernels (ppm)

Minerals	Gourd kernels					
	Cantaloupe		Sweet melon		Water melon	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Sodium	10.00	0.99	16.00	0.71	14.00	1.27
Potassium	114.00	1.41	72.00	2.83	50.00	2.12
Iron	5.72	1.02	3.03	0.42	2.55	0.71
Copper	0.79	0.11	0.87	0.28	0.56	0.14
Manganese	0.56	0.17	0.47	0.10	0.41	0.16
Zinc	2.20	0.56	8.00	0.57	3.40	0.71

$\bar{x}$: average of three determinations
±s: standard deviation

Table 2 shows that cantaloupe, sweet melon and water melon kernels are rich in sodium, potassium, iron and zinc. No great differences were found in the sodium, copper and manganese content of the investigated kernels. The potassium and iron

content of cantaloupe kernels was twice as high as in water melon kernels. At the same time zinc content in sweet melon kernels was about four times higher than in cantaloupe kernels. The results are comparable with those in water melon kernels (OYENUGA and FETUGA, 1975). It seems that gourd kernels could be a valuable source of the major elements and of iron, zinc, manganese and copper, particularly in diets for humans, in which no special provision is made for the supply of these important nutrients.

2.3. Protein fractions

The protein fractions of cantaloupe, sweet melon and water melon kernels, are given in Table 3.

Table 3

Nitrogen content of the protein fraction of gourd kernels in % of total N
(g per 100 g whole N)

Protein fraction	Gourd kernels					
	Cantaloupe		Sweet melon		Water melon	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Non-protein nitrogen	6.12	0.17	5.10	0.14	4.76	1.07
Protein nitrogen	93.88	1.24	94.90	1.27	95.24	1.41
Water soluble nitrogen	10.31	1.85	12.48	0.68	13.57	0.81
0.5 mol l^{-1} NaCl soluble nitrogen	14.65	0.92	15.74	1.05	16.82	1.15
1 mol l^{-1} NaCl soluble nitrogen	22.79	1.12	28.76	1.07	31.47	0.66

$\bar{x}$: average of three determinations
±s: standard deviation

Non-protein nitrogen fraction varies between 4.76% and 6.12% and shows a lower percentage compared with peach kernel flour (16.40%). As shown in Table 3 sodium chloride solution extracted more meal nitrogen than water. However, it is also apparent from such data that 1 mol l^{-1} NaCl extracted higher meal nitrogen than did 0.5 mol l^{-1} NaCl. This may indicate that the proteins are more soluble at higher concentration of NaCl.

2.4. Amino acid composition and chemical scores

There is not much data in the literature on the amino acid composition of cantaloupe, sweet melon and water melon (OYENUGA & FETUGA, 1975). Table 4 compares the total amino acid content of cantaloupe, sweet melon and water melon meal with those of whole hen's egg.

Table 4

Amino acid content of some gourd kernels meal compared with whole hen's egg
(g per 16 g N)

Amino acids	Gourd kernels						Whole hen's egg
	Cantaloupe		Sweet melon		Water melon		
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	
Essential:							
Threonine	5.79	1.12	7.54	0.76	8.62	0.88	5.12
Cystine	0.34	0.14	1.55	0.78	1.03	0.04	2.43
Valine	5.47	0.66	6.29	0.41	5.29	0.41	6.85
Methionine	2.34	0.48	3.27	0.38	2.92	1.30	3.46
Isoleucine	3.82	1.16	4.87	1.23	5.21	0.30	6.32
Leucine	10.12	0.17	10.09	0.13	7.87	1.23	8.79
Tyrosine	4.43	0.61	5.79	1.12	4.70	0.99	4.16
Phenylalanine	4.91	1.29	7.24	0.34	5.97	1.37	5.63
Lysine	2.89	1.26	3.05	0.07	2.52	0.73	6.99
Total EAA	40.11	6.89	49.69	5.22	44.13	7.25	49.75
Non-essential:							
Aspartic acid	9.73	1.03	8.29	0.41	9.88	1.24	9.02
Serine	3.22	0.31	4.77	1.09	4.25	0.35	7.65
Glutamic acid	26.31	0.44	19.43	0.61	18.37	0.52	12.74
Proline	3.71	1.00	3.59	0.83	3.23	0.32	4.16
Glycine	6.69	0.98	4.85	1.20	5.36	0.51	3.31
Alanine	3.72	1.02	4.37	0.52	5.85	1.20	5.92
Histidine	2.09	0.13	3.03	0.04	4.14	0.20	2.43
Arginine	5.38	0.54	6.03	0.04	5.68	0.96	6.24
Total Non-EAA.	60.85	5.44	54.36	4.75	56.76	3.39	51.47
Essential amino acids/Non-essential amino acids		0.66		0.91		0.78	0.97

$\bar{x}$: average of three determinations
±s: standard deviation

The total essential amino acids from cantaloupe and water melon kernels are lower than those of egg and consequently the proportions of the total nitrogen

derived from essential amino acids (E/T) are lower than other source. Meanwhile, the total essential amino acids of sweet melon, were found in comparable amounts to those of egg.

It could be seen that the major amino acids of cantaloupe are glutamic acid, leucine and aspartic acid, while the minor amino acids are cystine, histidine, methionine and lysine. Sweet melon was found to be rich in glutamic acid, leucine, aspartic acid, threonine and phenylalanine. While cystine and lysine had the lowest concentration of these amino acids. The water melon kernels meal was particularly rich in glutamic acid, aspartic acid, threonine and leucine. However, cystine and lysine recorded the lowest levels of all amino acids.

The total sulfur amino acid (methionine + cystine), valine, isoleucine and lysine contents of all studied samples were lower than in egg.

Threonine, leucine and total aromatic amino acid (phenylalanine + tyrosine) levels were higher in cantaloupe, sweet melon and water melon compared to egg.

The relative amino acid patterns to egg protein for the cantaloupe, sweet melon and water melon seeds calculated by the methods suggested by FAO/WHO (1982) are presented in Table 5.

Table 5

Amino acid scores of some gourd kernels flour[a]

Amino acid	Optimal amino acid content[b] (mg per g N)	Gourd kernel		
		Cantaloupe	Sweet melon	Water melon
Threonine	320	113	147	168
Valine	428	80	92	77
Isoleucine	395	60	77	82
Leucine	549	115	115	90
Lysine	437	41	44	36
Phenylalanine	352	87	129	106
Tyrosine	260	107	139	113
Methionine	216	68	95	85
Cystine	152	14	64	42
Methionine + cystine	368	82	159	127
First limiting A.A.		lysine	lysine	lysine
Second limiting A.A.		isoleucine	isoleucine	valine

[a]: amino acid score = $\frac{\text{mg amino acid in 1 g N}}{\text{mg amino acid in whole egg}} \times 100$

[b]: whole hen's egg

The data revealed that all studied samples have fairly low valine values compared with the whole hen's egg. The most marked deficiencies were, however, obtained in isoleucine, lysine and the sulfur amino acids (methionine + cystine).

The essential amino acids, threonine leucine, and the aromatic amino acide (phenylalanine + tyrosine) were found to be in greater quantities than in egg. Therefore, gourd kernels meal could be used as a good source of these essential amino acids in food products.

For the determination of the order of limiting amino acids, the amino acid scores were worked out taking in account the suggested pattern of whole hen's egg as a basis for calculation (Table 5).

Lysine was the first limiting amino acid in cantaloupe, sweet melon and water melon kernel meals, while isoleucine was the second limiting amino acid in cantaloupe and sweet melon and valine in water melon seed protein.

It would appear that the protein of gourd kernel meal is not likely to be a good supplement of cereals whose most limiting acid is also lysine. Sweet melon seeds having a higher cystine + methionine level than other gourd seeds might be reasonable supplements of pulse protein whose sulfuric amino acid content is low and lysine content is high.

2.5. Electrophoretic patterns of kernel protein

The polyacrylamine gel electrophoresis of cantaloupe, sweet melon and water melon kernel proteins in sodium phosphate buffer is shown in Fig. 1. There are four protein bands in cantaloupe kernel protein. Only one band, No. 3, is that of the major protein fraction in the kernel. The concentration of the other three bands was small.

On the other hand, sweet melon and water melon kernel proteins had typical banding patterns with respect to the number of bands (seven bands) and migration distances. At the same time, water melon protein bands had a relatively higher intensity compared to the sweet melon protein bands. Electrophoresis of the polyacrylamide gel showed both the low and high molecular weight fractions of the studied kernel proteins. It was found that the studied kernel proteins were not clearly separated, there were smears and diffused bands and mainly of the low molecular weight polypeptides appearing in the lower region of the gel. To the best of our knowledge electrophoresis of cantaloupe sweet melon and water melon kernel proteins has not been reported previously.

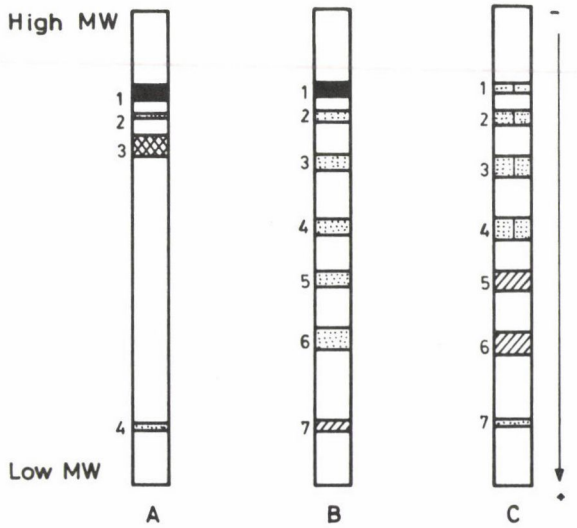
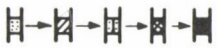


Fig. 1. Polyacrylamide gel electrophoresis of cantaloupe (A); sweet melon (B) and water melon (C) kernel proteins in sodium phosphate buffer of pH = 7.8. Note: colour density was increased as:



3. Conclusion

The conclusion of this study is that cantaloupe, sweet melon and water melon kernel meals could supply great amounts of total nitrogen required for the synthesis of dietary indispensable amino acids. It might also supply considerable amounts of threonine, leucine, phenylalaine and tyrosine. The major amino acids in which gourds kernels are deficient are those commonly present in limited quantities in cereals (lysine) and legumes (methionine, cystine). This might reduce its value in completing the proteins derived from these sources.

Moreover, gourd kernels would constitute a valuable source of the major elements and of iron, zinc, manganese and copper. The PAGE pattern shows that the studied kernel proteins contain both low and high molecular weight protein fractions.

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EFFECT OF COOKING ON THE FATTY ACID COMPOSITION OF SILVER CARP (*HYPOPHTALMICHTIS MOLITRIX*, V.)

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The fatty acid composition, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents of the muscle tissues of raw, cooked and deep fat fried silver carp were determined. Boiled fish lost about eight percent of its oil content, but there was no significant difference between the remaining part of its fatty acid composition and that of the raw fish. During deep fat frying, either in the form of fillets or of minced patties, the loss of fish oil was about 30–40%, and the frying oil was significantly absorbed. The polyunsatured fatty acids (PUFA), EPA and DHA except the cooking wastes were surprisingly stable. The total PUFA content of silver carp was lower than earlier reported. For keeping the n-3 PUFA level boiling is recommended.

Keywords: fish oil, cooking, polyunsaturated fatty acids

Highly unsaturated omega-3 (n-3) fatty acids in marine lipids can be important in preventing or reducing certain premature heart diseases and inflammations. Seafoods and their important lipid components are increasingly popular as part of human diet. PIGGOTT and TUCKER (1987) published a review of this topic. Fishing methods, food processing and preparation affect the quality of oil reaching the consumer. The highly unsaturated n-3s are easily oxidized, destroyed or reduced by industrial, commercial or home practices.

MAI and co-workers (1978) determined the effects of cooking, baking, pan frying and deep fat frying on the lipid content and composition of fillets of lake trout (*Salvelinus namacush*), white sucker (*Catastomus commersoni*) and bluegill (*Lepomis macrochirus*). Lipid changes were the smallest in the fish fillets containing relatively high levels of lipids, e.g. trout, while species containing small amounts of lipids absorbed more cooking oil. Trout loses about 30% of its oil content during baking, and the decrease of polyene fatty acid content, among others DHA is of this value.

The effects of baking, broiling, deep frying and cooking in a microwave oven on the fatty acid composition of grouper (*Epinephelus morio*), red snapper (*Lutjanus campechanus*), Florida pompano (*Trachinotus carolinus*) and Spanish mackerel (*Scomberomorus maculatus*) were determined by GALL and his co-workers (1983). The lipid content of low fat species was not significantly changed by baking, broiling

or microwave cooking. Deep-fried fillets absorbed the major fatty acids in the cooking medium, and as the lipid content of the fillet increased the absorption of fatty acids from the cooking medium decreased.

MAEDA and co-workers (1985) investigated the effects of cooking on the fatty acid composition, especially on EPA and DHA content in sardines. The fatty acid composition of sardines remained unchanged after grilling and broiling. Grilling reduced EPA and DHA in sardines by 17% and 15%, respectively. These decreases were almost directly proportional to the decrease (20%) of total lipids in sardines. In the case of boiled sardines, EPA and DHA contents did not decrease significantly. EPA and DHA were found to be unexpectedly stable.

FERNANDEZ (1986) studied the fatty acid profile of canned tuna in water, or in oil, canned salmon in water, deep-fried, battered and breaded fish sticks, and the effect of deep fat frying of fresh cod fish fillets. In the product combination of n-3 degradation, leaching and dilution took place.

HEARN and his co-workers (1987) investigated the stability of fish oils containing great quantities of polyunsaturated fatty acids (PUFA) in fresh fish of small, medium and big fat content cooked in microwave oven. Cooked and uncooked portions of butterfish, mullet, mackerel and sardines were extracted, the lipid recovered and fatty acid composition determined. The effect of cooking was small with no detectable difference between cooked and uncooked samples. PUFA were virtually unaffected, the cooked fish retained the original PUFA composition and content.

The fatty acid composition of raw silver carp was studied by CSENGERI and co-workers (1978). In Hungary the commercially available fresh table fish, silver carp, contains the largest amount of n-3 PUFAs. The oil of silver carps on natural diet was reported to contain 17% DHA and 8.3% EPA. According to CZUCZY and GAÁL (1992) the EPA content is 6.2%, the DHA content 12.5%. Different canned silver carp dishes contain 82 to 375 mg of EPA/100 g and 155 to 696 mg DHA/100 g.

It has been purpose of this study to investigate the effect of different cooking technics (boiling, deep fat frying in fillets or minced patties) and on the basis of the results to propose cooking methods for silver carp in order to preserve the biologically valuable n-3 polyunsaturated fatty acids.

1. Materials and methods

1.1. Raw material

Six fresh silver carps were bought at the market. The beheaded and eviscerated fishes were halved in length, as the fatty acid composition of individual fishes can significantly differ. After removing the unedible parts, one half was cooked, the other half served as control. The six fishes were kept in three groups of two for the following treatments.

1.2. Cooking methods

1.2.1. Boiling. Half of the fishes was filleted and the two samples (ca 500 g) were put into 1000 cm^3 of hot tap water, containing no salt or any spice. The fish fillets were cooked for 10 min in a dish under a lid on an electric oven. After cooling and removal of the bones the fillets were minced with a "Predom-Zelmer type 86" mincing-machine, applying a d = 4 mm disk to homogenize the sample.

1.2.2. Deep fat frying in fillet form. The filleted half fishes (sample weight ca 500 g) were fried in separate pans in 700 g of sunflower oil (HUNGARIAN STANDARD, 1983) for 15 min. The temperature of the oil was 160 °C. After cooling and removal of the bones the fillets were minced like the boiled fish.

1.2.3. Frying of minced patties. After mincing, patties were formed without eggs and bread crumb. They were fried according to 1.2.2. After cooling, the patties were homogenized by repeated mincing to homogenize the lipid content before sampling.

1.3. Preparation of the lipid samples

Lipids were extracted from raw and cooked meat after digestion with HCl (A.O.A.C., 1975).

1.4. Preparation of fatty acid methyl esters

After weighting and diluting with a known amount of chloroform, an aliquot of the lipid sample containing 7 mg of fish oil was transferred to a screw capped vial and the solvent evaporated under an N_2 stream. After saponification with 0.5 mol l^{-1} KOH/methyl alcohol, the sample was esterified with BF_3/methyl alcohol. The esters were salted out with saturated NaCl solution into n-heptane dried on sodium sulfate (HUNGARIAN STANDARD, 1979); 0.3 μl was used for chromatography.

1.5. Gas chromatographic investigation of fatty acid methyl esters

Chromatographic separation was performed on a Hewlett-Packard 5720A chromatograph equipped with FID and HP 3392A type integrator. The chromatographic conditions found best for fish oils were as follows:
Column: 0.75 mm i.d., 30 m long, wide bore borosilicate capillary column with 1 μm thick Supelcowax 10 film.
Carrier gas: hydrogen, linear velocity 30 cm sec^{-1}.
Temperature program: 180 °C – 4 °C min^{-1} – up to 220 °C final temperature.

1.6. Identification of the components

For the identification of fatty acid methyl esters standard FAMEs (Chrompack, the Netherland) and standard fish oil mixtures (Supelco, Inc.) were used. In addition the unsaturated components of silver carp oil were determined by hydrogenation, the number of double bonds of components was obtained by thin layer chromatographic (TCL) separation according to MANGOLD and ZWEIG (1984), by a modification of the eluent system. The collected TLC fractions were again rechromatographed on the GC. The log corrected retention time – carbon number plots were also taken into consideration.

2. Results

Twenty seven of the 44 components detected in fish oil extracted from raw silver carp meat were identified, amounting to 93–97% of the total oil. Results are summarized in Table 1.

Table 1 shows that the composition of the oils of individual fishes is different, so the effect of treatments can be easier detected by comparing the same individuals before and after cooking. The following tables contain the data of single fishes and not an average of parallel treatments.

The fatty acid composition of the pair of one raw and one boiled sample is shown in Table 2, while the pair of one raw and one deep fat fried sample in Table 3.

Table 1

Fatty acid composition of raw silver carp

Peak No.	Fatty acid	$\bar{x}$ (%)	$\pm s$ (%)	v (%)
3	C12:0	0.35	0.03	8.18
6	C14:0	4.30	0.26	6.04
8	C15:0	1.51	0.16	10.60
11	C16:0	20.17	0.81	4.04
12	C16:1	11.30	0.96	8.46
14	C16:2 + + C17:0	1.59	0.13	8.22
15	C17:1	1.42	0.09	6.24
15a	C16:3	1.10	0.19	17.38
16	C16:4	0.52	0.11	20.47
17	C18:0	3.33	0.27	8.24
18	C18:1	22.09	1.83	8.30
19	C18:2	2.98	0.20	6.68
23	C18:3	5.52	0.41	7.46
24	C18:4	0.42	0.01	2.94
26	C20:1	1.78	0.13	7.15
28	C20:2	0.59	0.07	11.11
30	C20:3	0.53	0.04	6.69
31	C20:4	2.08	0.27	13.10
32	C20:3	0.70	0.08	11.88
33	C20:4	1.18	0.08	7.01
34	C20:5 + + C22:0	4.03	0.43	10.76
36	C22:4	0.21	0.04	17.91
37	C22:5	0.86	0.14	16.60
38	C22:5	1.12	0.15	13.08
39	C22:6	3.46	0.62	17.96

$\bar{x}$: mean value of 6 individuals (48 determinations)
$\pm s$: standard deviation

The chromatograms of fatty acid methyl esters of raw, boiled and deep fat fried silver carp are shown in Fig. 1.

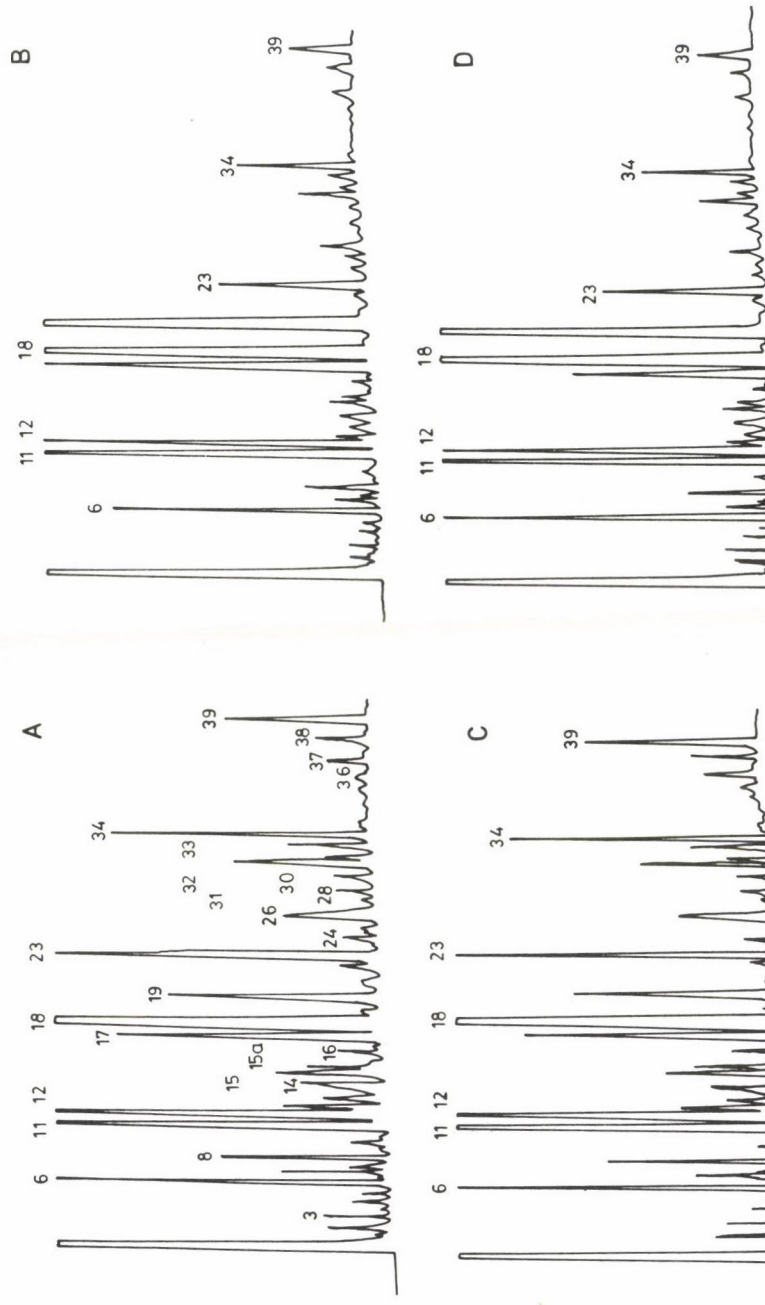

Fig. 1. Chromatogram of fatty acid methyl esters of silver carp. A: raw, B: fried in fillets, C: boiled and D: fried in patties. Peak numbers: see Table 1. GC conditions: 30 m long, 0,75 mm i.d. wide bore capillary column, coated with Supelcowax 10, temperature program: 180 °C – 4 °C min^{-1} – 220 °C

Table 2

The effect of boiling on the fatty acid composition of silver carp

Peak No.	Fatty acid	Raw 1			Boiled 1			t calculated
		$\bar{x}$ (%)	$\pm s$ (%)	v (%)	$\bar{x}$ (%)	$\pm s$ (%)	v (%)	
3	C12:0	0.33	0.04	12.98	0.25	0.03	13.10	−3.05*
6	C14:0	4.21	0.32	7.58	3.58	0.29	8.05	−2.91*
8	C15:0	1.36	0.07	5.09	1.20	0.06	5.25	−3.27*
11	C16:0	21.20	0.59	2.77	20.07	0.46	2.29	−3.04*
12	C16:1	12.87	0.51	3.99	11.89	0.17	1.43	−3.64*
14	C16:2 + + C17:0	1.36	0.07	4.87	1.34	0.03	2.49	−0.48
15	C17:1	1.39	0.03	2.12	1.36	0.02	1.76	−1.67
15a	C16:3	1.02	0.04	3.83	0.98	0.02	2.30	−1.67
16	C16:4	0.52	0.03	5.72	0.50	0.03	5.05	−1.36
17	C18:0	3.06	0.09	2.87	3.22	0.11	3.47	2.15
18	C18:1	24.14	0.37	1.53	24.61	0.68	2.75	1.22
19	C18:2	2.75	0.10	3.56	2.42	0.87	35.86	−0.74
23	C18:3	5.15	0.09	1.70	5.34	0.13	2.41	2.46*
24	C18:4	0.41	0.03	6.72	0.41	0.01	3.53	0.23
26	C20:1	1.60	0.30	18.61	1.89	0.07	3.53	1.92
28	C20:2	0.53	0.05	9.79	0.71	0.08	11.09	3.85**
30	C20:3	0.48	0.03	6.21	0.56	0.05	8.32	3.09*
31	C20:4	1.67	0.09	5.54	1.90	0.06	3.27	4.14**
32	C20:3	0.57	0.03	5.87	0.63	0.03	4.39	2.49*
33	C20:4	1.10	0.06	5.90	1.26	0.10	7.51	2.92*
34	C20:5 + + C22:0	3.50	0.23	6.58	3.88	0.10	2.68	2.98*
36	C22:4	0.18	0.04	20.93	0.28	0.11	39.65	1.71
37	C22:5	0.68	0.07	10.85	0.84	0.08	9.26	2.86*
38	C22:5	0.98	0.10	10.00	1.18	0.07	5.55	3.41*
39	C22:6	2.63	0.28	10.70	3.25	0.18	5.61	3.72**

$\bar{x}$: mean value of 8 deternibations

$\pm s$: standard deviation

$t_{5\%} = 2.45, t_{1\%} = 3.71, t_{0.1\%} = 5.96$

Table 3

The effect of deep fat frying on the fatty acid composition of silver carp fillets

Peak No.	Fatty acid	Raw 3			Fried 3		
		$\bar{x}$ (%)	±s (%)	v (%)	$\bar{x}$ (%)	±s (%)	v (%)
3	C12:0	0.35	0.05	15.55	0.10	0.01	13.35
6	C14:0	4.26	0.41	9.73	1.44	0.13	8.70
8	C15:0	1.38	0.10	7.30	0.47	0.04	8.14
11	C16:0	19.39	0.59	3.04	11.46	0.32	2.82
12	C16:1	10.41	0.28	2.74	3.52	0.26	7.48
14	C16:2 + + C17:0	1.67	0.01	0.79	0.57	0.04	7.75
15	C17:1	1.37	0.02	1.33	0.46	0.03	7.38
15a	C16:3	1.02	0.01	0.69	0.33	0.02	7.25
16	C16:4	0.47	0.01	1.12	0.14	0.02	14.07
17	C18:0	3.73	0.09	2.36	4.57	0.07	1.58
18	C18:1	21.88	0.58	2.63	22.32	0.18	0.83
19	C18:2	3.02	0.20	6.69	43.31	1.19	2.75
23	C18:3	5.31	0.19	3.67	1.79	0.13	7.37
24	C18:4	0.43	0.02	3.53	0.14	0.02	13.16
26	C20:1	1.94	0.08	4.19	0.74	0.05	6.40
28	C20:2	0.54	0.03	5.30	0.19	0.02	11.26
30	C20:3	0.58	0.02	3.48	0.18	0.02	12.86
31	C20:4	2.17	0.07	3.10	0.84	0.05	5.87
32	C20:3	0.70	0.04	6.08	0.23	0.01	5.25
33	C20:4	1.23	0.06	4.84	0.38	0.04	9.35
34	C20:5 + + C22:0	4.35	0.11	2.52	1.99	0.09	4.36
36	C22:4	0.27	0.05	18.15	0.09	0.02	20.67
37	C22:5	0.96	0.04	4.32	0.37	0.02	4.88
38	C22:5	1.24	0.06	4.54	0.59	0.02	4.02
39	C22:6	3.99	0.14	3.52	1.59	0.10	6.30

$\bar{x}$: mean value of 8 determinations
±s: standard deviation

Boiling caused an 8% loss of fish oil, but the remaining part did not show a marked difference in composition as determined by the Student's *t* test. Table 2 shows significant but unimportant changes. On the contrary, the fish oil in the cooking water has lost much of its polyunsaturated acids. Decrease of the concentrations of C16:3, C18:2, C18:3, C20:4, C20:5 and C22:6 acids is very strongly significant ($t_{99.9\%} = 5.96$, while the calculated *t* values are as follows: 26.6, 11.8, 38.5, 20.3, 16.1 and 12.5).

From the average quotient of concentrations of individual fatty acids in the oil of fried and raw fish not occurring in frying oil, the part of oil originating from the

fish was calculated. According to this calculation fish meat absorbs a very significant amount of frying oil both in slices and patties. About 50% of the oil content of fried fish came from frying fat.

Table 4 shows the EPA and DHA content and loss of different samples.

Table 4

EPA and DHA content of raw and cooked silver carp and losses during boiling and deep fat frying

Sample[a]	EPA (g per 100 g d.m.)	DHA (g per 100 g d.m.)	Loss of EPA (%)	Loss of DHA (%)	EPA (g per 100 g)[b]	DHA (g per 100 g)[b]
1. raw	1.55	1.16			0.41	0.31
1. boiled	1.57	1.32	–	–	0.47	0.40
2. raw	1.01	0.91			0.24	0.22
2. boiled	0.94	0.83	6.9	8.8	0.26	0.23
3. raw	0.97	0.77			0.26	0.20
3. fried fillets	0.73	0.59	25	23	0.22	0.18
4. raw	1.08	0.99			0.25	0.23
4. fried fillets	0.80	0.64	26	35	0.27	0.22
5. raw	1.12	1.04			0.24	0.23
5. fried patties	0.81	0.62	28	40	0.27	0.21
6. raw	1.21	1.01			0.31	0.25
6. fried patties	0.87	0.67	28	34	0.29	0.22

[a]: numbering refers to the individuals tested (see paragraphs 1.1 and 1.2.)
[b]: in the 100 g of the raw or cooked fish (not d.m.)

The decrease of EPA and DHA concentrations are the results of diffusion of fish oil into the frying medium. EPA and DHA in fish meat seem to be stable during normal cooking procedures.

3. Conclusion

From the 44 components detected in the oil of silver carp 27 were identified, amounting to 93–97% of the total oil. The fatty acid composition of the tested individuals has shown on the average a v = 11%. The EPA and DHA content was found to be much lower than reported by CSENGERI and co-workers (1978) but similar to the data of CZUCZY and GAÁL (1992).

As the effect of boiling an eight percent loss of fish oil was found but the remaining part did not differ remarkably from that of raw fish. The fish oil flowing from the fish slices into the water was less stable with respect to its polyunsaturated acid content. Deep fat frying caused an about 30–40% loss of fish oil, and the same decrease of EPA and DHA due to the diffusion of fish lipids into the frying medium. Fish meat absorbed the frying sunflower oil. EPA and DHA were relatively heat-resistant during cooking.

Considering the recommendations of WHO on daily polyunsatured fatty acid intake (1–2 g of n-3 fatty acids) it would be necessary to consume about 250–500 g cooked silver carp every day. This agrees with the results of CZUCZY and GAÁL (1992). In agreement with our experiments it is recommended to prepare the silver carp by boiling in order to conserve its valuable polyunsaturated n-3 fatty acids.

*

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MODIFIED CONDUCTOMETRIC METHOD FOR THE DETERMINATION OF THE FOAMING PROPERTIES OF FOOD PROTEIN PREPARATIONS

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The foaming properties of six protein isolates obtained from wheat barley and maize germs, pea, soy and lentil, and four commercial proteins (bovine serum albumin = BSA, casein, ovalbumin and lysozyme) were studied by conductometric and volumetric methods. A relatively small correlation (r = 0.67) was found between the foaming properties obtained by the conductometric method of KATO and co-workers (1983) compared with those obtained volumetrically. An improvement of correlation was achieved by the modification of the evaluation of conductometric curves. The ratio of the maximum value of the conductometric curve (c_{max}) and the time needed to reach the maximum (t_{max}) gives a significantly higher correlation (r = 0.93) compared to the data of the volumetric method.

The correlation of foam stability data obtained with the two methods can be improved also by the comparison of the ratio of volume (V_o) at maximum value and after 10 min rest time (V_{10}).

Keywords: protein, foaming properties, conductometric method of determination of foaming properties

In the processing of food products different protein preparations are widely used. Their application includes a wide range of substitutions, such as components that cause little or no change in functions of those which modify some textural and other properties of food products. The use of protein preparations requires a thorough knowledge of their properties and impact on the quality of products. As a result, much attention is paid to the investigation and measuring of functional properties. Foaming of whipping properties, i.e. the capacity to form stable foams with air (or other gas) is an important function of protein preparations. Many food products are foams from a colloid-chemical point of view (e.g. whipped cream and toppings, souffles, confectionary products, etc.). A variety of methods have been proposed to produce and characterize protein foams. Reviews (KINSELLA 1976, GASSMANN et al., 1987) describe mainly three procedures for the determination of the foaming capacity of protein including whipping, shaking or sparkling (WANISHEN & KINSELLA, 1979). One important difference between these methods is the amounts of protein required for foam production. The amount of protein ranges from 3% to

40% for whipping about 1% for shaking and 0.01% to 2% for spraying. The foamingbility of proteins is generally a measure of volume and stability. The effect of different conditions on foaming was also studied (e.g. GÁBOR, 1990; 1992). A new method was established to estimate the foaming properties of proteins with a simple apparatus consisting of a glass column and a conductivity cell (KATO et al., 1983). A close correlation was observed between the initial conductivity of foams and the foam volume of 11 proteins. In addition, a close correlation was also found between foam stability determined by changes in conductivity and foam volume. These data suggest that foam stability can be estimated from changes in the conductivity of foams, too.

The aim of our research was a comparative study of conductometric and common volumetric methods used for the determination of the foaming properties of protein preparations.

1. Materials and methods

Six protein isolates gained from wheat barley and corn germs, pea, lentil and soybean and from four commercial proteins (ovalbumin, bovin serum albumin = BSA, casein and lysozyme) were investigated. The barley and corn germ protein isolates were prepared by the method of NIELSEN and co-workers (1973), and the pea and lentil protein isolates by HSU and co-workers (1982). Soy protein isolate was prepared according to the method of CHARALAMBOUS and DOXASTAKIS (1989) and wheat germ protein isolate by the procedure of KORPÁCZY (1976). Ovalbumin, BSA, casein and lysozyme were products of Reanal Fine Chemical Factory, Budapest, Hungary.

1.1. Determination of foaming properties

The volumetric and conductometric methods of KATO and co-workers (1983) were slightly modificated. The same apparatus (Fig. 1) was used for both methods. Protein solutions of 0.1% (w/v) were dissolved in 0.1 mol l^{-1} phosphate buffer (pH = 7.4) and aerated for 2 min with an air flow of 90 cm^3 min^{-1}. The volume of the foam (V_o) was determined immediately after aeration and after 10 min.

The conductometric curves were observed under the same foaming conditions as above. The value of conductivity read from the recorded curve (c_i) immediately after stopping aeration was used for description of the foaming power (*FP*).

The foam stability (*FS*) of the volumetric method was expressed as follows:

$$FS = V_o \Delta t / \Delta V$$

where ΔV = decrease of foam volume as a function of time (Δt).

The *FS* for the conductometric method was calculated according to KATO and co-workers (1983) as follows:

$$FS = c_0 \, \Delta t / \Delta c$$

where c_0 = extrapolated value of conductivity to zero based on the linear segment of the conductivity–time curve (Fig. 2).

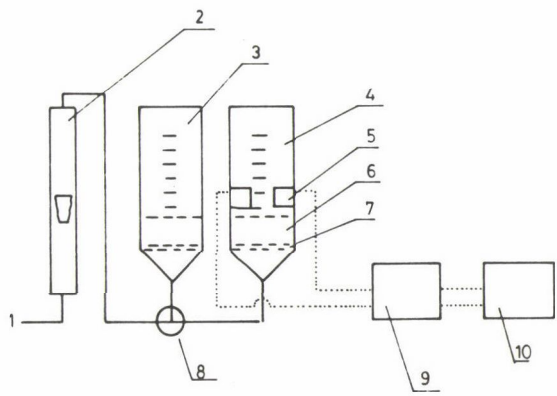

Fig. 1. Apparatus for measuring the foam properties. 1: Air (90 cm^3 min^{-1}; 2 min); 2: rotameter; 3: cylinder with water; 4: conductivity cell; 5: electrode (dist.: 1 cm); 6: protein solution (0.2%); 7: glass-filter (P4); 8: valve; 9: conductometer; 10: recorder

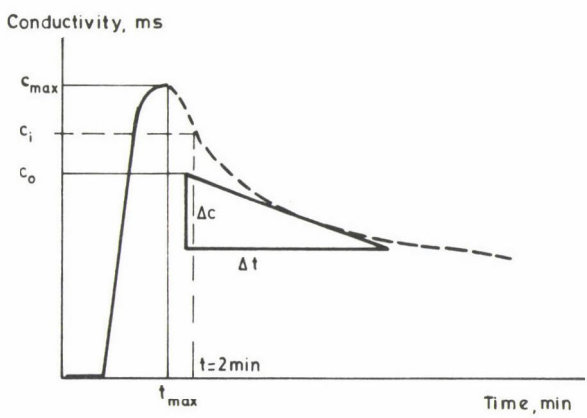

Fig. 2. General view of the conductometric curve. c_{max}: conductivity max during foam formation; t_{max}: time needed to reach c_{max}

2. Results and discussion

Table 1

Foam power of solutions of different protein preparations

Protein preparation	V_o (cm^3)		c_i(mS)		c_{max}/t_{max} (mS min^{-1})	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Barley germ protein	40.00	1.20	1.11	0.02	1.74	0.22
Pea protein	43.29	0.78	1.00	0.07	1.87	0.08
Wheat germ protein	15.86	0.11	0.59	0.09	0.30	0.5
Maize germ protein	46.42	1.22	1.24	0.06	2.09	0.20
Lentil protein	42.38	1.14	0.94	0.08	2.28	0.18
Soybean protein	45.86	2.18	1.37	0.08	1.99	0.09
Ovalbumin	31.32	1.14	1.12	0.08	1.40	0.11
Bovine serum albumin	40.39	2.62	2.90	0.20	2.42	0.20
Lysozyme	2.50	0.00	0.30	0.00	0.54	0.06
Casein	53.67	0.29	2.03	0.07	3.09	0.05
Correlation with volumetric method	– 93		0.67*		0.93**	

*: Significant at P = 95% probability level
**: Highly significant at P = 99% probability level

The FP values of protein preparations determined by volumetric and conductometric methods are summarized in Table 1. The volumetric method showed that casein and BSA have the best foaming properties. Good foaming properties were observed with maize germ, soy, pea and barley germ proteins and ovalbumin. Poor foaming properties were found with wheat germ protein and lysozyme. This ranking is of course valid only for the conditions used in experiments. With the conductometric method (c_i values) the protein can be similarly graded. Nevertheless, within the groups some changes in the order of proteins occurred. Calculation of the linear correlation between the data of the methods showed a value of 0.67.

The possibility of finding an improvement of correlation between the two methods was examined. The ratio of maximal conductivity (c_{max}) and the time needed to reach this conductivity (t_{max}) was used to characterize *FP* and compare it with the volumetric data.

The correlation coefficient comparing V_o and c_{max}/t_{max} values was high (r = 0.93). This is shown in Fig. 3.

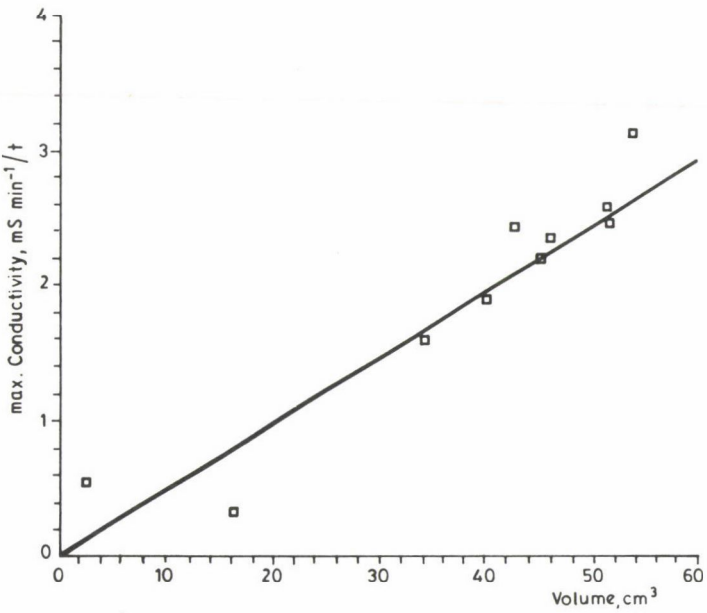

Fig. 3. Relationship between foam power of protein solution determined by modifyed conductivity and volumetric methods

The foam stability data are presented in Table 2. Maize germ, soy and lentil protein showed the best stability and wheat germ the worth properties. Stability data calculated on the basis of the volumetric method ($FS = V_0 \cdot \Delta t/\Delta V$) are also given in Table 2. The correlation between the data measured with the two methods is very small. We tried to find a way to improve correlation by means of a modified evaluation procedure. It was found that using a volumetric method for characterization of FS a ratio of V_0 and V_{10} (foam volume ten minutes after stopping aeration greatly improves correlation (Table 2)).

Table 2

Foam stability (FS) of six protein preparations determined by conductometric and modified volumetric methods

Protein	Foam stability					
	Conductometric $FS = c_0 \Delta t/\Delta c$ (mS)		Volumetric $FS = V_0 \Delta t/\Delta V$ (min)		Volumetric $FS = V_{10}//V_0$ 100 (%)	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Barley germ protein	0.98	0.11	3	1.5	92	3
Pea protein	0.83	0.07	82	6	80	7
Wheat germ protein	0.38	0.05	10	3	28	4
Maize germ protein	0.95	0.15	98	7	98	5
Lentil protein	1.06	0.08	61	4	42	4
Soybean protein	1.2	0.09	68	6	92	6
Ovalbumin	0.65	0.08	71	3	65	4
Bovine serum albumine	0.7	0.09	63	9	70	5
Lysozyme	0	–	0	–	0	–
Caseine	0.7	0.05	67	5	75	3
Correlation with conductometric method			0.62 $^-$		0.82*	

$^-$: not significant at 95% level

*: significant at P = 95% probability level

Nevertheless, further experiments are needed to find a reliable comparison of *FS* values determined with volumetric and conductometric methods.

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ABSTRACTS
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PRODUCTION AND CONTROL OF NEW NATURAL FOOD PRODUCTS

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Foods consumed these days differ greatly from those used a century ago and even from those consumed a couple of years ago.

In the protection of human health against environmental damages and prevention against pollution caused illnesses the movement "back to nature" having started in the eighties in Western countries, has lead to the production of natural foods creating a new kind of food concept.

Akadémiai Kiadó, Budapest

This paper deals with the principles of quality regulations and of control of raw materials of vegetal and of animal origin usable in the production of natural foods, and with the permitted food-technological procedures, as part of the laws for food.

It is necessary to work out the definitions of these new products and label them according to European specifications.

PRODUCTION OF CEREAL PRODUCTS WITH FULL NUTRITIVE VALUES

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During milling (husking and polishing i.e. milling to a white flour as end-product) the most valuable components from the aspects of nutrition-biology, the high vegetable oil, vitamin and mineral content of the corn, the skin rich in fiber are all lost. A significant part of the biologically very valuable aleuron protein fractions is removed with the bran. There is a further loss in nutritives during the application of cooking technologies.

To protect health, it is highly important that the basic food, the cereals, should be protected as much as possible during transformation, in order to preserve their original, natural nutritives. In milling technologies new product variants can be produced with the utilization of the possibilities of grading and with the propagation of sound nutrition, enriching the former cereal product assortment. The results obtained with the simplification of milling should be maintained.

The whole milled cereal products, broken particulate products, darker flours with high fiber content, various flour mixtures, are of much higher biological value than the white refined flour products. With the use of new processing technologies, new product types with a presentation differing from the traditional appearance but containing almost fully the valuable components of the grain, cereal flakes, puffed cereal products, extruded goods can be prepared. The special processing procedures applied in the milling industry and their effects on the composition of cereals are dealt with.

MODERN METHODS FOR THE MIXING AND CONDITIONING OF COARSE AND FLOURY CEREAL MATERIALS

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Mixing of bulk solids is one of the most frequently applied operation in the manufacture of foods and feedstuffs. Aim of the mixing is to produce a final product of appropriate homogeneity from raw materials of various particle sizes and properties. The significant differences in the physical-chemical properties of basic materials and in their application have resulted in a wide range of technological and technical solutions. The development trends of this field can be characterized by a better utilization of the results of electronics and computers, with a wider application of new structural materials, and at the same time, the introduction of improved equipment based on new technical solutions.

The results of research and development activity of the Departments of General and Analytical Chemistry and Agricultural Chemical Technology of Technical University of Budapest, provide examples for this trend of development. The new solutions realized up to now can be grouped around three topics, as follows:

1. Changing to computer aided manufacture (CAM) from traditional mixing in the field of large-scale industrial feed production. This change can be carried out at low cost and within a short time, and facilitates the control of feed mixing plants using different recipes, diet formulas, reducing the possibilities of human errors to a minimum, and resulting in a 20–80 percent growth of capacity.

2. The planning and realization of new computerized mixing plants appropriate to solve very sensitive mixing tasks working with various basic materials and formulas, mainly in food industry. New equipment has been developed for the transport, dosage and packaging of bulk materials.

3. The development of patented continuous mixing equipment, and the elaboration and realization of related (patented) operations for the continuous production of a solid multi-component mixture with dosage according to weight, and for the continuous transport of one or more liquid components to the surface of a solid carrier in an appropriate quantity, while the end-product should preserve its solid state. These kinds of tasks are met more and more frequently in food industry as well as in feed production: enrichment with fat, with molasses, with lecithin, introduction of flavouring and fragrante substances, adjustment of humidity content (conditioning).

TRANSMISSION NEAR INFRARED SPECTROSCOPY (NIR) FOR THE TECHNOLOGICAL CONTROL OF THE PROCESSING OF CORN GERMS

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For monitoring the industrial processing realized for the mild transformation of corn germs produced by wet procedure (De Smet Rosedowns), technological quality control was carried out by NIR spectroscopic measurement. Selective and combined calibrations were developed for each group of material to determine the chemical components (moisture, protein and oil content) of the whole germ, of press cakes and of intermediates. With help of calibration models, the three main constituents could be determined with an accuracy of 0.1, 0.3 and 0.5%, corresponding to a relative error of 1.2–2.3%. By optimizing the sampling and measuring conditions, a quality control system has been elaborated producing analytical results after some minutes of measurement and thus, the technological control of raw materials, intermediates and final products can be monitored semi continuously. The economical advantages of the use of infrared technique have been discussed.

HEAT TREATMENT OF SOY BEANS WITH RADIO FREQUENCY

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In 1986, a research project started to increase the domestic protein feed bases with the help of the National Committee for Technical Development (OMFB) by dielectric radio frequency treatment of soy bean. In this paper the test results and laboratory investigations are described.

The followings have been proved by technological tests and laboratory essays:

– The trypsin inhibitor activity of soy bean can be reduced by radio frequency treatment below the permitted limit (10 TIU mg^{-1} value), when its protein nutrient content changed favourably.

– This technology needs the least specific energy supply compared to other methods used commercially in Hungary and shows various other beneficial characteristics, too.

Based on the successes the establishment of a reference plant, constructed of home made elements, was decided with the help of the Central Food Research Institute, the OMFB, the Ministry of Agriculture and the Cereal Industrial Trust.

The paper continues by describing the reference plant of the soy treatment, the experiences with radio frequency equipment, the main economic and technical parameters and the general experiences with dielectric radio frequency heating applied to other technological processes.

ANALYSIS OF PROCESSED MAIZE AND POTATO STARCH BY MEANS OF HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

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HPLC analysis of mixtures containing cyclodextrins (CD), glucose, maltose, short chain dextrins and malto-oligosaccharides of polymerization degree (Dp) > 10 have been studied. The aim was the characterization of bioconversions involving a multistep process, namely prehydrolysis of starch by alpha-amylase, the subsequent cyclization by cyclodextrin-glycosiltransferase and then the physical separation of CDs from substrate and by-products using ultrafiltration and crystallization.

On the basis of earlier data the separations of oligosaccharides of Dp > 3 and CDs were performed on gel beds, mostly on ion-exchange resins. Separation was based on ion exchange, etc. effects.

Bio-Rad aminex HPX-42A column was used for the analysis of the processed starch composition, that is the CD and malto-oligosaccharide (up to Dp = 10) content.

The chromatographic equipment was an LKB-Pharmacia modular HPLC system, with differential refractometer detector. The chromatographic column operated at ambient temperature (70 °C), with eluent glass distilled water, flow rate 0.5 $cm^3\ min^{-1}$. The samples did not require any pretreatment, they could be directly injected into the column after a double distilledwater dilution. Total analysis time was less than 30 min.

Data characteristic of chromatographic performance and separation parameters N_{eff}, H_{eff} corresponded with separation effectivity data in the literature.

The system proved to be suitable for the qualification of starch solutions, having cyclic and linear carbon-chain dextrin content.

PRODUCTION OF ESSENTIAL OIL BY SUPERCRITICAL EXTRACTION

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Supercritical extraction is based on the observation, that the solving capacity of gases is much higher and the selectivity much finer in the domain above their critical pressure and temperature. From the starting material in solid or liquid state, mainly the desired components are extracted at an appropriately chosen pressure and temperature. For the extraction, mostly carbon dioxide is used as solvent due to its most favourable characteristics (not harmful to health, cheep, non-inflammable, non-polluting). The great advantage of supercritical extraction is the obtaining of much purer products of higher quality, compared to the extraction carried out with traditional aromatic or chlorinated solvents. It is possible to obtain with it numerous natural substances which are all decomposed by the traditional separation operations. Due to these beneficial properties the supercritical extraction is industrially used in the highly developed countries in various domains of food, pharmaceutical, cosmetical and chemical industry.

The production of essential oils is traditionally carried out with steam distillation. The heat-sensitive components are partially decomposed as an effect of the relatively high temperatures. Essential oils with components nearer to the natural composition can be produced with an extraction made with carbon dioxide solvent at a medium temperature (max 40 °C). Different plants including lavender, thyme and fennel were extracted at different conditions, in a high pressure apparatus with 5 dm^3 extractor vessel volume.

OXIDATIVE STABILITY OF VEGETABLE OILS REFINED BY VARIOUS TECHNOLOGIES

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The oxidative stability of sunflower oils refined with alkaline technology and with up-to-date physical refining were compared during heat treatment at high temperature in the presence of air.

Heating effect was studied at two characteristic temperatures (180 and 190 °C), potato chips as model as either fried continuously or in batches. The processes were monitored with modern GC, HPLC, spectroscopic and classical methods. According to the results, the oxidative degradation of oils refined with various technologies were similar during heating and frying. The total polar compound content of 25%, being admissible from nutritional aspects, was attained in both kinds of oils as an effect of heating for 24 h at a temperature of 190 °C. In the continuous frying procedure the degradation of oils has been observed to be significantly lower than when frying is interrupted for some time.

REFINING OF VEGETABLE OILS WITH SILICA ADSORBENTS

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The application of synthetic silica adsorbents in vegetable oil processing has been studied. It was found in the extensive laboratory model trials that the silica adsorbents removed with high efficiency the phosphatide, soap and metal content of vegetable oils. For these accompanying and contaminating substances, the capacity of the silica adsorbent surpassed the adsorptive capacity of acid-activated bleaching earth used in the traditional technology. The silica adsorbent – fitted appropriately into the technological process – can be effectively used both in traditional alkali and in physical refining technologies.

In contrast to bleaching earth the silica adsorbent is not suitable for the complete removal of plant colourings, thus e.g. in the refining of rapeseed oil containing chlorophyll the combined use of the two kinds of adsorbents is needed.

The refining carried out with silica adsorbent is a gentle treatment and the oxidative stability of the oils in case of those susceptible to autooxidation is not damaged even in case of efficient bleaching.

SOME EXPERIENCES WITH THE ROASTING OF COFFEE MIXTURES

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In order to substitute the expensive and imported coffee, and due to a more advantageous price, coffee mixtures (the mixture of roasted coffee beans and coffee substitutes) are popular in the circle of consumers and of processors as well, and consumption is rising steadily. An important characteristic of coffee mixture product is, that a coffee drink should be prepared without any change with the traditional coffee machine.

The effect of technological parameters influencing the quality of barley-based coffee mixtures were studied (pre-treatment, roasting, grounding). It has been found, that during roasting, the starch content of barley gets decomposed and becomes soluble in cold water. This affects the viscosity of the extract made of this material.

The particle distribution of roasted ground barley depends – presumed an identical breaking energy – on the roasting time, and this is one of the most critical parameters playing role in the preparation of the coffee drink. A method has been elaborated with derivatographic tests and model roasting trials for the shortening of the roasting time of barleys.

It has been found that the greatest part of the heat used for roasting is required for the evaporation of bound water. Separating the drying process and applying the remainder heat, the roasting can be effectuated during half of the time needed in the normal procedure, ensuring the same high product quality.

DEVELOPMENT AND TECHNOLOGICAL APPLICATION OF A SOFTWARE SYSTEM IN HEAT TREATMENT

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A unique, communicative software system has been discussed, summarizing the numerous different softwares developed to solve various tasks related to heat processing. The programs written formerly in Commodore and IBM BASIC as well as in Turbo Pascal computer languages, were improved to Lotus 1-2-3 R3.1 MACRO language. The new system gives more emphasis on the aspects of practical use, as well.

A part of the system is the program which generates different technological alternatives on heat processing equipments. This part is completed with the most up-to-date divided hydrostatic sterilizer types. Beside the former 6 Hunister types the s.c. "large can" Hunister and the "Novoster" are included. The principles of rapid generation of technological alternatives for tunnel pasteurizers and retorts are also given.

An other part of the system is the program for evaluation of the heat penetration data measured and collected by fix or mobile type of heat penetrometers. Data presentation are in table forms and in graphs.

The program for the simulation of the effects of different heat processing technologies on the microbiological and quality status of heat treated food is now fitted into the package.

A very important goal is to prepare a uniform data bank usable in practice for the technological engineer until the national network joining the computer system of the individual companies would be built, and to elaborate a comprehensive program system for heat treatment of foods.

MODERNIZATION OF THE TECHNOLOGY OF PRE-COOKING

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In the Department of Refrigeration and Livestock Products Technology of the University of Horticulture and Food Industry, as a comprehensive research project, the modernization of blanching technology has been studied. A great progress in the development of pre-cooking technologies could be observed in recent years. Beside new technical solutions, milder technological procedures also aim at the improvement of quality. The following studies aimed at this goal:

- the effect of the blanching method on product quality,
- the limit of these parameters (temperature, time) in pre-cooking can be decreased, with consideration of quality and of shelf-life.

The samples for the trials were prepared under industrial conditions in the Békéscsaba Frozen Enterprise. The raw materials were: green peas, cauliflower and carrot. Blanching was carried out with water and with steam and the quality of vegetables studied in a Cabinplant equipment (IQB blancher). The blanching parameters used in industrial practice were applied, but there were trials for the testing of quality parameters of the products blanched at lower temperature and with shorter treatment periods as well.

The enzyme inactivating effect of blanching the degree of bleaching with the change of dry matter content, and for carrots the reduction of nitrate content were tested. From the quality characteristics colour and texture were measured instrumentally. Sensory properties were judged by organoleptic testing.

Test results proved, that the blanching parameters used in industry are guaranteed. The reduction of blanching temperature and shortening of blanching period are realizable and the combination of these two factores can be applied. Water blanching bleaches soluble substances more than steam, and in consequence, water gets more contaminated. The favourable effect of blanching with steam is lost with the cooling of the product in water flow.

The measurements after blanching and freezing showed some differences as a function of blanching, but during storage these values approach each other.

The reduction of blanching parameters can be done only where the technical level of the equipment used enables the rapid adjustments, the control of the whole process. Favourable effects were experienced with the Cabinplant equipment made

in Denmark, where temperature and treating period could be reliably controlled. It would be most beneficial for the quick frozen food industry, if this type of equipment could be used in a much wider circle.

USE OF REACTION KINETIC EQUATIONS FOR THE DESCRIPTION OF CONCENTRATION CHANGES OF 5-HYDROXY-METHYL-FURFURAL IN GRAPEFRUIT JUICE

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The increase of 5-HMF concentration in grapefruit juice was studied at four temperatures (90, 100, 110, 120 °C), and after five time periods (20, 40 ... 100 min), and with 5 – 12 parallel determinations.

Determination was carried out according to the Hungarian Standard (1974, MSZ 14842-74). After mixing the contents of two simultaneously heated ampoules, 5 – 12 parallel determinations were carried out from the mixture.

The results of the measurements were evaluated with relatively new methods. The object of the investigations was not the analysis of the chemical steps of 5-HMF formation, but the determination of kinetic constants belonging to the so-called empirical reaction kinetic equations with the use of new methods.

Evaluation methods were the following: search for a transformation, by minimizing the quotient of residual and error variances so that the time dependent change of the transformed concentration can be considered linear (F-test, test for linearity); the Durbin-Watson test (with the means of parallels); the test for homogeneity by Barlett's method; weighted and unweighted regression analyses with various conditions in the transformed initial value. From transformations $y = (A - A_0)^{1-n}$ (A = concentration, A_0 = characteristic value, n = exponent of reaction order, y = transformed concentration), the selection of $A_0 = 1$ mg dm^{-3} and $n = 0.31$ seemed to be the most appropriate.

The version determining a common, previously not known initial value with temperature dependent weight was found the most suitable of all regression analyses.

Thus, at four temperatures, four rate constants and one transformed initial value (near to zero) have been obtained. The correlation coefficients were not lower than 0.993. The mean activation energy between extreme temperatures was $E_a = 129.66$ kJ mol^{-1}, with ± 1.34 kJ mol^{-1} confidence intervals, and

$|k_r| = 1.0485.10^{-2}$ (mg dm^{-3})$^{0.69} \times$ s^{-1} rate constant at the reference temperature (T_r = 100 °C).

These values were obtained by the special application of the least squares method (KÖRMENDY, 1991, *Acta Alimentaria*, *20*, 269–283).

Retransformation of a value on the regression line, if it is considered to be the expectation value of a symmetric (e.g. normal) distribution, will give the median of the distribution of the original concentration.

The concentration curves are characteristic of a slightly auto-catalytic chemical process. The power functions were slightly shifted to the positive direction of both the time and the concentration axes.

UNDAMAGING HEAT TREATMENT OF FRUIT CONCENTRATES IN TUBULAR STERILIZER EQUIPPED WITH STATIC MIXERS

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In the food industry, aseptic technology is not a novelty. Heat treatment at high temperature for a short time has been used for about 40 years in the processing of liquids, as it was found that high quality products can be manufactured with permanent microbiological safety only by a continuous system. The study of sterilization of viscous liquids or of liquids containing pieces of solid particles has been up to now a goal of research.

During the heat treatment of concentrates containing sugar and fiber the heat treated particles can burn on the surface inhibiting thereby the transfer of heat and at the same time caramellization could harmfully influence the colour, taste and odour of the final product. During cooling the viscosity of the product increases and the adhesion of the film layer on the walls can also grow, causing a decrease of heat transfer and at the same time inhomogeneity of the temperature along the cross-section. For continuous sterilizers (scraped-surface heat exchanger) the cleaning of the heating surface and the removal of the product layer be carried out either mechanically, or by the increase of flow turbulence. Applying FixMix static mixers developed in the Institute, an experimental equipment in large laboratory size has been constructed for the testing of procedures like solution, homogenization, heat treatment and sterilization in semi-continuous and continuous ways. The equipment

is constructed from two modul units, from a semi-continuous homogenizing heat treating unit and from a continuous tubular sterilizer.

By using the equipment the composition of food industrial products and their heat treatment were studied. Based on the measurements data were given for the planning of plant technologies. Results obtained during the sterilization of ketchup, fruit juices and fruit puree concentrates are discussed.

APPLICATION OF NUCLEAR MEMBRANES FOR MICRO- AND ULTRAFILTRATION IN THE FOOD INDUSTRY

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The decisive importance of micro- and ultrafiltration in various fields of the food industry is well known. The nuclear filters – membranes produced by nuclear methods, mainly by heavy ion irradiation – are usually 5 to 20 μm thick plastic foil layers with 0.1 to 10 μm pore diameter when used for micro- and ultrafiltration. These membranes can be applied to ensure microbial safety thereby increasing the shelf-life of liquid foods and drinks.

The question of applying nuclear filters with 0.2 and 0.6 μm pore diameter, and of 6.5% porosity, produced in the Joint Nuclear Research Institute (Dubna) is analyzed. The experiments were carried out to study the application possibilities in brewing, distilling soft drink and dairy industries.

ROLE OF ULTRAFILTRATION AND DIALYSIS IN THE SEPARATION OF PROTEINS

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Ultrafiltration and dialysis membranes with different apparent pore diameter enable the fractionation of macromolecules of different molecular weights. The

ultrafiltration experiments were carried out at constant pressure while dialysis (simple bag or electrodialysis) at atmospheric condition.

At different pressures protein macromolecules change their secondary and tertiary structure depending on the ionic strength, proteolitic conditions and on the protein concentration of the aqueous solution. The apparent molecular size is changing too, while the retention and transport properties of the given UF membranes are very uncertain.

Considering carefully the separation of protein fractions of fixed molecular weight a technology was elaborated for hypoallergenic protein production. For this purpose ultrafiltration, dialysis (ED) and gel-chromatographic separation were carried out in laboratory and pilot plant scales.

Ultrafiltration experiments were performed at 1–4 bar pressure with DDS polysulfone membranes of 500–50 000 Dalton cut-off values, dialysis was carried out with bags, circulating plan and heamodialysing capillar modules and electrodialysis. As analytical method gel-chromatography was applied.

It was found that product qualities were the best when combined ultrafiltration and dialysis was applied. Ultrafiltration is the most economic solution.

DETERMINATION AND TESTING OF LACTIC ACID BACTERIA IN BEER

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In the technological processes of brewing the harmful microorganisms are of an importance of first order and therefore the destruction of lactic acid bacteria and the maintenance of the infections at a minimum is essential.

In beer hexose, amino acids, salts, purins and pyrimides and various factors ensure a suitable nutritive medium for the multiplication of lactic acid bacteria.

Wort, fermented beer can also be considered a selective nutrive medium due to its low pH value, reducing rH value and its carbondioxide, ethylalcohol and hop content.

Consequently the determination and study of multiplication and destruction characteristics of the lactic acid bacteria species found most frequently in the brewing industry, is a basic condition of safe protection.

Lactobacillus brevis was found the most frequently met species in brewery samples containing lactic acid bacteria. The characteristics of multiplication of this

species were determined at 25 °C with pH values of 3.5, 4.5, 5.5. The testing of heat destruction was carried out at a 4.5 pH.

Results

- The optimum of the multiplication was found at ph 4.5.
- The thermal resistance factor, the Z-value was characterized by 8.13 °C.

GRANULATION AND COATING IN THE PROCESSING OF PLANT RAW MATERIALS

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Granulation and coating are being used in more and more domains of the food industry. Thus, a majority of "instant" products is sold in form of granulates. Coating is applied in the sweet industry (sweets in dragée form, chocolates, etc.). Evaporation and drying are widely used methods in the processing of plant raw materials and have been supplemented in the last decades by finishing operations as granulation and/or coating.

From the concentrates obtained by the evaporation of fruit juices, solid products can be produced only by relatively expensive operations (e.g. spray, microwave, freeze-drying). The use of fluidization spray drying operation enables a batch-like or a continuous production of "instant" granulates made from fruit juice concentrates, suitable for the preparation of beverages by a one-step process.

The ground products of spices or their mixtures should be granulated not only for a more aesthetic appearance and a better convenience of their use, but with an appropriate granulation and/or coating, the conditioning and preservation of aroma and flavour substances can be achieved in a much better form. By microcapsulation the unpleasant odours can be covered (e.g. garlic). Among others, these were the problems solved by the so-called rotofluidization procedure and equipment.

The dried pieces of vegetables are not always popular among customers. From the ground products of these vegetables (e.g. mushroom, cellery, horseradish, onion, etc.) or from their mixtures, granulates can be produced in a fluidized form. These products are with appropriate binders and auxiliaries suitable for the instant preparation of soups, gravies, etc. In this above-mentioned form sometimes certain dried fruit products are also needed (e.g. apples, maroons). Actual and prospective results in this field are also discussed.

TO THE MARGIN OF SULFITE ALTERNATIVE LICENCE

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It is well-known that enzymatical and non-enzymatical brownings influence the quality, the nutritive value and the safety of foods. The use of sodium-sulfite applied very effectively in a wide circle, has been forbidden or limited for various foods by the foods law (FDA 1986, 1987), due mainly to the increased sensitivity of asthmatic patients to sulfites. Our tests have proved that from the various "sulfite-alternatives" recommended in the pertaining literature or by commercial experts inhibiting browning (ascorbic acid, potassium sorbate, citric acid, Sporix, salicylic acid) none can approach the effectivity of sulfite. The carefully studied and widely tried substituted amino acids containing SH-group, were found to have similar effect to that of sodium sulfite in an equal mol concentration.

PARTIAL ENZYME HYDROLYSIS WITH ALCALINE PROTEASES OF SOY PRODUCTS WITH DIFFERENT PROTEIN CONTENT

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Plant proteins having an advantageous amino acid balance particularly soy protein, are in human nutrition potential protein sources in addition to animal proteins. The utilisation of plant proteins in food industry is inhibited by their unfavourable techno-functional properties. To improve these properties chemical or physical pretreatments are applied. From the nutritional aspect the most suitable of these treatments is the partial enzymic hydrolysis as a special biotechnological procedure.

Laboratory experiments were carried out in the study of partial enzymic hydrolysis of soy products with different protein contents (defatted soy flour, soy concentrate and soy isolate) with microbial and animal origin proteolytic enzyme (Alcalase and Neopancreatin).

The change of soluble protein content and degree of hydrolysis was followed as a function of enzyme concentration and reaction time.

The chemical and physico-chemical properties of the partially hydrolyzed soy proteins were tested as a function of the degree of hydrolysis.

NEW TECHNOLOGIES FOR THE PRODUCTION OF FERMENTED DAIRY PRODUCTS

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The biological advantages of the consumption of milk and of dairy products are widely known. Nevertheless, it is a fact, that about 30% of the population can only consume a limited variety of dairy products due to a sensitivity to lactose. Those, who suffer from "milk sugar sensitivity" are sensitive – to a higher or lower degree – to the consumption of lactose and/or galactose. Various procedures are applied in the production of milk and dairy products with reduced lactose content, but there is no safe method for the manufacture of lactose-free galactose-reduced fermented dairy products.

Based on our researches, a "two-stage" fermentation technique has been developed by means of lactose-free and galactose-reduced, or lactose-free and practically galactose-free fermented dairy products can be prepared.

The principle of the procedure is that in a main fermentor completed with an auxiliary fermentor from a basic material of reduced lactose content the pre-calculated lactic acid quantity is neutralized with a physiologically harmless alcaline mixture in the first fermentation stage. The second fermentation stage is carried out in the package container or in a post-ripening tank by spontaneous acidification. Production can be carried out batch-like or in a continuous way. The fermentation step can be fitted into a practiced technology.

The fermented dairy products prepared by this technology are similar to the original products and can be aromatized, foamed, etc. in the same way.

AERATION IN AIRLIFT REACTOR

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In aerobic fermentation technologies the molecular oxygen required for the growth of microorganisms should be supplied in a form of solution. However, oxygen is soluble in aqueous media only very slightly and the oxygen consumed by the microbes has to be substituted continuously. Aeration is often combined with agitation for several reasons. First, the intensive turbulation of the liquid due to mixing splits the air bubbles and thus the limiting gas-liquid surface is enlarged. This brings about the increase of the absorption of oxygen. Secondly, as an effect of mixing, the introduction of nutrients into the cell is accelerated, and the formation of macroscopic concentration gradient is inhibited in the fermentor. The removal of the products of metabolism gradient formed during the fermentation and the conduction of the generated heat can also be promoted by mixing. At repeated feed batch and continuous fermentation the aim is to ensure a homogeneous environment for the microbes.

In case of large fermentors (50 – 100 m^3), these requirements are more and more difficult to satisfy. Though with the increase of the number of revolutions of the mixer an ideal state can be approached this can be obtained only by a high energy input. On the other hand, the growing shearing force due to the increase of the number of revolutions the blades of the mixer damage the microorganisms.

These disadvantages are partly eliminated by airlift reactors of various types. These show relatively good mass transfer and their energy demand is smaller compared to the reactors equipped with mechanical agitators. There are no moving parts in it and it can be built in large sizes, too. They can be used not only for fermentation process but also for the biological waste water treatment. With static mixers of various types placed into the draft tubes, the dispergation of the air in liquid can be theoretically intensified.

The effect of empty draft tube, draft tube filled with FixMix static mixers and vertically slit draft tubes on the mixing and mass transfer were compared. The measurements were carried out in an air – water system in three devices of various diameters. The diameter of the draft tube was not changed.

The results of the investigations are discussed in the paper.

INHIBITION OF CELL RELEASE IN CHAMPAGNE PRODUCTION WITH IMMOBILIZED YEAST

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In classic champagne production the release of the lees, clarifies the fermented and ripened champagne. It is the most labour requiring and most expensive part of the production. To accelerate this operation, trials were carried out with the use of immobilized yeasts.

The yeasts were immobilized in Ca-alginate gel beads and the kinetics of fermentation, as well as the clarity of the champagne were studied and compared to the classic (not immobilized) control.

Because of the results of previous trials it was expected that part of the cells proliferating in the Ca-alginate gel would be released during fermentation. To inhibit this phenomenon a bio-catalyzer coated with cell-free Ca-alginate, was prepared. The not coated and the alginate coated bio-catalyzer were prepared with two kinds of cell concentrations. Fermentation was carried out in a cellar at a temperature of 12 °C.

Based on sugar consumption, it was found that compared to the control the process of fermentation was slower when treated with immobilized yeast. Nevertheless, technologically this means no disadvantage. After the end of fermentation, no essential difference was found between the analytical values of the samples.

Evaluation of the clarity showed a small turbidity after treatment with immobilized yeasts without coating. The tested method wants to eliminate totally shaking off or filtering, but the results were not satisfactory. At the same time, it was possible to reduce the outwash of cells from coated bio-catalyzer to a slightly perceptible degree. The mechanism of cell release was studied by electron microscopy, too. The results suggest that the process with alginate coating could be an effective method for the preservation of the clarity of the champagne provided that coating and the adhesion of alginate covering can be improved.

The evaluation of trial treatments is continued during the aging of the champagne, in special consideration of the autolysis of immobilized cells.

MONITORING OF BETA-GLUCANES AND THEIR DECOMPOSING ENZYMES IN THE PREPARATION OF BEER WORT

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From the aspect of enzyme reactions in the preparation of wort hydrolysis is characteristic. The aim of the operations in the brewhouse is to turn the polysaccharides and protein macromolecules of basic materials and adjuncts into water-soluble substances and to decompose them by enzymes. The beta-glucanes are non-starchy polysaccharides and as such characteristic components of the cell wall of the barley endospermium. The appropriate decomposition of these macromolecules ($\geq 5 \times 10^4$ dalton) is first an increased amount of extract followed by improved wort separation and the colloidal stability of beer.

Object of the work was to follow the enzymic decomposition of beta-glucanes in malt mashes (with low or suitable enzymic activities) and/or at the mashing of unmalted barley adjunct.

Some of the beta-glucanase enzymes of malt origin get synthetized during malting (beta-1,3-glucanase; beta-1,3,1,4-glucanase), while the other part is activated (e.g. beta-1,4-glucanase, solubilase).

It can be proved by laboratory-scale mashing that above 60 °C the beta-glucanases of malt origin are rapidly inactivated. Taking into consideration that the water-soluble part of beta-glucane is solved only above 63 °C even the excellent quality beta-glucanases of malt cannot be utilized for the hydrolysis of beta-glucane of poorly modified malts. With 20 min previous boiling of unmalted barley adjunct, the beta-glucane of barley origin can be turned into solution and can be decomposed with the beta-glucanase enzyme system of malt.

Microbial beta-glucanases preserve their activity even at a temperature of 75–85 °C, thus causing a prolonged hydrolysis of the solved substrate after the separation of the wort up to hop-boiling. In case of mashes of poor quality malts, this temperature tolerance is favourable by utilizing the barley adjunct, the enzymic activity of malt is increased.

It has been observed that the technological problems due to the solved and unbranched beta-glucane content of mashes produced with the complete or partial lack of first-class malts can be solved with the boiling of adjunct material and/or with the addition of industrial beta-glucanase enzyme. A very rapid ($\leq$ 10 s) method for the determination of beta-glucanase based on the principle of flow injection analysis is suitable for the measurement of beta-glucanase enzyme activities, and thus, the

beta-glucane level of large-scale beers can be held below the limit (250 mg dm^{-3}) as recommended in the pertaining literature.

ALCOHOL PRODUCTION OF *ZYMOMONAS MOBILIS* AND *SACCHAROMYCES CEREVISIAE* ON MOLASSES AS RAW MATERIAL

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The alcoholic fermentation of two strains of *Zymomonas mobilis* (ATCC 10988, ATCC 29191) was studied on molasses as raw material. The results of the trials were compared to the similar data of the industrial strain V_{30} of *Saccharomyces cerevisiae*.

During the batch-like superfilled fermentations, the following observations were made.

– The biomass yield of *Z. mobilis* strains from molasses was lower than from *S. cerevisiae* (about 1/3 from strain ATCC 10988, and more than the half from ATCC 29191 not corrected for levan formation).

– The alcohol tolerance of the bacterium strains tested was smaller than that of yeast. The final concentration was raised to 9.16 %(v/v) in the case of *Saccharomyces* and to 7.58–8.80 %(v/v) in that of *Zymomonas*.

While the fermentation time of ATCC 10988 (192 h) was much longer than that of V_{30} (120 h), the fermentation of ATCC 29191 was finished in 70 h.

Optimization tests made with ATCC 10988, showed that multiplication had been promoted by the low concentration of the molasses (100–150 g dm^{-3}), the addition of yeast extract (2.5 g dm^{-3}) or vitamin enrichment (pantothene acid + biotin 5.0–5.0 mg dm^{-3}). Optimum temperature of the strain was 30 °C and the pH-optimum was 7.0. Fermentation was carried out at 5.0 pH, as more concentrated acid medium reduced the probability of infection hazard which was greater on industrial scale. The spirit produced by the molasse fermentation of *Z. mobilis* contained a higher amount of a volatile contaminating component than the spirit fermented with *S. cerevisiae*. This can be easily eliminated during the refining process. Advantage of the spirit obtained by bacterial methods compared to that produced with yeast is the smaller amount of fusel oils.

Summing up the findings, it can be stated that with *Z. mobilis* a spirit of industrial quality can be produced from molasses with low biomass formation. The volumetric productivity (g $alcohol^{-1}$ h^{-1}) can be increased with the use of strains of

better alcohol tolerance and by the elaboration of an optimal technology suitable for bacterial spirit fermentation. The practical realities of these findings are supported by published data.

ENERGY SPARING AGRICULTURAL DISTILLERIES

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Energy consumption and CO_2 emission has been growing exponentially since the turn of the century. The use of solar energy and of biomass have appeared. FAO analyses suggest that the developing countries might come up to date by the turn of the millenium in a degree so that only half of the now agriculturally cultivated surface of the Earth will be needed for the food supply of the human population.

Experts suggest that on half of the freed area oil used now could be substituted 30 to 40% and with this CO_2 and NO_xPb emission would be significantly reduced – as the CO_2 formed in the motor from ethyl alcohol would be used in a circulation due to photosynthesis. The mass production of bio-ethanol has been technically solved. The gasoline–ethanol mixture is generally used in a proportion of 9:1 as a mean with the exception of Brasilia.

The octane number rising effect of the mixture is important as 10% ethanol rises the octane number by a value of 4. In Hungary 920 t of lead was emitted into the air with the gasoline used in 1990. Budapest became terribly polluted by 86.5 t.

The climatic conditions of Hungary and the structure of its agriculture suggest the conversion to bio-ethanol gained from the cereals which are available the whole year. This is supported by over-production as a consequence and therefore by the spreading of reduced demand for cereal products and of uncultivated land.

For the treatment of rapidly growing unemployment, the repatriation of agricultural distillery plants based on the economy of the past regime, on agricultural and animal breeding farms, on a modern and higher technical level, seems to be reasonable.

Distillation technology of our days enables with an internal recirculation the high solid substance of stillage (20–25%), and the low energy consumption being 30% of the classic procedure.

The modern plant produces from the cereals ground to flour in a hammer mill with stillage recirculation, with continuous enzymatic hydrolysis and with a 2–3 days fermentation period from beer with a capacity of 5–10 m^3 24^{h-1} raw spirits of 85 %(v/v). This is further processed by greater distilleries by membrane technology (30–50% energy sparing compared to azeotropic distillation) to a dewatered spirit.

With high recirculation of the stillage, the heat needed for hydrolysis can be provided from the heat used for distillation. From this, the heating of the fermentors of the bio-gas plant treating the liquid manure of the animal breeding farm, can be provided, as well. The decanted liquid stillage above balance (about 15% of the nonfermentable solid substances) assures on the bio-gas plant fermented together with the liquid manure, the steam needed for the distillation of the raw spirit.

Taking into consideration, that the water requirement of this procedures makes 30% of the water consumption of classic agricultural distilleries, it can be admitted that when the state will give up the tax imposed on gasoline, new working places in the country and a fuel being tolerable by the environment could be obtained due to the agricultural distilleries of the future.

CONDITIONS FOR THE MANUFACTURE OF NATURAL MEAT PRODUCTS

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The International Federation of Organic Agriculture Movements (IFOAM) founded in 1982, published basic specifications for the conditions of the production of foods fully valid from the aspect of nutrition biology.

As to the natural products of animal origin three domains i.e. three components of the production can be distinguished. The establishment of a correct feeding system is of fundamental and essential importance. A feeding basis suitable for the breeding of animals serving as raw materials of natural products has to be created. After the solution of these two problems meat industry has to solve numerous other questions of the production of really natural products.

Up to now the trials and efforts in Hungary have given only partial results but even these should be appreciated. The first steps are very important, as activities and changes will produce significant results only if used together. This work can be realized only step by step, and needs more time for producing final result.

A new feeding system has already started where feeds and premixes are produced with suitable microelement and vitamin contents and free of antibiotics.

For the breeding of animals which will be raw materials of natural meat product, technical directives have been published by the Hungarian Office of Standardization. Based on these directives, products branded as "natural" were filed for permission by the Budapest Meat Industrial Company. This has started the

manufacture of natural products in Hungary. The problems to be solved in the technological process for the manufacture of natural products are wellknown.

COMPOSITION OF THE FOOD OF PIGS OF A NEW GENOTYPE

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A new pig genotype has bred from the crossing of 25% of "mangalica" and 75% of Hungarian big white varieties. The new genotype was bred in an isolated farm system, under free natural environmental conditions up to a body weight of about 160 kg. Neither any yield-increasing agent, nor any antibiotic was administered. The feed had a commercial composition containing less pesticide residues than the limiting values.

As a control, meat samples coming from pigs fattened among large-scale farming conditions and of pigs of the Hungarian big white variety were studied. Compared to the control the malondialdehyde level characteristic of lipid peroxidation decreased significantly in the chop, spare rib, and ham regions of the new genotype. Consequently superoxide dismutase, representing the enzymatic protective system against free radials, was much more active in the ham and chop samples of the new genotype.

In meat samples of the new genotype a much higher iron and copper content was found among the essential trace elements, than in the control, and these value were higher than those published in the international food composition tables. Thiamin levels were found to be higher, too. One of the essential fatty acids (arachidonic acid) was on a significantly higher level in the liver of the new genotype.

As far as protein and fat contents are concerned no significant difference was found between the two groups of pigs (12 – 12) tested. For the detection of the causes of the differences mentioned above further investigation has started.

"GOOD MANUFACTURING PRACTICE" IN THE MEAT PROCESSING PLANTS

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The realisation and correct functioning of "Good Manufacturing Practice" (GMP) and "Good Hygienic Practice" (GHP) are of great importance. In case of suitable documentation the adaptable requirements of the standard series ISO 9000 can be fulfilled. By this, both the consumers' requirements and the legal requirements such as good food quality, appropriate food safety and correct labelling can be satisfied, as producers are forced to face keener and keener competition on the market.

A guidance is given for the attitude of the management and of the workers within this system of quality control, and also for the technology meeting the hygienic requirements, covering the raw material control, the inspection of process control and the testing of finished products. A systematic and periodic testing with the determination of frequency is proposed. Chemical and bacteriologic tests should be carried out by monitoring or – in an increased number – according to the HACCP-conception. An active functioning of a team trained in sensory testing can facilitate the elimination of defects and of the lack of food safety.

EFFECT OF pH, IRRADIATION AND HEAT TREATMENT ON THE SURVIVAL OF *CLOSTRIDIUM SPOROGENES*

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The effect of heat treatment and irradiation was investigated on two strains of *Clostridium sporogenes* (V. 1240 and PA 3679), at neutral and acidic pH. The thermal death curves were determined at 100 °C. D_{10}-value was 3.26 min for strain V.1240 with a heat treatment period of 0 to 1.5 min, and 24.47 min when heat treatment lasted 30 to 60 min. The corresponding values for strain PA 3679 were 1.92 min and 96.46 min, respectively. The average radiation D_{10}-values were 5.12 kGy for strain V.1240, and 2.39 kGy for PA 3679. Irradiation with 3 kGy prior to heat treatment increased the heat sensitivity of spores in the range of 107 to 119.5 °C (strain V.1240).

From the response surface analysis (RSA) it can be concluded that the responses of the two strains to the combined treatment are different. The sensitization effects of irradiation were higher at 95 °C than at 115 °C for strain PA 3679, while in case of strain V.1240 this effect was more obvious at 115 °C. Effectivity was further influenced by the low pH value.

MEDICAL AND SPORT FOOD WITH HIGH PROTEIN CONTENT

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Medical and sport foods are products with high protein contents. The Central Food Research Institute developed the technology of protein concentrates 15 years ago, and in cooperation with the Dairy Factory of Country Vas an enzymatically pre-treated milk protein concentrate, the "SPORTROBI" was worked out. This product has been on the market already for ten years, and is used by hospitals in the intensive wards for the rehabilitation of patients with burns or in a weak condition. A wide circle of sportsmen, too, take this product.

The product has improved to meet more efficiently the essential demands of the organism. A great advantage of the protein content of SPORTROBI is that during production it has been treated with proteolytic enzymes and as a consequence it is digested more rapidly and absorbed more completely. Its biological value when combined with other proteins is 86 to 98. The protein of high biological value was combined with minerals and vitamins. This granulate is flavoured and dissolves rapidly. Minerals, mainly K and Mg are added to meet the needs of the human organism. A complex vitamin product of Hoffmann La Roche is added in an amount ensuring by the consumption of 50 g of the protein concentrate, half of the daily vitamin need.

The chocolate-flavoured product of 50% protein content is recommended to teenagers, to sportsmen and to people in poor health condition.

THE FUTURE OF BIOTECHNOLOGY

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The results of up-to-date molecular biology made it likely that after the informative society, a biological society should be created where multidisciplinar biotechnology based on the results of biological sciences would play the main role. Biotechnology takes part – even in our days, but will probably more intensively in the future – in the solution of the basic problems of our world (production of raw materials from renewed substrates, plant improvement and plant protection, production of medical substances, environmental problems). Of course, biotechnology cannot fulfill the hopes shortly, the unfounded exaggerated expectations brought disappointment. It can be expected with good reason, that concrete industrial results will appear in this field. Production statistics have shown, that the value of modern biotechnological products nearly reaches that of products produced by classical fermentation. It has been proved that small biotechnological undertakings can survive only when their activity can be joined to a R + D of a great industrial firm or when their activity concentrates on diagnostics.

Biotechnology proved to be a significant motive in reactor development, too. After the fermentor with mixed aeration – as in the first generation reactor – the development of the second generation bio-reactors was introduced. These are on one hand characterized by an immense volume increase (Biohoch, ICI, Deep-Shaft Reactors), on the other hand, bioreactors for special purposes were developed. These are the reactors operating in more than one phase (gas–solid phase, 2 water phases, water and organic phase, fluid-bed, immobilized bio-catalyzer reactors), special membrane reactors (integrated systems), bubble-free reactors and bioreactors functioning with co-enzyme regeneration.

IMPROVEMENT OF BREWER'S YEAST WITH GLUCOAMYLASE ACTIVITY

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About 15–25% of the carbohydrate content of beer wort consists of maltooligomer (dextrin) molecules which are bigger than the maltotriose. These molecules cannot be fermented with traditional brewer's yeast strains. For the reduction of the dextrin content residue, industrial glucoamylase enzyme products are added or dextrin fermenting yeast strains are used. In our breeding activity the STA gene of an extracellular glucoamylase producing yeast strain, belonging to the Saccharomyces cerevisiae race diastaticus, was tried to transfer to brewer's yeast strains by the method of protoplast fusion. The glucoamylase activity of the initial strain was increased ten-times via the isolation of meiotic segregants, that were markered with auxotrophy mutation, then the plasmids coding for the killer phenotype were introduced into the cells by protoplast fusuon. The brewer's yeast and the killer strains producing glucoamylase were hybridized by protoplast fusion and the fusion products were selected with the help of the killer toxin production. From the hybrids, the clones not producing phenol aroma (POF^-) were further analyzed and karyotyped by pulsed-field gel electrophoresis. Thus, a possibility was open to select the hybrids with the chromosoma patterns mostly similar to brewer's yeast, approaching best the genetical and physiological properties of the brewer's yeast strains.

POST HEAT TREATMENT OF FERMENTED DAIRY PRODUCTS

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The consumers demand for fermented dairy products is rising all over the world. Their favourable dietetic effect, their palatable taste and their texture made them popular in Hungary, too. For a safe distribution, there is a demand for products with a relatively long shelf-life. The longest period of the products made with the

traditional technology is generally 3–5 days, with a post-treatment by heat, 10–30 days and with complete sterilization 30–120 days.

For lengthening the shelf-life of fermented dairy products, almost exclusively the post-treatment technology has been used. Aim of the technology is to kill in the product the most of the possible microorganisms and thus, to assure a longer period of palpability without damaging the taste, and texture of the finished product. This kind of treatment can even improve these quality attributes.

Heat treatment is carried out in plate- or tubular heat exchangers, combinators or cutters generally at a temperature of 74 to 90 °C. Part of the products is packed semi-aseptically an other part cooled to 4–8 °C, or – in case of foaming – to 8–12 °C. The product has to be stored up to the end of the "best before" time at a temperature of 10 °C.

During post-treatment by heat, first of all the protection of product against heat (to inhibit change of the texture) and the solidification of the texture of the finished product should be solved. To this technologic operation various hydrocolloids of plant and/or of animal origin, can be applied. Manufacturers prefer the combination of these agents and the proportion of the components, being a result of their researches, is kept a secret.

The most important factors being decisive from the point of view of realization of the post-treatment and shelf life of the finished products are the followings:

- heat and treating period used for the post-treatment procedure
- fat and carbohydrate content of the product
- speed and temperature of cooling
- pH-value of the product.

Decisive factor from microbiological points of view:

- microbiological state of the product to be treated (quantity of coliforms, of yeasts and moulds, and of aerobe spore-carrier bacteria).

FACTORS INFLUENCING THE MULTIPLICATION AND DEATH OF STARTER CULTURES

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Two opinions are in contrast concerning the starter culture in the meat industry. One of them is of the view that with one-component s.c. protective culture the safe production of rapidly ripening sausage products can be realized, as the aroma components are produced by the active proteolytic and lypolytic enzymes of

meat. The other theory is, that there is a need for aroma-producing microorganisms too and it is recommended to use mixed cultures (acid- and aroma-producing microbae).

Aim of this study was the investigation, how the combined effect of water activity, pH-value and ripening temperature influences the multiplication and death of a widely applied protective cutlture strain of *L. plantarum*.

In the trials, the determination of optimal factors was not based on the obtainable maximal cell number, but on the maximal speed of multiplication (S_{max}). Therefore the curve of multiplication was plotted (5 measuring points). Special culture bouillon medium was used for the tests, the water activity was adjusted with glycerine, the pH-values with Mc. Ilvain buffer. The tests were made for five various water activities, four pH-values and four incubation temperatures in various combinations with 3–3 parallel determinations at five points of time, corresponding to 1200 determinations of vial cell numbers.

It has been stated, that though the *L. plantarum* was a typical "cold fermenting" microorganism, it was multiplied in a pure culture even at a temperature of 41 °C. It grew at the initial temperature, water activity and pH-value combination of salami ripening but due to a further reduction of water activity (after one week of ripening), it reached the death phase.

A difference observed between the vital conditions optimum determined on the basis of maximal vial cell number and on the basis of maximal speed of multiplication. The results can be used in the production of rapidly ripening sausage products.

PHYSIOLOGICAL STUDY OF LACTOCOCCI IN THE CHEMOSTAT SYSTEM

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The ability of lactococci to ferment lactose and citrate are encoded in the plasmids, which can be unstable properties and cause problems in the fermentation of milk due to variations in starter activity.

The stability of the lactose and citrate plasmids in *Lactococcus lactis* subsp. *lactis* var. *diacetylactis* was examined under different carbon limited conditions in a chemostat culture at $D = 0.2\ h^{-1}$ and pH = 5.8.

Under carbon limitation with 12.5 mol l^{-1} lactose and 10 mol l^{-1} citrate present in the medium mixed end-products (lactate, acetate, formate, ethanol) were formed. When the carbon source was changed to 12.5 mol l^{-1} lactose the organism produced over 95% lactate. Changing the carbonhydrate source to 25 mol l^{-1} glucose in the end-products other than lactate became significant.

In the last step of the fermentation process the conditions were switched back to those given in the first part of the experiment (12.5 mol l^{-1} lactose and 10 mol l^{-1} citrate) which also caused a change in the end-products.

The ability of *Lactococcus diacetylactis* to utilize lactose and citrate were stable functions when cells were grown in lactose and citrate containing medium. Changing of the carbohydrate source to glucose resulted in the loss of ability to ferment lactose. At the same time, the absence of the citrate in the medium (either lactose or glucose) the organism did not give the selection of citrate negative variants.

These results indicate that LAC plasmid is an unstable genetic element and CIT plasmid in *Lactococcus lactis* subsp. lactis var. diacetylactis is remarkably stable.

CONCENTRATION OF HEAT SENSITIVE SUBSTANCES IN THIN-FILM EVAPORATOR

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In the concentration of heat sensitive substances (e.g. milk, whey, blood plasma, blood, etc.) the wall temperature of the evaporator plays an important role in contrary to the processing of other heat sensitive substances such as fruit or vegetable juices, for which this factor is less important. To avoid the coagulation or the scale formation, the evaporator has to be heated with saturated steam of less than 100 °C temperature. For this a film evaporator was developed, equipped with fixed blade rotor and heated with low pressure steam. The temperature of the steam varied between 50 and 100 °C. Froth formation could be prevented by additives and by the alteration of design of the rotor.

The paper deals with the advantages of the developed evaporator and its main running conditions, for example the concentration of frothing, heat sensitive substances such as dilute aqueous cellulase enzyme solutions, methanolic solution of vitamine B_{12}, whey, blood plasma and blood.

The monitoring of damage and the testing of its degree have been carried out from the protein content determined by the biuret-method and by agar- and

immunelectrophoresis. It was proved that these methods of analysis were suitable for the judgement of the heat sensitive properties of thin-film evaporator, too.

FERMENTATION AND DOWNSTREAM PROCESSING OF GLUCOAMYLASE ENZYME

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Pilot plant scale technology has been developed for the production of glucoamylase enzyme of mould fungus origin. *Aspergillus niger* ATCC 22343/65 mutant strain was chosen for the production of the enzyme. The submerged "batch" fermentation technology required the optimization of the composition of the media. The optimization was realized by factor scheme of orthogonal central arrangement. As a result 12–15 GAU cm^{-3} enzyme activities were achieved. (One unit glucoamilase (GAU) activity corresponds to the formation of 1 g glucose per h at 60 °C, pH = 4.2.) These values were reproducible in 100 and in 200 litre fermentation volumes. The effectiveness of enzyme production can be increased by the application of "fed batch" fermentation technology.

Alternative methods (vacuum evaporation, ultrafiltration and their combination) were applied for the concentration of the ferment broth and the increase of specific enzyme activity. A two-step procedure has been recommended for the economical production of glucoamylase with 100 GAU cm^{-3} specific activity where vacuum evaporation (to 1/2, 1/3 volume) is the first step. The desired specific activity can be obtained by ultrafiltration. The formulation of the enzyme product was made by the addition of glycerol for the adjusment of the suitable water activity value and the microbiological spoil was inhibited by sodium-benzoate.

Storage properties and stability of the product made by this method are excellent. In a one-year cooled storage the degree of inactivation did not reach 5%.

DEGRADATION OF LIGNOCELLULOSIC SUBSTRATES BY A WHITE-ROTTING FUNGUS

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Xylan-degrading enzymes were induced when *Phanerochaete chrysosporium* was grown at 30 °C in shake flask media containing xylan, Avicel PH 102 or corn stalk. The highest xylanase activity was produced in the corn stalk containing medium, while xylan based fermentation resulted, contrary to expectation, in the lowest induction. Analytical and preparative isoelectric focusing were used to characterize xylanase multienzyme components.

Preparative focusing was performed only with cultures grown on Avicel and corn stalk.

Of over 30 protein bands separated by analytical focusing from the Avicel and corn stalk containing media, each of the three main groups (I, II, III) of about 5 isoenzymes showed xylanase activity according to zymogram technique using xylan overlay.

Enzyme assays revealed the presence of 1,4-β-endoxylanase and arabinofuranosidase activities in all the three isoenzyme groups separated by preparative focusing.

β-Xylosidase activity appeared in the first peak and also as an independent peak between peaks II and III.

Denatured molecular weight for the three isoenzyme groups were found between 18 and 90 kD, pI values in the range of 4.2–6.0. β-Xylosidase with an apparent molecular weight of 20, 30 and 90 kD (peak I) and 18 and 45 kD (independent peak), indicating a trimer / dimer structure, respectively, with pI values 4.2 and 5.78. Three more minor xylanase groups were produced on corn stalk: a double peak in the acidic range (pI 6.25–6.65 and 6.65–7.12) and two minor peaks in the alkaline range: pI 8.09–8.29 and pI 9.28–9.48, respectively.

The profile of xylanase separated by isoelectric focusing (zymogram) of culture filtrate from cells grown on corn stalk were more complex than that of culture supernatants from cells grown on cellulose.

The optimal values of the three major xylanase groups are equally in the range of pH 4–5.5.

The activities of some purified xylanase components were not stable even at 37 °C after 10 min incubation.

PARTIAL OXIDATION WITH *GLUCONOBACTER OXIDANS* STRAIN IN FED-BATCH FERMENTATION

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The *Gluconobacter oxidans* strains are capable of partial microbial oxidation of various compounds. With *Gluconobacter* species sorbose from sorbit, glucuronic acid from glucose, dihydroxiacetone from glycerol, acetic acid from ethanol can be fermented. The last of the mentioned reactions different from the *Acetobacter* specii which oxidize acetic acid to carbon dioxide.

Dihydroxiacetone (the product of which was studied) is used as aroma enhancer and antioxidant in the food industry and as artificial browning agent in the manufactor of cosmetics. It has an important role in the production of plastics, in the production of surface-active compounds, of organic metal and of other compounds.

Partial oxidation can be realized with resting cells, when cell mass is produced by direct fermentation and the obtained not growing cell mass acts as an enzyme carrier in the a second step of the wanted reaction.

The separation of cultivation and of oxidation has the advantage that from the fermentation liquid a product of higher purity can be directly produced, though it requires double cell separation, two fermentation steps and the multiple of fermentation time.

The fermentation carried out in one steep with multiplying cells have much less time, labour- and cost requirements, but the purity of the product and the conversion (according to substrate inhibition) are not satisfactory in some of the cases.

S.c. substrate inhibition can be eliminated with the substituting of batch fermentation by fed-batch technique.

The products made directly from fermentation liquid are contaminated with metabolytes, amino acids, proteins and peptides which have been accumulated during fermentation. Therefore fed-batch fermentation with resting cells proved to be more successful. This was made possible by dihydroxiacetone fermentation belonging to the I. type according to the Luedeking-Piret model, where the product formation does not depend on the speed of multiplication.

$$\frac{dp}{dt} = Bx$$

With this method, final product concentration of 25% was obtained in the 50th hour of the second fermentation step with 621/H *Gluconobacter oxidans* strain, of a conversion of 90–95%. Authors want to introduce feed-back control, or feed-back superfilling so, that glycerine concentration should be measured by NIR (Near Infrared Reflectance) technique and superfilling adjusted to the glycerine conversion. The determination of glycerine and dihydroxiacetone with NIR technique (determination of derived spectra, choice of mathematical models, calibration, etc.) has been carried out in the Department for Physical Analysis of the Central Food Research Institute in Budapest.

PRESERVATION OF BY-PRODUCTS OF THE MEAT INDUSTRY BY LACTIC FERMENTATION FOR FEEDING PURPOSES

GY. SZAKÁCS

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A new type of wet preservation of the by-products of slaughter houses and of the meat industry has been worked out where the main elements of the traditional lactic acid ensilage was applied. To the autoclaved by-products of protein-character, 7–10% (w/w) of cereal flour (corn, fodder wheat), amylolytic enzyme (Termamyl 24 OL, BAN 360) and lactic inoculum (e.g., *Lactobacillus plantarum*) were added. The slurry with 30–40% d.m. was fermented at 37 °C in anaerobic conditions at least for 2 days. During this time, the initial pH-values of 6–6.5 of the substance was reduced to 4–4.5 (3–4% lactic acid is produced), resulting in a good preservation effect. The "meat pulp" ensilaged in closed containers can be stored for weeks without any deterioration. After mixing with appropriate feeding components, it can be applied in dry and in wet pig feeding systems. Advantages of wet conservation method compared to the drying technologies (production of meat meal) are the followings:

- Considerable cheaper, as drying is an energy-consuming procedure;
- Practically no sewage is produced in this technology, as compared to the production of meat meal, where a waste water purification plant has to be installed;
- The fermented meat pulp is a favourite meal of the pigs, they like this taste.

A pilot plant has been established in 1980 at the Kisalföld State Farm. Then, in 1987, a large-scale plant was built at Pápa State Farm with a capacity of 20 tons per

day. The flow diagram of the two plants are shown. More than 300 000 pigs have been raised with feeds containing well-preserved "meat pulp" till now.

CONSTRUCTION OF GENOMIC LIBRARY OF GLUCOAMILASE PRODUCING *ASPERGILLUS NIGER*

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The aim of our work is to enhance the productivity of the *Aspergillus niger* ATCC 22343/65 strain producing glucoamylase by increasing the copy number of the glucoamylase gene.

DNA was isolated from the lyophilized *Aspergillus niger* mycelium. The molecular size of DNA was appropriate for digestion by restriction endonucleases. As the DNA sequence of the glucoamylase gene of *Aspergillus niger* is known, the EcoRI restriction enzyme was chosen which has no restriction site inside the glucoamylase gene.

The genomic library was made in lambda-phage insertion vector, which suits the cloning of fragments in the 2 and 10 kD range. The DNA fragments were separated by sucrose density centrifugation. The insertion of the fungal DNA fragments took place in the only EcoRI restriction site of the vector. Via in vitro packaging phages were regenerated and the ratio of the recombinant phages was above 98%. The *E. coli* Y-1090 strain was infected by the recombinant phages. Clones containing the glucoamylase gene were selected by the method of plaque hybridization. Synthetic oligonucleotides were used as hybridization probes.

WASTE WATER TREATMENT IN THE FOOD INDUSTRY WITH INTENSIVE ANAEROBIC BIOREACTORS

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A short survey was given about the causes inhibiting the use of traditional aerobic biological organic substances removal methods with special consideration to

concentrated food industry effluents. Practical problems limiting the spreading of the use of intensive immobilized cell bio-reactors solving this situation are outlined.

One of the most successful processes is the upflow sludge bed system. Research results of the authors related to the intensification of this method and to directed microbial selection are described.

Industrial technologies for waste water treatment worked out in industrial scale or in pilot plants in the distillery, canning and sugar industries and the complex solution of pig manure treatment are outlined.

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G. TÖRÖK MEMORIAL MEETING
organized by
THE CENTRAL FOOD RESEARCH INSTITUTE
Budapest, Hungary
15 June 1992

Dr. Gábor Török
(1902–1966)

Dr. Gábor Török was the first director of the Central Food Research Institute and a great organizer of Hungarian food research.

Dr. Török was born at Abrudbánya in 1902. Having finished his secondary schools in Kolozsvár he graduated in Budapest in the Faculty of Chemical Engineering of the Technical University.

As a young engineer he was employed by the Agricultural and Chemical Works in Budapest. He spent almost fifteen years there working on fermentation processes, pesticides and finally was appointed director of the Works. The establishment of the Canning Factory, Paks made him deal with different aspects of food preservation, which later became his favourite project. In Hungary he was one of the first experts of theoretical and experimental aspects of this subject.

In 1941 Dr. Török was assistant professor of the Physical Chemistry Department of the Technical University where his research activities were focussed on quick-freezing technology. His effort to establish the Hungarian deep-freezing industry was realized when the first deep-freezing plant was built in 1943. After the World War a new plant was set up with his assistance. At the same time, Dr. Gábor Török was managing the "Helio" Canning Factory in Kaposvár and become vice-president of the National Union of Canning Manufacturers. He was aware of the lack of quality control in the Hungarian industrial food technologies and initiated the standardization of the canning industry.

There was no organized food research in Hungary up to the fifties. As councillor of the National Office for Public Supply Dr. Török was in a position in which he could do a great deal for technical development and became the first organizer of Hungarian industrial food research.

His efforts as research manager were acknowledged when he was appointed director of the Canning-, Meat- and Refrigeration Research Institute in 1949. During

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these years, which was the most remarkable period of his life, Dr. Török not only created a modern research institute but established the network of Hungarian industrial food research, too. Under his direction a number of new research centres came into existence representing different branches of the food industry. In 1959 he was appointed director of the Central Food Research Institute where he worked till the end of his life.

In his last years he actively initiated the establishment of the Technical School for the Food Industry and was head of the Chemistry Department.

Dr. Török's scientific activity covered a wide range of food science, but his main interest was always focussed on deep-freezing. His preference for this subject was inspired by the fact that he had the change of working at the famous Max Planck Institute in Karlsruhe.

Dr. Török was vice-president of both the Agricultural Industrial Scientific Society and of its successor, the Hungarian Scientific Society for the Food Industry in which he acted as honorary president till his death in 1966.

Dr. Gábor Török was a great personality of Hungarian food research. His outstanding abilities made him tutor of the younger generations of food scientists. In recognition of his activities he received the Palm Order of the French Academy, The Kossuth Prize and a number of other awards and decorations.

BIOPROCESSING AND NUTRITION OF FOODS

C. ERIKSSON

SIK – the Swedish Institute for Food Research and Department of Food Science, Chalmers University of Technology Göteborg, Sweden

Bioprocessing as a mean to preserve food and make it palatable goes back to ancient times. Though modern societies produce and consume large amounts of foods like cheese, beer, wine, yoghourt and sauerkraut, etc. according to biotechnological principles, the impact of these procedures on the nutritive value is very little studied. On the other hand, there are a lot of claims on the great healthiness of fermented foods. These claims, however, are based mainly on weak or non-existent evidence. This situation is rapidly changing since the interest in food manufactured according to biological and natural principles has increased. Therefore, health claims should also be based on scientific evidence demanded also by food and health authorities.

This lecture will focus on our work about mineral availability and how it is affected by simple bioprocessing principles as malting and lactic acid fermentation.

Special attention is paid to the role of enzymatic degradation of phytic acid in cereals and its effect on the absorption of zinc and iron by human beings.

We studied fermented vegetables both in solid and liquid form when some interesting observations were made regarding lowering of blood glucose and insulin response after a meal containing fermented vegetables. Lactic fermentation produces factors both in vegetables and soy sauce which raises greatly the absorption of iron in humans. There is evidence about organic acids such as citric and malic acids but other factors must still be investigated.

Examples of how bioprocessing can be used to produce physiologically functional food ingredients are certain peptides produced from red blood cells in a continuous enzymatic membrane process. The resulting heme peptides are almost as good iron sources as hemoglobin and technically easier to use. Other examples are phospholipids where omega-3 fatty acids were introduced into 2-position by a reversed enzymatic hydrolysis technique in low moisture medium.

ASEPTIC PROCESS AND PACKAGING TECHNOLOGY

E. W. WARTENBERG

Tetra Pak Int., Lausanne. Switzerland

Aseptic process and packaging technology have reached a high level of sophistication and are gaining increasing significance since they allow food to be stored and transported over great distances without refrigeration without being spoiled, so that the nutritional physiology of the food is maintained to a high degree.

These techniques are becoming more and more widespread due to the use of new food products. This fact forces product-related variations in technology, both in food sterilisation and in packaging materials. The general trend involves higher quality, new foods and energy-saving, as well as environmentally acceptable production processes. In addition this, aseptic technology is ideally suited to great role in the solution of world food supply problems. Tests have shown that the nutritional value of the food after a mild UHT treatment and the use of aseptic packaging suited to the product is essentially comparable with that of pasteurised products.

This study described the basic principles of the aseptic process and packaging technology. The concept of "commercial sterility", so important from the point of view of marketing is explained. The sterilisation and packaging of food is treated, and the importance of bacterial loading of food and packaging material for the efficiency of the aseptic techniques is explained by examples of figures obtained in practice. Migration and residue characteristics are described and the fact that these techniques meet the requirements of food legislation is demonstrated.

Acta Alimentaria, Vol. 22 (1), pp. 87–88 (1993)

BOOK REVIEWS

Food taints and off-flavours
M.J. SAXBY (Ed.)

Blackie Academic and Professional, London. Glasgow, New York, Tokyo, Melbourne, Madras, 1993, 260 pages

Taints: "unpleasant odours and flavours imparted to food through external sources" and off flavours: "unpleasant odours or flavours imparted to food through internal deteriorative change" – as defined by contributor of this book – causing quality deterioration make foods unacceptable for consumers. Although parallelly with flavour research a lot of work was done also in studying of off-flavours nevertheless the full clarifying of ways of such contaminations and methods to avoid such problems need further studies and also exchange of informations.

This book covers the sources of taints and off-flavours, their methods of identification, and methods by which they can be minimised or eliminated. Each of the nine chapters included in the book is written by specialist author. The first three chapters give; (1) an overview of the methodology of sensory evaluation of taints and off-flavours ; (2) a survey of chemicals causing taints and off--flavours in foods; (3) summarizes problems connected with detection and determination of such compounds. The following three chapters deal with taints in some important areas (water, dairy products, fat and oils and high fat foods) of food production. The seventh chapter deals with packaging materials as a potential source of taints. Retailers' problems with unacceptable products are treated in the next part of the book. Finally, in the last chapter the emphasis is on off-flavours and their microbiological formation. Unfortunately this chapter – being very important – is a very brief outline and too short.

The book presents the problems in a way which is understandable to academic, technical and commercial staff. It will be a good source of knowledge for production and quality control managers and due to the very extensive literature survey for academic and research institutions.

R. LÁSZTITY

Akadémiai Kiadó, Budapest

Principles and applications of modified atmosphere packaging of foods

R.T. PARRY (Ed.)

Blackie Academic and Professional, London, Glasgow, New York, Tokyo, Melbourne, Madras, 1992, 305 pages

Modified atmosphere packaging has proved to be one of the most significant and innovative growth areas in retail food packaging of the past decades. Today there is a substantial wholesale market for bulk packaged fresh vegetables and fruits, various meat and poultry products, bakery goods and pasta, ready meals and dried foods.

The book provides a comprehensive survey of the technological (including quality assurance and materials), scientific and commercial aspect of the modified atmosphere packaging (MAP) of foods. The eleven chapters ((Introduction, The market, Packaging machinery, Films for MAP of foods, Quality control of MAP products, Fruit and vegetables, Bakery products, Miscellaneous applications: dairy products, ready meals, coffee, snacks, delicatessen/multi-component products, beverages, Fish, Meat products, Modified atmosphere storage of fresh meat and poultry covers all aspects of technique for quality assurance and shelf-life extension of foods. The lists of references are organic parts of chapters and they help the readers to become immersed in details. The index of subjects completes this well--illustrated publications.

The book is written for food technologist, packaging engineers, packaging machinery manufacturers, film suppliers and production managers in the food and packaging industries.

I. VARSÁNYI

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Effect of extrusion temperature and initial moisture content on the protein solubility and distribution in full fat soybean
HORVÁTH, E. & CZUKOR, B.

"VAS KÁROLY" Memorial Session, 1992

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

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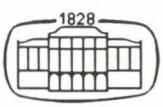

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Acta Alimentaria, Vol. 22 (2), pp. 89–95 (1993)

A STUDY ON THE DURABLE RESISTANCE TO RICE BLAST (*PYRICULARIA ORYZAE* CAV.) DISEASE UNDER EGYPTIAN CONDITIONS

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(Received: 18 June 1992; accepted: 2 December 1992)

In Egypt, the most destructive disease of the crop is blast, *Pyricularia oryzae* Cav. It causes from moderate to severe losses in production varying from one year to another. Thus, breeding for blast resistance is one of the most important objectives of the rice breeding program in Egypt. The program concentrated on utilization of major genes for resistance until 1984, when a severe blast epidemic occurred. Then the need for different types of resistance became apparent and currently, durable resistance has become a chief concern in Egyptian rice breeding.

This investigation was carried out at the Rice Research and Training Center (RRTC) Sakha, Kafr El-Sheikh, Egypt in the 1989 and 1990 growing seasons to study the durable resistance to rice blast under Egyptian conditions. Ten local and exotic rice varieties, namely: Giza 171, Giza 172, Giza 175, Giza 176, Giza 181, IR28, IR36, IR50, Pi No. 1 and Toride 1 were used. Five measurements of partial resistance, i.e. type of and number of lesions, lesion length, lesion width, lesion size (length × width) and diseased leaf area, were used as indicators for durable resistance.

The data showed that all the indica rice varieties were completely immune in both seasons of study. On the other hand, high level of infestations were recorded for the Egyptian japonica rice varieties Giza 171, Giza 172 and Giza 176. In average, the highest diseased leaf area was found in Giza 172 while the lowest was recorded for Pi No 4. The rice variety Giza 175 showed the lowest lesion type number, size and diseased leaf area among the other Egyptian varieties under the same conditions.

This study showed a wide variation for all measurements used between the tested varieties. Even though the indica rice varieties IR36 and IR50 are known to be susceptible to blast disease in several countries, they and other indicas all showed complete resistance in Egypt. The rice variety Giza 175 (indica × japonica) was the most promising variety among the other Egyptian varieties inasmuch it possessed a type of durable resistance.

Keywords: durable resistance, rice blast, Egyptian varieties

In Egypt the most destructive disease to the rice crop is blast caused by *Pyricularia oryzae* Cav. It causes from moderate to severe losses in the rice production varying from one year to another. Breeding for blast resistance is one of

the most important objectives of the rice breeding program in Egypt, which concentrated on utilization of major genes for resistance until 1984, when a severe blast epidemic attacked the Reiho variety. Then the need of more durable type of resistance became apparent and this is now one of the most important concerns in rice breeding in Egypt. JOHNSON (1981) has defined durable resistance as resistance that has remained effective while a cultivar possessing it has been widely cultivated in an environment favoring the disease. The characteristics of durable blast resistance have not been extensively studied (BONMAN & MACKILL, 1988), the available information indicates that durable resistance to blast is associated with partial resistance.

While breeding rice for blast resistance is of high priority in Egypt comparatively little study has been made on partial and durable resistance. This research aimed to study the durable resistance to blast of some Egyptian and exotic rice varieties utilizing some partial resistance measurements.

1. Materials and methods

The work was carried out at the farm of Rice Research and Training Center (RRTC), Sakha, Egypt, in the 1989 and 1990 rice growing seasons. Ten varieties were used in this study: Giza 171, Giza 172, Giza 175, Giza 176, Giza 181, IR28, IR36, IR50, Pi No 4 and Toride 1.

A randomized complete block design experiment with three replicates was conducted in the two seasons. Entries were individually transplanted at 30 days after seeding, in 5 m rows with 20 × 20 cm equidistance in each replicate. Fertilizer was applied (150 kg N ha^{-1}). Borders of highly susceptible varieties (Giza 159 and Sabeiny) were grown as spreaders. A spore suspension prepared from affected leaves was sprayed twenty days after transplanting as another source of inoculum.

Four components of partial resistance were determined under field conditions as indicators of durable resistance. Observations were collected 7–10 days after inoculation on the leaves of 10 random plants per variety. Data were collected on lesion type and number, lesion length (mm), lesion width (mm), lesion size (mm^2) and diseased leaf area. Classification of lesion type was made according to the international classification (OU, 1965). The length and width of each lesion were multiplied to produce lesion size in mm^2. Results were then presented as averages of 10 lesions. Diseased leaf area was expressed in %. Results were statistically analyzed according to PANSE and SUKHATME (1967).

In order to calibrate the Egyptian results on durable resistance with observations in other geographic areas, results from 1983 to 1990 were obtained from the International Network for Genetic Evaluation of Rice (INGER, Table 4). Among the entries not displaying immunity in Egypt, clearly the highest relative frequency of

occurrence of resistance rating (0–3) was recorded for Toride 1 and Pi No 4 (japonica) and a high value of the same rating was observed for Giza 175 (indica × japonica). However the highest estimates of susceptible rating (7–9) were scored for by Giza 172. Among the varieties immune in Egypt, IR36 and IR50 (indica) showed good levels of resistance in INGER results, while Giza 181 had the highest frequency of 7–9 ratings.

2. Results and discussion

Table 1 shows mean square values of the tested characters for the ten varieties grown in the 1989 and 1990 seasons. It is clear that the tested varieties varied significantly for all characters. This leads to the conclusion that all these varieties differed significantly in susceptibility to blast under the same conditions.

Table 1

Mean square values of the studied traits of the ten varieties grown in the 1989 and 1990 seasons

Source of variance	D.f.	Mean square									
		No. of lesions		Lesion length (mm)		Lesion width (mm)		Lesion size (mm^2)		Diseased leaf area (%)	
		1989	1990	1989	1990	1989	1990	1989	1990	1989	1990
Replications	2	0.013	0.121	0.323	0.317	0.145	0.0774	0.009	0.013	0.107	0.227
Variations	9	8.738**	7.122**	1.815**	2.094**	0.911**	0.986**	1.779**	2.373**	12.261**	11.794**
Error	18	0.513	0.662	0.135	0.203	0.082	0.1093	0.194	0.225	1.584	1.666

** Significant at P = 0.01% probability level

Type and number of lesions are presented in Table 2 for each variety. Of the indica rice varieties none showed any lesion whatever, while the japonicas varied greatly. There were also notable differences in infection levels between the 1989 and 1990 growing seasons.

Lesion sizes in 1990 were lower than those of 1989 for all varieties (Table 3) indicating the effect of the environmental conditions on the degree of the susceptibility to blast disease. Type 4 lesions prevailed over the other types. The highest number of type 4 lesion was found on Giza 172 rice variety in 1989. Pi No 4 and Toride 1 showed the lowest number of type 1 and type 2 lesions. The variety Giza 175 (indica × japonica) was the most resistant variety among those Egyptian

rice varieties which did not, through immunity, escape scrutiny of their durable resistance.

Table 2

Type and number of lesions of the studied varieties under the field conditions, 1989 and 1990

Varieties	Number of lesions											
	Type 1			Type 2			Type 3			Type 4		
	1989	1990	Aver.	1989	1990	Aver.	1989	1990	Aver.	1989	1990	Aver.
Japonica												
Giza 171	31.2	46.3	38.7	7.4	11.9	0.6	36.5	20.6	28.6	69.1	56.3	62.7
Giza 172	11.4	20.8	16.1	28.7	20.2	24.5	46.5	30.6	38.5	82.3	66.3	74.6
Giza 176	13.3	20.8	17.1	20.2	15.1	17.6	23.5	36.4	29.9	42.3	41.2	41.8
Pi No 4	17.3	11.0	14.2	5.6	4.6	5.1	0.0	0.0	0.0	0.0	0.0	0.0
Toride 1	22.6	10.1	16.4	7.2	5.0	6.1	1.0	0.0	0.5	0.0	0.0	0.0
Indica												
Giza 181	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
IR28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
IR36	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
IR50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ind. × jap.												
Giza 175	15.0	11.1	13.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 3

Lesion length, width, size (length × width) and diseased leaf area of the studied varieties under Egyptian conditions, 1989 and 1990

Varieties	Lesion length (mm)			Lesion width (mm)			Lesion size (mm^2)			Diseased leaf area (%)		
	1989	1990	Aver.	1989	1990	Aver.	1989	1990	Aver.	1989	1990	Aver.
Japonica												
Giza 171	10.3	11.0	10.7	2.6	2.0	2.3	26.5	21.1	23.8	46.3	39.2	42.7
Giza 172	12.3	11.9	12.1	3.1	2.7	2.9	36.4	25.7	31.1	53.4	48.8	51.1
Giza 176	8.5	9.4	9.0	2.5	2.0	2.3	16.3	18.3	17.3	44.1	32.5	38.3
Pi No 4	1.3	0.9	1.1	1.1	0.7	0.9	3.2	2.0	2.6	0.7	1.0	0.9
Toride	2.1	2.5	2.3	1.5	1.3	1.4	4.6	3.5	4.0	0.9	1.5	1.2
Indica												
Giza 181	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
IR28	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
IR36	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
IR50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Jap. × ind.												
Giza 175	1.6	0.9	1.3	1.0	0.6	0.8	2.1	1.4	1.7	0.9	0.9	0.9

Lesion length showed a trend largely similar to lesion number (Table 3). Giza 172 and Giza 171 gave the longest lesions while the shortest ones were found on Giza 175 and Pi No 4. Moreover, lesion width followed the same ranking as did lesion length. The widest lesions were found on Giza 172 and the narrowest ones were found on Giza 175. Lesion size ranged from a low of 1.62 mm^2 (for Giza 175) to a high of 31.06 mm^2 (for Giza 172). The diseased leaf area of the different varieties ranged between 0.73% to 53.41% and from 0.86% to 48.77% in 1989 and 1990 seasons respectively. In average, the highest diseased leaf area (51%) was found in Giza 172.

This study showed a wide variation for all measurements used for the tested varieties. Even though the indica rice varieties used in this investigation had various levels of susceptibility in other countries, they all showed complete resistance under Egyptian conditions. In a study on the components of partial resistance using some Egyptian rice varieties and promising lines, KAMEL and EL-SHARKAWY (1987) likewise reported that the varieties IR1626-203 (Giza 181) and IR28 could not be evaluated as no pathotype of the fungus has been found. The INGER results agree with BONMAN and co-workers (1986), who found IR36 to possess partial resistance to blast and concluded that this partial resistance is associated with durability.

It is important for the breeding program that variety Giza 175 showed the lowest values for lesion type, number, length, size and diseased leaf area among the other Egyptian varieties under the same conditions. KAMEL and EL-SHARKAWY (1987) found that the line Gz 1394-10-1 (=Giza 175) had a high level of partial resistance, as it showed the smallest and lowest number of lesions, sporulation capacity and relative infection efficiency; however, it showed race-specific sporulation as it is susceptible to some pathotypes and resistant to others, indicating the presence of both complete and partial resistance. The report of INGER (1990) clarified that none of the Egyptian rice varieties including Giza 181 (indica) have worldwide immunity to blast, while the Egyptian rice variety Giza 175 (indica × japonica) was resistant in all nurseries except in three locations (Table 4). This leads to the conclusion that the latter variety may have durable resistance according to BONMAN and MACKILL (1988). They stated that durable resistance is associated with partial resistance controlled by undetermined number of genes. These results confirm the conclusion that it is difficult to study the durable resistance to rice blast for the indica varieties under the Egyptian conditions because of the absence of the compatible pathotypes of the fungus, while this test is more suitable for the japonicas under the same conditions together with other sites.

In the comparison of the results from this study with INGER results, those results that can be verified largely corresponded with the findings in Egypt. Therefore, the INGER results may also allow speculation of the level of partial resistance to be expected in the currently immune Egyptian indica varieties should

virulent races of blast enter the country. In that eventuality Giza 181 would have a high probability of being susceptible.

Table 4

Relative frequency of occurrence of blast ratings in sites of International Rice Blast Nursery (IRBN), 1983–1990[a]

Varieties	Sites	Blast scores (%)		
		0–3	4–6	7–9
Japonica				
Giza 171	51	31.4	43.1	25.5
Giza 172	76	23.7	40.8	35.5
Pi No 4	279	61.3	25.4	13.3
Toride 1	53	75.5	18.9	5.7
Indica				
Giza 181	5	20.0	20.0	60.0
IR28	169	60.9	30.0	8.9
IR36	375	68.5	25.1	6.4
IR50	307	52.8	30.0	17.3
Ind. × jap.				
Giza 175	5	60.0	20.0	20.0

[a] Courtesy INGER

Thus it can be concluded that it is useful to utilize the indica varieties such as IR36 and the indica × japonica variety, Giza 175 that possess durable resistance under the Egyptian conditions in hybridization program together with the local varieties to combine durable resistance with the other characters such as early maturity and good grain quality. However, based on these INGER results, if Giza 181 is to be used as a parent in breeding programs care should be taken to include other donors, for durable blast resistance.

It would be useful to test promising indica lines of the Egyptian program systematically in INGER or at other places, where blast races virulent on indica varieties make testing for durable resistance possible.

*

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FERMENTATION AND PROPERTIES OF THERMOSTABLE PROTEINASE

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The fermentation characteristics and properties of a heat stable proteinase produced by a newly isolated Bacillus strain were investigated in shake flask and laboratory scale fermentors. Optimal growth and production temperature and pH were 55 °C and 7, respectively. The time course of laboratory fermentation showed that the growth was completed in the first 12 h while enzyme production continued further in the late growth and sporulation phases for 24 h. Some characteristic values of the enzymatic casein hydrolysis were also determined: optimum pH was 9.5 and optimum temperature was 60 °C. Both values were significantly different from the optimal fermentation conditions. Analysis of temperature dependence (E_a and Q_{10}) of the enzymatic breakdown of casein showed that the enzyme was a thermostable alkaline proteinase.

Keywords: fermentation, production, proteinase, thermostable

There have been extensive studies regarding the extracellular enzyme producing ability of the genus Bacillus. A number of research works has been dealt with the influence of various environmental conditions on the production of thermostable proteinase (CHANDRA et al., 1980; OKADA et al., 1984; CHO et al., 1987; FUJIWARA & YAMAMOTO, 1987). Some reports were focused on the effect of suitable C-sources and other culture media components for high enzyme excretion as well as on enzyme production kinetics (CANTERO, 1990). Other authors reported data on the role of amino acid composition of the protein on the thermophilic character of these enzymes (ZEIKUS, 1979). Activity and heat stability of alkaline proteinases were examined and characterized under extreme conditions (pH and temperature) by many authors (TSUCHIDA et al., 1986; MANACHINI et al., 1988; TAKAMI et al., 1989). Importance of these studies is obvious because of the increasing industrial exploitation of thermostable enzymes. They are attractive candidates for several applications in the field of leather manufacturing (UNDERKOPFLER, 1976) and as laundry detergent additives (AUNSTRUP et al., 1972).

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In this study the proteolytic enzyme production of a newly isolated thermophilic Bacillus strain was investigated. Some data are also presented regarding the properties of the crude enzyme solution.

1. Materials and methods

1.1. Materials

1.1.1. Bacterium strain. The strain used in this work was *Bacillus sp* No. HC5 isolated from a Hungarian compost sample (SEVELLA et al., 1991). Some morphological and biochemical characteristics of this strain were examined by the methods described by BAJPAI and BAJPAI (1989).

1.1.2. Maintenance and inoculation. The microorganism was maintained at 5 °C on nutrient agar containing 1% Bacto Trypton, 0.25% Bacto Yeast Extract and 3% Bacto Agar (pH 7) after incubation at 55 °C for 24 h. For inoculating shake flask and lab fermenter experiments, 50 cm^3 culture was prepared in shake flask of 250 cm^3 using the same medium as for fermentation experiments.

1.1.3. Cultivation conditions. 1.1.3.1. Shake flask experiments – Microbial cultivation was carried out at 300 r.p.m. and 55 °C for 12 h in a G–25 Rotatory shaker (New Brunswick Sci. Co.). The culture medium was a modified medium (ELFADALY et al., 1991) that of used by MANABE and co-workers (1985). The medium composition (in g per 100 cm^3 tap water) was as follows: 80 cm^3 soybean meal extract (filtrate of 1 h digestion of 50 g soybean meal with 300 cm^3 of 0.5 *N* NaOH at 50 °C), 0.8 soluble starch, 0.03 KH_2PO_4, 0.05 $MgSO_4 \cdot 7H_2O$, 0.05 KCl, 0.2 $CaCl_2$, and pH 7.0–7.2 (adjusted before sterilization).

1.1.3.2. Bioreactor operation – Batch cultivation experiments were run in a Biostat M bioreactor (B. Braun Melsungen) applying 1.2 dm^3 working volume and 10% inoculum. Operation conditions were as follows: agitation 1000 r.p.m. aeration rate 1.5 vvm, temperature 55 °C ±0.2 and initial medium pH was 7. Foaming was automatically controlled by adding Glanapon DG–100 antifoam agent.

1.2. Analytical methods

1.2.1. Bacterial growth was measured as optical density of a 25-times water dilution of culture broth at 620 nm in Ultrospec Plus (Pharmacia LKB) spectrophotometer.

1.2.2. Total nucleic acid content (TNA) of the cellular material as alternative cell growth monitoring parameter was determined according to LEVINE and COONEY (1973). This method applies a perchloric acid extraction followed by

spectrophotometry at 260 nm. For quantitative assay, standard nucleic acid solution from either RNA (bakers yeast) or DNA (chicken erythrocytes) were used.

1.2.3. Determination of carbohydrate. Total carbohydrate consumption was measured using the colorimetric Anthrone method (PLUMMER, 1987) with glucose standard.

1.2.4. Enzyme activity. Quantitative assay of proteinase activity was carried out using the modified casein digestion method (LUPIN et al., 1982): one cm^3 of appropriately diluted enzyme solution ($10-100$ U cm^{-3}) was preincubated at 60 °C for 2 min. One cm^3 or similarly preincubated 1% casein solution (Hammerstein quality) (prepared in phosphate buffer pH 7.5 for neutral proteinase (NP) or in carbonate-bicarbonate buffer pH 9.5 for alkaline proteinase (AP)) was added and incubated for further 10 min. The enzyme reaction was terminated by adding 3 cm^3 5% trichloroacetic acid (TCA) solution. The precipitated undigested casein was separated by centrifugation at 3000 r.p.m. for 10 min. Absorbance of the supernatant was measured against distilled water at 280 nm 1 cm cell using Ultrospec Plus (Pharmacia LKB) spectrophotometer. An UV-absorbing background was taken into account subtracting the absorbance of another test which was prepared by the same way in reverse order not allowing the development of enzymatic breakdown of the casein. The resulting reading difference was calibrated to the reading of standard tyrosine solution. One unit (TU) proteinase was defined as the quantity of enzyme which deliberates 1 μmol tyrosine-equivalent in the form of 5% TCA soluble casein fragments, under the above-mentioned reaction conditions in 1 min.

2. Results and discussion

2.1. Strain characterization

Microscopic examinations showed that the organism belongs to the spore forming, long rod shaped, motile, and Gram positive bacteria. The microorganism showed positive catalase, caseinase, gelatinase, and amylase activities. The cells formed chain, the spores were spherical and located at the center of the cells proving that they are closely related to the genus Bacillus.

2.2. Microbial growth

2.2.1. Temperature effect. To determine the optimum temperature of growth and enzyme production, shake flask experiments were carried out at 50, 55 and 60 °C. The results in Fig. 1 show that 55 °C was the most favourable in respect of the growth and enzyme activity produced. Maximum growth was reached at 12 h at 50 °C and 24 h at 55 °C and 60 °C (Fig. 1a). At 50 °C the final neutral proteinase activity was

half of the value obtained at 55 °C (Fig. 1b) while the alkaline proteinase activity showed about 60% increase at the higher temperature (Fig. 1c). Data showed also that no enzyme activity was observed at 60 °C, corresponding to poor growth.

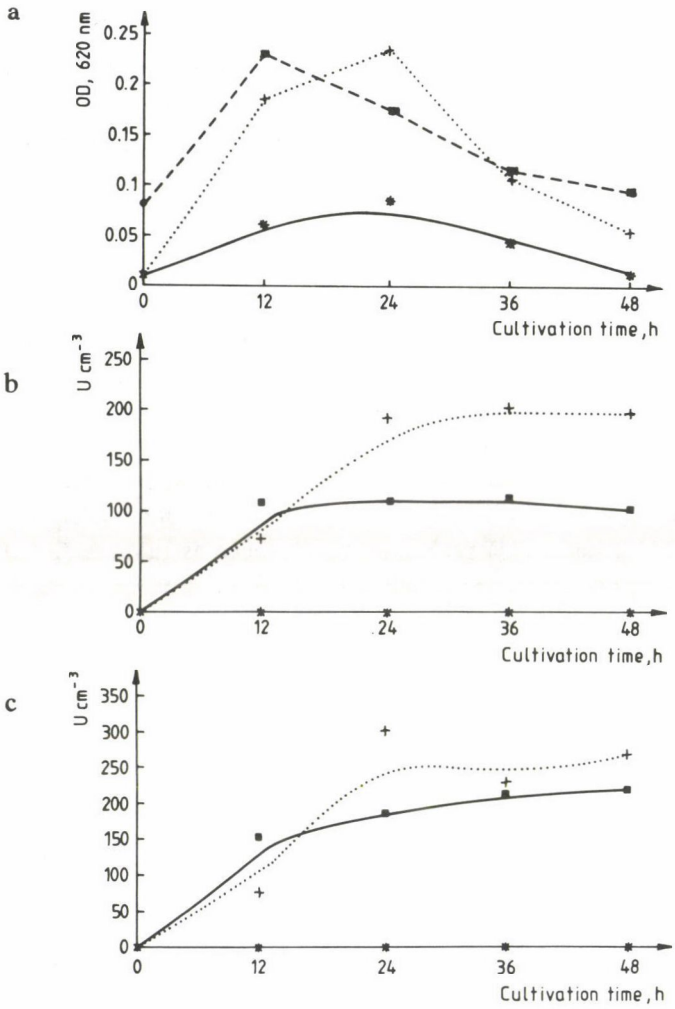

Fig. 1a–c. Effect of temperature on growth and enzyme production. a: Optical density at 620 nm; b: Neutral proteinase (NP) at 280 nm; c: Alkaline proteinase (AP) at 280 nm. ■ : at 50 °C; + : at 55 °C *: at 60 °C

Nevertheless this strain can be considered as a thermophilic Bacillus according to the definition, viz. any organism having an optimum growth temperature above 45 °C (SONNLEITNER, 1984).

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TUDOMÁNYOS AKADÉMIA
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From graphed data, it can be also seen that no lag phase was observed because the same culture medium composition was used in preparing the inoculum which gave the same physiological state for the microbial growth.

According to these data, the forthcoming experiments were only focused on the alkaline proteinase since it showed higher activity than the neutral one.

2.2.2. Effect of pH. According to the preliminary experiments concerning the effect of pH on the enzyme production, the initial pH of growth medium was adjusted to various levels (pH 7–10) with 0.1 *N* HCl or NaOH solution. Experiments were run at 55 °C for 48 h. Figure 2 illustrates the influence of initial pH on alkaline proteinase production. Increasing the pH value resulted in an almost linear and steep decrease in the final activity while the final pH of the culture broth at every case was in the range of 8.0–8.2. These data also suggest that the strain HC5 used in this work is a thermoneutrophile Bacillus species according to BROCK (1978) who pointed out that this group of microorganisms has a pH optimum between 5.8 and 8.5.

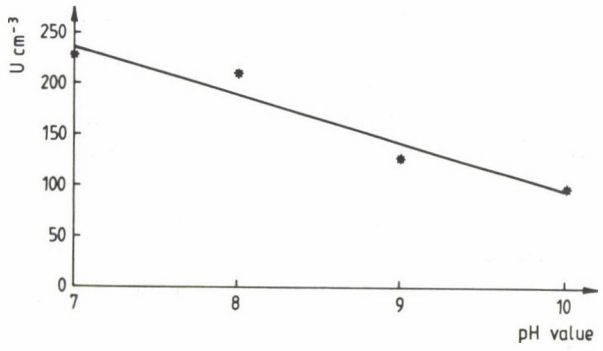

Fig. 2. Effect of initial pH of the culture medium on alkaline proteinase production

2.3. Enzyme production in bioreactor

Laboratory scale cultivation experiments in Biostat M reactor were carried out to examine the time course of the growth and enzyme production. One of these experiments with pH control at pH 7 (adding 20% sulfuric acid) is shown in Fig. 3. The growth phase was completed after 6 h. The lowest dissolved oxygen tension (about 30% saturation level) was reached also at that time, and followed by a slow gradual increase in DO and simultaneous decrease in the bacterial growth. It is noteworthy that whereas the total cell mass decreased after 6 h there was a further remarkable carbohydrate consumption which proved the energy requirement of the proceeding enzyme synthesis and/or excretion outside the cell. These data are in

accordance with the results of CANTERO (1990) or MOON and PARULEKAR (1991) who also observed that enzyme production was started in the growth phase but continued in the period of lysis and sporulation too.

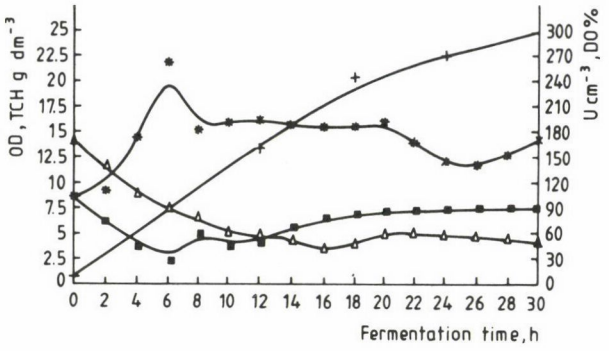

Fig. 3. Time course of pH-controlled lab scale fermentation. *: Optical density at 620 nm; Δ: Total carbohydrate concentration, g dm^{-3}; ■ : Dissolved oxygen, %; +: Enzyme activity, U cm^{-3}

Due to the incomplete solubility of the culture medium components as well as the partial coagulation, there arose a question whether the optical density was a good measure of the cell growth. To clarify this problem, total nucleic acid content of the cell material was measured and the results are shown in Fig. 4. According to the figure, it could be concluded that the OD can be used for bacterial growth monitor even in opaque media. The figure showed also that the decline in the growth curve after the first 6 h of cultivation was caused by the autolysis of the cells.

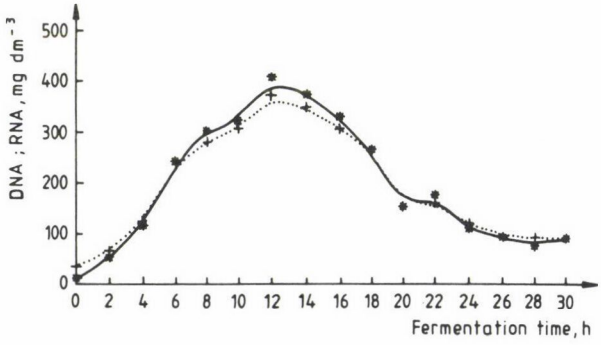

Fig. 4. Growth monitor during the course of fermentation. *: RNA, mg dm^{-3}; +: DNA, mg dm^{-3}

2.4. Properties of enzyme reaction

Extracellular enzymes are biosynthetised and excreted into the surrounding environment to break down the high molecular weight proteins and carbohydrate to make their cellular uptake possible. From this fact it would be logical to suppose that the optimal pH and temperature of the action of these enzymes are likely the same as those of growth and enzyme production.

2.4.1. Temperature. The proteolytic activity of the cell free culture broth of strain HC5 was examined in the range of 45–70 °C. Figure 5 shows a relatively flat optimum curve between 55 and 65 °C and sharp decrease before and after that range. The maximal enzyme activity was found at 60 °C which was significantly different from the 50 °C optimal growth temperature.

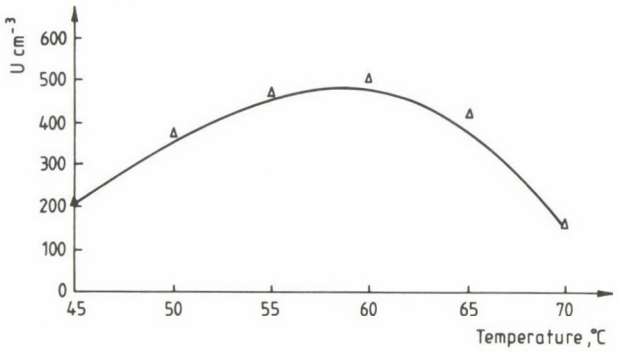

Fig. 5. Effect of temperature on enzyme activity

2.4.2. pH. The effect of pH on the enzyme activity was also compared in a wide range of pH: 7–10.5. Figure 6 proves that the pH optimum was 9.5 with a moderate decrease before and a very steep decrease after that point. This optimum value was again significantly different from both the optimal initial pH value (7) and the final culture broth pH values (8–8.2) as well.

2.4.3. Activation energy. Figure 7 shows the Arrhenius plot of the casein breakdown by crude enzyme solution based upon initial reaction rate determinations. According to the 60 °C optimum temperature, the plot consists of two linear fragments. From the straight line below 60 °C the activation energy was found to be E_a = 50.63 kJ mol^{-1}. E_a values of enzymes from thermophiles are much higher than those from meso- and psychrophiles, e.g. JACKMAN and co-workers (1983) pointed out that E_a for mesophiles was three times higher than for psychrophile strains (50.22 kJ mol^{-1} and 7–13 kJ mol^{-1}, respectively).

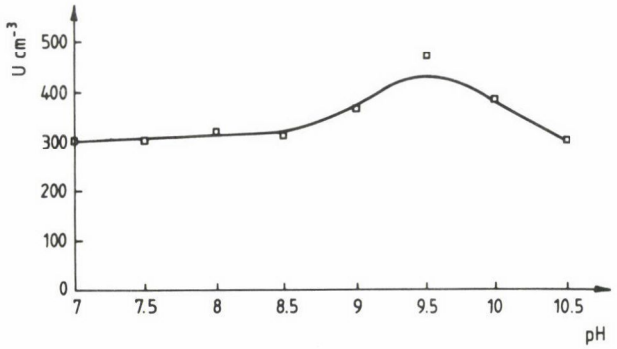

Fig. 6. Effect of pH on enzyme activity

The measured Q_{10} value was 1.5 and it is in good agreement with the results of ZEIKUS (1979) who pointed out that Q_{10} values for thermophilic enzymes lie in the range 1.4 to 1.9 which considerably depends on the molecular properties and nature of the enzymes.

On the other hand the straight line with positive slope over 60 °C suggests a reversible thermal deactivation of the enzyme in this range. From this latter straight line the deactivation enthalpy (ΔH_d) was approximately 124.58 kJ mol^{-1} (BAILEY & OLLIS, 1986).

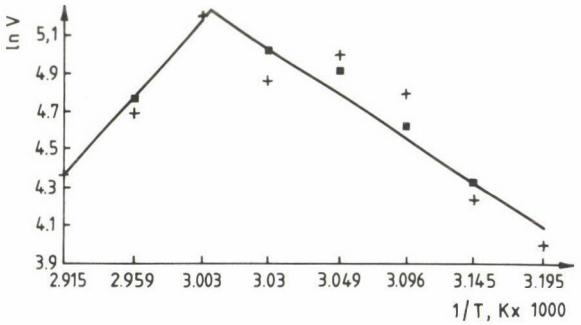

Fig. 7. Effect of temperature on the rate of enzymatic casein hydrolysis. T: absolute temperature; V: velocity of the enzyme reaction; ■: Predicted values; +: Experimental observation; Right straight line: $\ln(V) = 8.517 * 10^3 * (1/T) + 31.247$, $r = 0.89$; Left straight line: $\ln(V) = 16.477 * 10^3 * (1/T) - 43.946$, $r = 0.99$

3. Conclusion

As a part of our programs, this work showed the possibility to produce a thermostable alkaline proteinase by a bacterial strain isolated from local material.

It can be concluded that the rapid growth (12 h in shake flask and 6 h in bioreactor) of thermophiles resulted in greatly reduced cultivation time which can be useful from economic point of view.

The technical application of thermophilic bacteria could be highly advantageous as compared with classical bioprocesses which require temperature ranging between room temperature and about 37 °C. Thermophilic processes have also a great benefit in reducing contaminations by other widespread microorganisms. Furthermore, cooling is cheaper and easier for the reduced cooling cost requirement and higher temperature difference of the heat exchange.

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COMPARATIVE STUDY OF PROTEINS OF SOME RYE AND TRITICALE CULTIVARS GROWN IN HUNGARY, USING HPGLC

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Five rye cultivars and five triticale cultivars grown in Hungary were investigated. Protein content, protein fractions according to Osborn were measured. The albumin, globulin, prolamin and residue (glutelin) fractions were separated by high performance gel liquid chromatography (HPGLC).

Triticale cultivars had higher protein content and lower proportion of albumins and globulins than rye cultivars. Using HPGLC characteristic elution profiles were obtained containing generally four peaks. Mathematical statistical evaluation of results revealed significant correlations between total protein content and fraction distribution.

Keywords: rye, triticale, protein, HPGLC

Comparing the literature of wheat and rye proteins it can be stated that our knowledge concerning rye proteins is much poorer than wheat proteins. Such a situation may be explained first of all with two facts: (1) standing decreasing production and use of rye in bread making, (2) the less important role of rye proteins in formation of dough and bread structure compared with wheat proteins (LÁSZTITY, 1984; POMERANZ, 1987). In case of wheat flour dough and wheat bread a well-expressed continuous protein matrix is characteristic and the starch granules (partly gelatinised in bread) are embedded in this matrix. In rye dough the starch granules are surrounded not only by proteins but also by non-starchy polysaccharides, the protein matrix is not so expressed and discontinuous. In the bread crumb starch granules are highly gelatinised and have a looser structure (WINKEL & FLAMME, 1983; SEIBEL et al., 1983; POMERANZ & SEIBEL, 1984).

The triticale, a new cereal species synthesised by man combining the genomes of wheat and rye, plays at this time a very small role in the cereal production. Nevertheless the newer achievements in triticale utilisation confirm the views that triticale might be successfully used both in human nutrition and animal feeding (LÁSZTITY, 1984; TSEN, 1974).

The aim of the present work was to investigate and compare the protein composition of some rye and triticale varieties grown in Hungary by HPGLC.

1. Materials and methods

Table 1 shows the rye and triticale varieties investigated. Samples were made available by the Research Institute of Plant Growing, Kecskemét (Hungary).

Table 1

Rye and triticale varieties investigated

Serial number	Name	Species
1	BL	Triticale
2	KT-13	Triticale
3	KT-15	Triticale
4	KT-84	Triticale
5	KT-200	Triticale
6	Kisvárdian 1	Rye
7	Lovászpatonian	Rye
8	Halo	Rye
9	D. Zeote	Rye
10	D. Nowe	Rye

Fifteen g of sample were comminuted to a particle size below 0.2 mm in a laboratory mill (Type QC 114 hammer crusher, LABOR MIM Budapest, Hungary) and the break obtained was for the investigations.

The moisture content of the samples was determined after drying at 105 °C for 4 h. The relative standard deviation was 1.2%.

The protein content of the whole meal and their extracts by sodium chloride solution, ethanol and sodium hydroxide was calculated from the quantity of ammonia formed by Kjeldahl's method, using a factor of 5.7. The quantity of ammonia was determined with a "CONTIFLO" automatic analyser, using Berthelot's reaction. The relative standard deviation was 2%.

Fractionation of proteins was realised as shown in Fig. 1. The sample (0.5 g whole meal) was extracted with 0.5 mol l^{-1} sodium chloride solution and then with 70% (v/v) ethanol. The given 20 cm^3 72% (v/v) ethanol with the water content of samples gives 70% (v/v) ethanol concentration. For the quantitative determination of proteins the residue was extracted with 0.1 mol l^{-1} sodium hydroxide and for the purpose of HPGLC separation with 10 g dm^{-3} SDS (sodium dodecil-sulfate, product of Reanal, Hungary) and 10 g dm^{-3} mercaptoethanol (product of Fluka A. G., Germany) mixture at 60 °C. The supernatant was separated from the residue on a

centrifuge (type LC 425, Labor MIM, Budapest, Hungary) centrifuging for 3 min at a speed of 3500 min^{-1}.

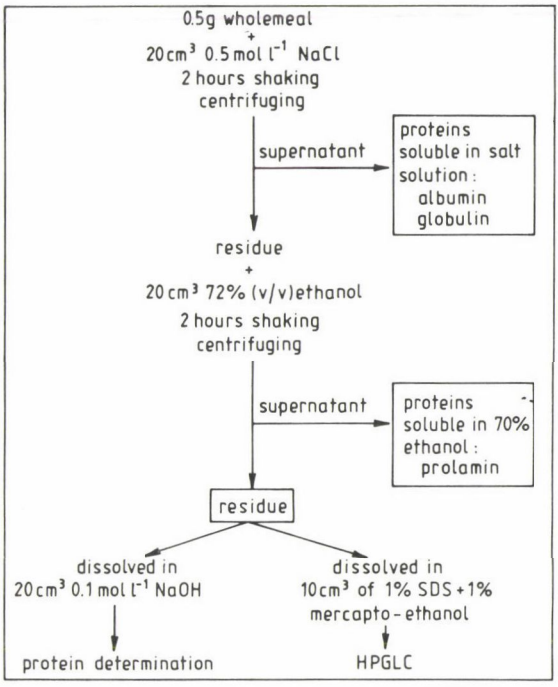

Fig. 1. Separation pattern of proteins

The HPGLC separation of soluble macro fraction was characterised by the following parameters:

Equipment	Waters liquid chromatograph M45 pump M440 photometer U6K sample injection unit
Separation column	300 × 8 mm MicroPack TSK G3000SW
Eluent	2 g dm^{-3} SDS + 0.2 mol l^{-1} NaH_2PO_4, pH 4.5
Flow rate	1 cm^3 min^{-1}
Temperature	22 °C
Injected volume	20 mm^3
Detector	UV 280 nm
Sensitivity	0.02 – 0.1 absorbance unit/20 mV

The calibration curve for determination of molar mass from retention time was made by proteins known molar mass: the next equation gives valid result in the range 10 – 300 kD with ± 4 kD standard deviation.

$$\log(M) = 5.555 - 0.52095\ V_R$$

M = molar mass, kDalton

V_R = retention volume, cm^3

Mathematical-statistical evaluation of the result: The investigated samples were characterised as vectors with 9 variables. The correlation matrix of variables and principal components of samples was determined by computer program Statgraphic.

2. Results and discussion

The moisture and protein content of samples are summarised in Table 2.

Table 2

Moisture and protein content of rye and triticale samples

Serial number	Name	Moisture (%)	Protein (%)	Protein referred to dry matter (%)	Average and standard deviation of protein (%) $\bar{x}, \pm s$
1	BGL	10.84	17.77	19.33	
2	KT-13	11.19	16.13	18.16	
3	KT-15	10.23	17.64	19.65	19.2
4	KT-84	10.00	15.01	16.68	±1.8
5	KT-200	10.21	19.17	21.35	
6	Kisvárdian 1	10.31	15.35	17.11	
7	Lovászpatonian	10.98	15.56	17.48	
8	Halo	10.44	13.42	14.98	16.2
9	D. Zeote	10.44	13.71	15.30	±1.1
10	D. Nowe	8.82	14.49	15.89	

As it is shown, the total protein content is relatively high. The triticale is in every case richer in protein, than the rye.

The distribution of soluble macro fractions is shown in Table 3.

The total quantity of the fractions corresponds to the quantity of total protein obtained with Kjeldahl's process with an accuracy of ±10%. Similar differences were found also concerning the percentage distribution of the fractions between rye and triticale. The average quantity of albumin + globulin fraction was in triticale 40±0.3%, while that of rye samples was 50±0.8%, that is significantly higher by about 10%. The quantity of prolamin was in triticale 25±1.6%, while in rye samples only 20±0.9%, that is significantly less by about 5%. The average quantity of residual protein of glutelin character was in triticale 35±1.5%, in rye 31±1.1%.

Table 3

Quantity of protein fraction

Serial number	Name	Total protein (mg/g)	Albumin + globulin (mg/g)	(%)	Soluble in alcohol (mg/g)	(%)	Residue (mg/g)	(%)
1	BGL	174	71	41	42	24	61	35
2	KT - 13	170	67	39	45	26	58	34
3	KT - 15	184	71	39	48	26	65	35
4	KT - 84	161	64	40	37	23	60	37
5	KT - 200	181	73	40	49	37	59	33
6	Kisvárdian 1	154	76	49	30	19	48	31
7	Lovászpatonian	158	79	50	31	20	48	30
8	Halo	155	76	49	30	19	49	32
9	D. Zeote	154	78	51	29	19	47	31
10	D. Nowe	145	73	50	30	21	42	29

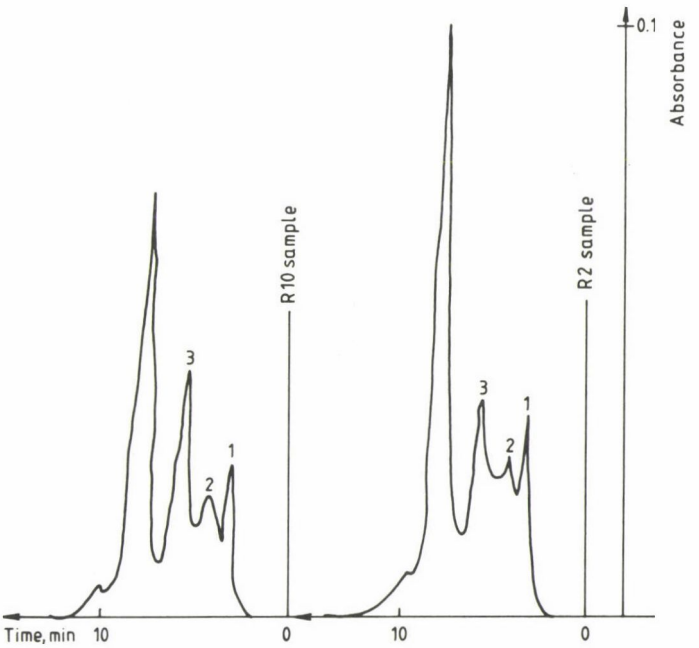

Fig. 2. Separation of proteins soluble in 0.5 mol l^{-1} sodium solution by HPGLC. R2: KT 13 Triticale; R10: D. Nowe rye. The proteins signed by 1, 2 and 3 correspond according to the checked calibration curve to molar masses of 300, 100 and 14 kD, respectively. The fourth peak represents the low molecular mass components of non-protein character of the sample, absorbing in UV

The glutelin : prolamin ratio was found in triticale to be 1.39 ± 0.15, while in rye 1.57 ± 0.12, that is significantly higher.

The solution of protein fractions obtained by extraction was further fractionated with HPGLC. Figure 2 shows the chromatograms of triticale sample No. 2 and rye sample No. 10.

Generally three components were separated. These correspond according to the checked calibration curve to molar masses of 300, 100 and 14 kDalton, respectively. The fourth peak represents the low molecular mass components of non-protein character of the sample, absorbing in UV. The rye sample is characterised by a considerable surplus of peak 1.

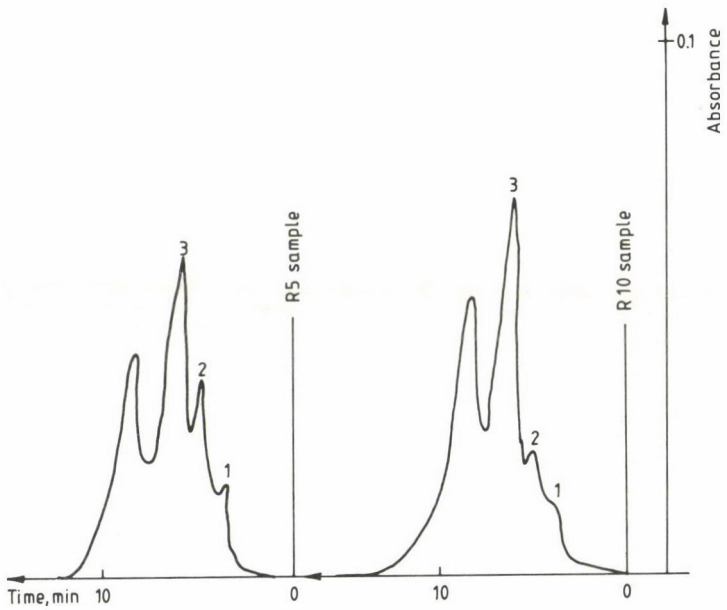

Fig. 3. Separation of proteins soluble in 70% (v/v) ethanol. R5: KT 200 Triticale; R10: D. Nowe rye. Proteins signed by 1, 2 and 3 correspond to molar masses of 200, 43 and 10 kD, respectively

Figure 3 shows the separation pattern of protein fractions soluble in 70% alcohol for triticale sample No. 5 and rye sample No. 10. In triticale a considerably higher quantity of the medium fraction can be observed. Proteins correspond to molar masses of 200, 43 and 10 kDalton, respectively.

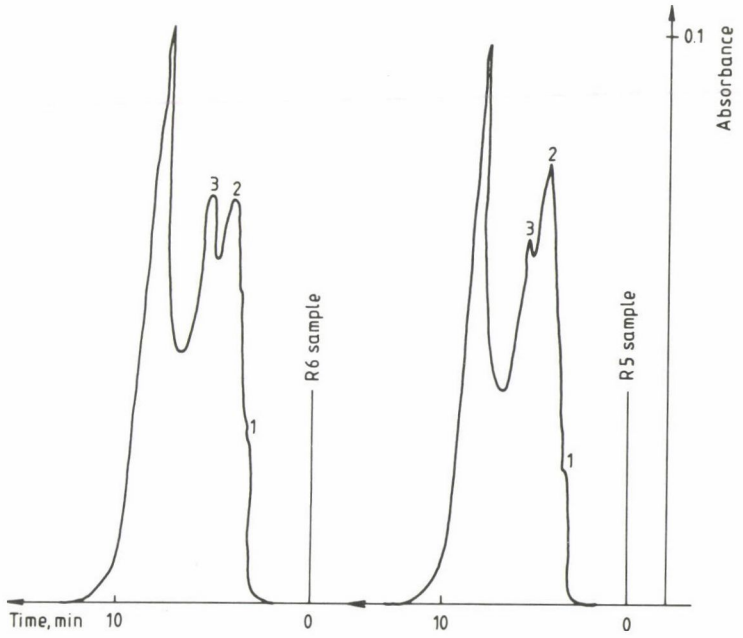

Fig. 4. Separation of the residue proteins (solved in SDS-mercapto-ethanol solution). R5: KT 200 Triticale; R6: Kisvárdian rye. The proteins signed by 1, 2 and 3 correspond to molar masses of 200, 37 and 21 kD, respectively

Figure 4 shows the separation of the fraction of residual proteins, dissolved in SDS and mercaptoethanol. These proteins can be separated into three fractions, too, corresponding to molar masses of 200, 37 and 21 kDalton, respectively. In triticale fraction 2 exceeds fraction 3, while in the case of rye an inverse proportion can be observed. The distribution of the protein fractions of the samples investigated is summarised in Table 4.

Table 5 contains the calculated average values ($\bar{x}$) and standard deviation ($\pm s$) for triticale and rye samples. The differences between the two species are also indicated.

Data in Table 5 support that these species significantly differ in the fraction distribution. It is characteristic that triticale contains more of fraction of higher molecular mass, while rye is richer in the fraction of lower molecular mass.

For the complex mathematical-statistical evaluation of composition the relationships among the various fractions were investigated. Since the results of protein separation by HPGLC were given as percentile proportion of the separated peek field to the total area, only two of three were taken into consideration since the sum of three is always 100%.

Table 4

Distribution of protein fractions (%) separated by HPGLC

Serial number	Name	Albumin + globulin fractions			Prolamin fractions			Residue fractions		
		1	2	3	1	2	3	1	2	3
1	BGL	18	29	53	5.6	22	73	5.5	52	42
2	KT-13	17	30	53	5.7	28	66	9.5	52	39
3	KT-15	15	32	53	5.9	28	66	6.9	54	39
4	KT-84	16	33	51	5.7	25	69	7.1	55	38
5	KT-200	15	33	52	7.4	27	66	5.1	55	40
6	Kisvárdian 1	11	28	62	4.2	12	84	6.4	49	44
7	Lovászpatonian	10	24	67	4.0	11	85	3.6	48	48
8	Halo	8.8	22	69	4.0	11	85	3.6	47	50
9	D. Zeote	9.1	23	68	3.3	10	86	6.6	47	46
10	D. Nowe	12.5	25	63	3.5	9.9	87	6.5	46	48

Table 5

Average and standard deviation values of fraction distribution (in %)

Serial No. of samples	Name of species	Albumin + globulin fractions			Prolamin fractions			Residue fractions		
		1	2	3	1	2	3	1	2	3
1-5	Triticale									
	$\bar{x}$	16	31	52	6.1	26	68	6.8	54	40
	$\pm s$	±1.3	±1.8	±0.9	±6.8	±2.6	±3.1	±1.7	±1.5	±1.5
5-10	Rye									
	$\bar{x}$	10.3	24	66	3.8	11	85	5.3	47	47
	$\pm s$	±1.5	±2.3	±3.1	±0.4	±0.9	±1.1	±1.6	±1.1	±2.3
	Difference	5.7	7.0	-14	2.3	15	-17	1.5	7.0	-7.0

The significant correlation coefficients were summarised in Table 1.

With the exception of fraction 1 of the residue, close significant correlations were found between the variables. The quantity of the fraction soluble in sodium chloride solutions is negatively correlated with the quantity of total protein and other fractions except fraction 1 of rest protein.

Owing to the many significant correlations, it is worth to investigate the data by principal component analysis as fewer variables may also be sufficient for the description of the multivariable system. In Table 7 the weight hand separations of two principal components are shown according to the original variables.

Table 6

Correlation between protein fractions (100-fold of correlation coefficient)

No. of variable	Name of variable	Number of variable							
		2	3	4	5	6	7	8	9
1	Kjeldahl protein	−73	83	69	73	86	75	7	75
2	Salt soluble total		−93	−89	−89	−91	−99	−47	−93
3	Alcohol soluble total			85	83	91	94	42	84
4	Salt soluble fraction 1				82	77	86	53	79
5	Salt soluble fraction 2					89	90	47	95
6	Alcohol-soluble fraction 1						92	21	93
7	Alcohol-soluble fraction 2							49	94
8	Residue fraction 1								32

$R_{95\%}$ = 0.631 least significant correlation coefficient

Table 7

Variance of the first two principal components and the weight of variables

Principal component	Variance (%)	Weight (%) of variables								
		1	2	3	4	5	6	7	8	9
I	80	9.5	13	13	11	12	12	13	3	13
II	12	16	0.3	0.1	3.0	0.1	6.0	0.4	72	1.0

Table 7 shows that the first two principal components contain 92% of the variance of the original system with 9 variables, so that the two principal components are very suitable for the adequate description of the system. While the first principal component contains all variables with almost identical weight except No. 8 (first

fraction of rest). The second principal component contains exclusively variable No. 8 with highest weight. Figure 5 shows the projection of the original variables on the plane of the first two principal components. Three groups of variables can be distinguished: group 1: variables 1, 3, 4, 5, 6, 7 and 9 take the positive direction of the first principal component; group 2: variable 2 directs to the negative field of the first principal component because of its negative correlation with most of the other variables; group 3: variable 8 takes the positive direction of the second principal component.

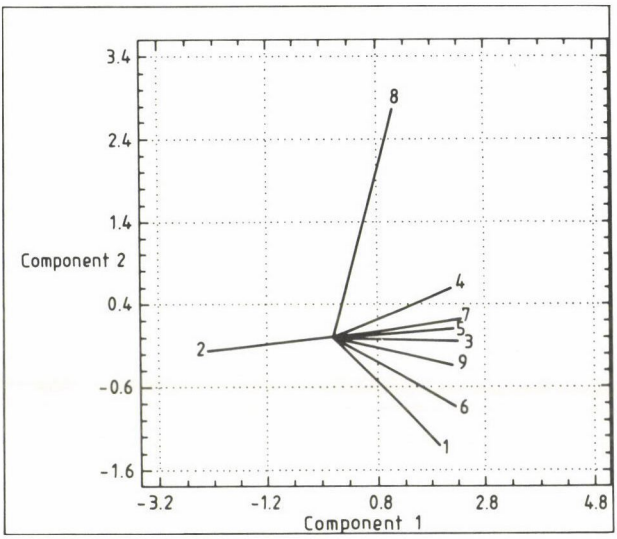

Fig. 5. The projection of the original variable on the plane of the first two pricipal components.

Data vectors:	
1. Kjeldahl protein	%
2. Alb – Glob total	% of Kjeldahl protein
3. Prolamin total	% of Kjeldahl protein
4. Alb-Glob 1 fraction	% of Alb-Glob total
5. Alb-Glob 2 fraction	% of Alb-Glob total
6. Prolamin 1 fraction	% of Prolamin total
7. Prolamin 2 fraction	% of Prolamin total
8. Glutelin 1 fraction	% of Rest total
9. Glutelin 2 fraction	% of Rest total

In Fig. 6 the samples were plotted in the plane of the first two principal components.

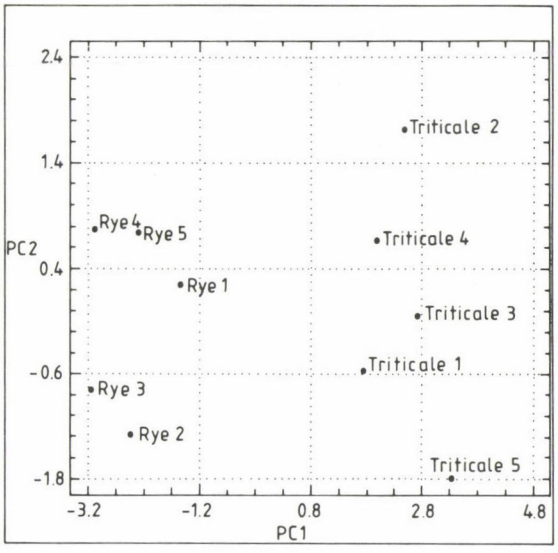

Fig. 6. Samples plotted in the plane of the first two principal components

Figure 6 shows that triticale samples No. 1–5 and rye samples No. 6–10 are clearly separated and can be distinguished on the basis of their characteristic protein composition. Rye samples are located in the direction of the high albumin + globulin fraction (variable 2) while triticale samples are at the opposite end as their albumin + globulin proportions are lower then in the rye and they have higher quantity for the majority of the protein fractions. Along the second principal component the individual differences of the samples are primarily reflected by the characteristic variable No. 8 (the glutelin fraction of high molecular mass).

Summarizing the results it can be established that HPGLC of protein components combined with traditional fractionation according to Osborn is a suitable tool for investigating and distinguishing rye and triticale.

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IMPORTANCE AND UTILIZATION OF CHICKPEA IN CEREAL TECHNOLOGY

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Two cultivars of chickpea (*Cicer arietinum*) grown in Slovakia and Iraq were investigated. Chemical composition (moisture, ash, petroleum ether extract, total nitrogen, P, Ca, Mg, Na, K, Cu, Fe, Mn, Zn, amino acids, fatty acids, thiamine, riboflavin) was determined and compared. Some physical characteristics of grain such as colour, shape, surface properties, weight of 1000 kernels and bulk density, were also studied. Finally rheological properties of dough and sensory characteristics of baked goods containing wheat- and chickpea flour were investigated.

Relatively high protein (23–27%) and lipid content (5.8–6.2%) was observed in comparison to other leguminous plants. Phosphorus and potassium were the main mineral components. High lysine content (over 7 g/16 g N) and a considerable amount of essential amino acids (34–36%) were characteristic. The main fatty acid component is linoleic acid (about 70% of total fatty acid content). The whole grain contains significant amounts of vitamin B_1 and B_2. Chemical composition of the two cultivars is similar, only the protein contents differ significantly. Baked goods (bread, biscuits) containing 10–20% of chickpea flour had acceptable quality and higher nutritive value.

Keywords: chickpea, chemical composition, physical characteristics, use in bread and biscuits production

The chickpea is one of the agricultural plants grown in warm, dry and semi-dry regions. Only the variety *Cicer arietinum* is cultivated in more extensive areas.

The first reports of cultivation of the chickpea came from India (1st century A. D.), where it was used similarly to pea. It is mainly grown in the Mediterranean countries, and in some areas of the Community of Independent States (former USSR) in dry, non-irrigated regions.

It is most widespread in India and in Asia. It has bean cultivated in the warmest and dry regions of Slovakia and Moravia, nowadays it can be sporadically found in gardens.

It grows equally well in light, medium and heavy soils. It is also a very good pre-farm plant.

In the steppe- and subtropical-regions it is grown as a leguminous plant, but also as a vegetable. From the point of view of cultivation and harvesting it has the advantage that it does not drop and so it can be harvested by machines. For food industrial purposes varieties with pale coloured seeds are predominantly used. Other (e.g. black) cultivars are used for feed (SINSKY et al., 1985; HRUSKA, 1955; HOLOVLASKY, 1971; JAYA & VENKATARAMAN, 1979, 1980; TIWARY & SHARMA, 1977). Varieties cultivated in Slovakia yield 2 t ha^{-1} in average and under more favorable conditions up to 3 t ha^{-1}. In Slovakia chickpea showed higher yields than peas, especially in dry climate conditions. Its resistance to dryness surpasses other main types of leguminous plants.

Among leguminous plants, the chickpea occupies the second place in the world (in world production) to the beans. The seeds of chickpeas are used for preparing soups, as relish, and also coffee surrogate. In some countries it is ground to flour and then used in bread and pastry or meat spreads and sausages. The seeds can also be fried.

At this time, in Slovakia, a renaissance of leguminous plant cultivation can be observed. Chickpea belongs to the partly forgotten plants like earthnut pea and bean.

In comparison with other farm plants, the leguminous plants have some advantages. First they are classified among agricultural plants with high protein content and nutritional value. The presence of lectins and other antinutritional factors are disadvantageous.

To increase the consumption of leguminous plants it is necessary to ensure the production of sufficient amount of attractive, ready-to-cook and innovative products from these plants. The search for new suitable ingredients for the cereal industry led to the choice of non-conventional raw materials.

In this paper we summarize the results of our investigations concerning characteristics, chemical composition and possible uses of the chickpea in cereal processing.

Materials and methods

Whole grain flour of chickpea (*Cicer arietinum*) grain cultivated in Czecho-Slovakia on experimental fields in the Research Institute of Plant Production in Piešťany (sample A) (seeds originating from Syria) and that of imported from Iraq (sample B) were studied. The whole-grain flour was produced by milling in a laboratory impact-mill (VEB Nossener Maschinenbau). For bread and biscuits commercial white wheat flour (ON, 1977) was used.

The following primary characteristics of the grain were determined: bulk density, weight of 1000 kernels, sensory properties according to SMELIK and co-workers (1985).

Moisture, ash, petroleum ether extract, total nitrogen and titratable acids were determined as described by SMELIK and co-workers (1985). Fibertec apparatus was used for fiber determination (PRIBELA et al., 1979; DAVIDEK et al., 1981). Particle size distribution was studied by sieving through a silk sieve (230 μm, 160 μm). The mineral substances were determined by using atomic absorption spectrometry, thiamine and riboflavin were measured flourometrically by thiochromic and luminoflavine method (CZECHOSLOVAK STANDARD, 1970, 1972).

Amino acids were determined on the automatic amino acids analyzer AAA-T-339, Mikrotechna, Prague (MIKROTECHNA, 1983) using the modified method of Speckmann, Moor and Stein. The ninhydrine reaction was measured photometrically. The data from photometric detection were recorded on a TZ 4 100 recorder as absorbance.

The hydrolysate was prepared using 6 M hydrochloric acid, for 24 h at 105 °C. Following evaporation the residue was dissolved in 100% acetic acid and transferred to 25 cm^3 volumetric flasks containing 10% acetic acid.

The analysis of the amino acid mixture proceeds in the column containing ionex in Na^+ cycle. After automatic sample dosing the amino acids are eluated from the column with a series of sodium citrate buffer solutions, pH 3.5–4.25–9.5 at temperatures t_1 = 50 °C and t_2 = 60 °C.

Tryptophan was determined using the method of Roth and Schuster (PRIBELA et al., 1979; DAVIDEK et al., 1981).

Fatty acids were determined using the gas chromatograph CHROM 5 (Laboratorni pristroje, Praha) according PRIBELA and co-workers (1979).

The effect of addition of chickpea whole grain flour to wheat flour on the amount and characteristics of wet gluten (extensibility, elasticity, swelling) and the amount of dry gluten were studied using standard methods. Rheological characteristics of doughs were examined using Farinograph and Extensograph (SMELIK, 1985).

The laboratory microbaking test including sensory evaluation as well as the determination of specific density, moisture, content of titratable acids was also realized according to standards (SMELIK, 1985; DODOK, 1989). Experimental production of biscuits was made in Factory Sered according to DODOK (1989).

In all cases 10 and 20% whole grain chickpea flour was added to the wheat flour.

Results and discussion

Some physical properties of chickpea grains and whole grain flour are summarized in Table 1.

Table 1

Some physical characteristics of grains and whole grain flour of two chickpea cultivars

Characteristic	Cultivar grown in Slovakia	Cultivar grown in Iraq
Colour	bright, beige	bright, beige with rose tinge
Shape	round, with small characteristically sticking radicale	round, with marked characteristically sticking radicale
Surface	wrinkled	furrowed
Weight of 1000 kernels	303 g	367 g
Bulk density	0.816 g cm^{-3}	0.796 g cm^{-3}
Particle size distribution of whole grain flour		
smaller than 160 μm	70.2%	71.2%
over 230 μm	15.4%	13.2%
Colour of flour	bright creamy	creamy
Odour of flour	natural	similar to pea
Taste of flour	natural	sweetish

As it is shown, the special physical characteristics of grain need adaptation of a specific milling technology. The average chemical composition of A–B samples is shown in Table 2.

From the data of chemical analysis and organoleptic characteristics of flours from both cultivars of chickpea only small differences were observed with the exception of protein content being higher in cultivar grown in Slovakia.

Fibrous matter has been present in about half of the amounts found by other researches (VALIČEK et al., 1989).

A relatively high content of lipids was found. In the chickpea this figure is three times higher than other in leguminous plants, namely 5.8 and 6.2%, respectively compared to that of edible peas (1.39%), edible lentils (1.20%) and beans (1.60%) according STRMISKA and co-workers (1988).

Table 2

Chemical composition of the grains of two chickpea cultivars
(% dry matter basis)

Component	Cultivar grown in Slovakia	Cultivar grown in Iraq
Moisture	8.38	7.42
Dry matter	91.62	92.58
Ash	3.26	3.08
Petroleum ether extract	5.8	6.2
Total N	4.38	3.71
Protein (6.25 × N)	27.37	23.18
Fibre	3.20	2.89
P	5.19	3.18
Ca	1.01	1.61
Mg (g kg^{-1})	1.40	1.59
K	11.01	10.04
Na	0.05	0.16
Cu	11.74	11.04
Fe	82.4	114.09
Mn (mg kg^{-1})	16.47	32.95
Zn	26.94	33.41
Vitamin B_1 (mg kg^{-1})	5.4	5.8
Vitamin B_2 (mg kg^{-1})	5.8	15.8
Flour acidity (mmol kg^{-1})	88.6	82.4
after 6 weeks	89.0	84.5

Amounts of titratable acids in whole grain flours increased only slightly during 6 weeks (the period of our experiments). No changes of their organoleptic characteristics were observed.

Relatively high Ca and Mg content was found (Table 2). In mutual comparison of both samples of chickpea there are marked differences for other elements. A lower phosphorus content was found in the sample from Iraq (about 40%), but substantially higher values were found for Na, Fe and Mn.

Vitamin content data (Table 2) showed higher amount of riboflavin in sample from Iraq.

Amino acid analysis data are collected in Table 3.

High lysine content is characteristic for both cultivars, and higher amount of essential amino acids in comparison with wheat flour. The differences between chickpea samples A and B were not so significant. However they were higher than some of those reported in literature.

Table 3

Amino acid content of chickpea

Amino acid	Sample A		Sample B	
	g kg^{-1}	g per 16 g N	g kg^{-1}	g per 16 g N
Lysine	18.52	7.39	16.38	7.66
Leucine	16.73	6.67	14.49	6.74
Isoleucine	10.20	4.06	7.14	3.32
Phenylalanine	18.46	7.40	13.18	6.13
Methionine	1.56	0.62	1.36	3.63
Threonine	8.39	3.35	7.72	3.60
Tryptophan	5.04	2.01	5.07	2.36
Valine	12.18	4.85	8.82	4.10
SEAA	91.08	36.35	74.26	34.54
Alanine	10.34	4.12	9.16	4.26
Arginine	19.13	7.63	17.96	8.35
Cystine	+	+	+	+
Glycine	9.62	3.84	8.21	3.82
Histidine	10.54	4.20	8.24	3.83
Aspartic acid	29.86	11.91	27.79	12.93
Glutamic acid	35.33	14.09	33.22	15.45
Proline	10.74	4.28	11.36	5.28
Serine	11.51	4.60	10.38	4.38
Tyrosine	8.06	3.21	6.83	3.18
SOAA	145.13	57.88	133.15	61.93

SEAA: sum of essential amino acids
SOAA: sum of other amino acids
Sample A: whole chickpea flour from Piešťany
Sample B: whole chickpea flour from Iraq

Table 4

Composition of fatty acids in whole grain flour from chickpea

Fatty acid	Sample A	Sample B
	(% from total fatty acid amount)	
Myristic	0.01	0.01
Palmitic	6.32	5.08
Palmitoleic	0.01	0.002
Stearic	0.09	0.05
Oleic	17.37	28.44
Linoleic	70.39	66.00
Linolenic	0.64	0.3

Linoleic acid content is the highest in both samples, and higher than that of cereals and soybean oil (DAVIDEK et al., 1983). Considerable difference in the oleic acid content between the chickpea samples was noticed (Table 4).

The effect of addition of chickpea flour to the wheat flour was examined on the gluten content and rheological characteristics of wheat flour doughs by farinograph and extensograph and the results are summarized in Tables 5–7.

Table 5

Evaluation of gluten characteristics

Characteristics	Wheat flour	Substitution of wheat flour by chickpea whole grain flour			
		Sample A		Sample B	
		10%	20%	10%	20%
Quantity of wet gluten (% in d.m.)	34.9	32.8	29.5	32.6	29.0
Elasticity	elastic	middle elastic	little	middle elastic	little
Elasticity number	4	3	2	3	2
Extensibility	middle extensible	middle extensible		middle extensible	
Extensibility number	3	3	2	3	2
Swelling (cm^3)	19	18	16	18	15
Quantity of dry gluten (% in d.m.)	11.6	10.6	9.3	10.8	9.0

Table 6

Evaluation of farinograms

Parameters	Wheat flour	Substitution of wheat flour by chickpea whole grain flour			
		Sample A		Sample B	
		10%	20%	10%	20%
Absorption	59.5	60.3	21.3	60.5	61.0
Dough development time (min)	5.5	5.0	4.0	5.0	4.0
Weakening degree (BU)	50	55	90	45	80
Stability (min)	9.0	8.5	5.0	8.0	4.5
Mechanical resistance index (BU)	20	30	75	40	60

Table 7

Evaluation of extensograms

Parameters	Rest time (min)	Wheat flour	Substitution of wheat flour with chickpea whole grain flour			
			Sample A		Sample B	
			10%	20%	10%	20%
Extensographic energy (cm^2)	20	84.7	72.7	55.4	73.8	56.4
	65	107.9	83.5	68.5	85.5	67.5
Extensographic resistance (EU)	20	470	370	310	380	350
	65	520	430	370	430	380
Extensographic extensibility (mm)	20	120	115	107	120	116
	65	133	127	110	130	113
Resistance extensibility	20	3.91	3.21	2.89	3.16	3.01
	65	3.90	3.38	3.36	3.30	3.36

Wet gluten values of doughs decreased with the increase of the amount of chickpea whole grain flour. Elasticity of gluten was markedly lower only in case of 20% replacement. No significant differences were found between the doughs prepared with flours from different chickpea cultivars.

With the increase of wheat flour replacement by chickpea whole grain flour the water binding capacity of mixed flours increased, time of dough development and stability values decreased, especially at 20% replacement. Weakening values increased. Gradual weakening of dough consistency took place. Higher the amount of chickpea flours is, greater the changes are. Farinographic results are very similar for both samples of chickpea, no significant differences were observed (Table 6).

A decrease in extensographic energy, extensographic resistance and extensibility was found, with no significant difference between the samples grown in Slovakia and Iraq (Table 7).

The results of baking tests (microbaking experiments) are collected in Table 8.

It was found that with the increase of the amount of chickpea whole grain flour, the volume of bread rolls decreased. In comparison with the sample without chickpea flour, the average specific volumes decreased from the starting value of 282 cm^3 (average 8%), at 10% replacement; to 241 cm^3 (−15%) at 20% replacement in the case of sample A. A similar decrease took place in sample B at 10% replacement (to the value of 250 cm^3 (−12%)). Organoleptic characteristics of loaves at 10% replacement were not affected; at 20% replacement taste and smell changed,

but not disagreeably. They resembled the taste and smell of peas. The colour of crumb was yellow, caused by the natural colour of whole grain flours. Sample A from Iraq had similar characteristics to sample B from Pieštany. In practice, we recommend here to use the lower replacement of wheat flour with chickpea whole grain flour where such marked changes of shape and colouring do not occur. From this view it appears that the 10% replacement is optimal.

Table 8

Evaluation of microbaking experiment
(scores 1 to 5 scale)

Characteristic	Wheat flour	Substitution of wheat flour with chickpea whole grain flour			
		Sample A		Sample B	
		10%	20%	10%	20%
Shape of product	4	3	3	3	3
Colour of crust	4	4	4	4	4
Thickness, hardness of the crust	4	4	4	4	4
Elasticity of crumb	4	4	4	4	4
Porosity of crumb	4	3.6	3	3.8	3
Colour of crumb	4	3.6	3	3.5	3
Odour	4	3.8	3	3.8	3
Taste	4	3.8	3	3.6	3
Resistance at bitting, not chewing	4	4	4	4	4
Stickiness (to palate)	4	4	4	4	4
Sum of values	40	37.8	35	37.7	35

After having obtained primary information on the chemical composition of chickpea whole grain flour and its effect on the rheological characteristics of mixed doughs, we prepared a laboratory experiment of biscuits production and then a commercial-scale experiment in a bakery.

We observed that the dough mixed with chickpea whole grain flour was more fatty, required more energy and time for treatment. When chickpea is used, it is necessary to take the higher amount of protein and saccharides in chickpea flour into account, as well as the lower amount of starch and its characteristics. Considering these facts, less fat was applied in the experimental formula.

The advantage of using this whole grain flour in these cases is its yellow colouring, especially in products, where synthetic colouring is used.

Production of biscuits on commercial-scale was performed only with whole grain flour from chickpea cultivated in Pieštany. The dough was worked in kneading

machine MW 14 with horizontal kneading arms at 18 min^{-1} (r.p.m.). During preparation no negative phenomena appeared. The dough was thoroughly mixed with fat as the technology requires, short crushing, well formable, did not stick to the rollers and the dough easily moved on to the conveyor belt. The parameters (with the exception of fat), kneading time, temperature and time of baking were the same as in the production of Club type biscuits (DODOK, 1988).

The effect of whole grain chickpea flour does not appear negative in any of the examined characteristics (colour, shape, consistency, odour, taste). In comparison with bread rolls the production of pressed out biscuits is different. Here the replacement of wheat flour with chickpea whole grain flour even at 20% did not cause any adverse effect on the shape, organoleptic characteristics of the product and therefore, replacement of 20% is better regarding the nutritive value and weakening of strong flours gluten. The products have been evaluated by a group of experts, and all criteria were favourably regarded.

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ISOLATION AND PARTIAL CHARACTERIZATION OF SERINE PROTEINASES PRESENT IN THE LATEX OF *MACLURA POMIFERA* ("OSAGE ORANGE") FRUITS

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Latex provided by superficial incisions of *Maclura pomifera* fruits contains several serine proteinases. Maximal activity of crude preparations is reached at alkaline pH (more than 80% of maximal activity between pH 9.2 and 10.8 on casein and between pH 8.0 and 9.9 on azocoll), but when ionic strength is higher than 0.15 M an abrupt falling of enzyme activity is noted. Thermal stability of crude preparations is a remarkable fact, as enzyme activity is high even after 25 min at 65 °C, but decreases in the case of the main purified proteolytic fraction (III). Acetone fractionation followed by ion-exchange chromatography (DEAE- and CM-Sepharose CL–6B) affords four active fractions with closely related molecular sizes (63–71 kD, SDS–PAGE). The main proteolytic fraction (III, 70 kD) shows higher affinity for N-α-carbobezoxy-L-alanine p-nitrophenyl ester.

Keywords: *Maclura pomifera* (Moraceae), serine proteinases, enzyme purification, plant proteases, osage orange

Many proteases, both endopeptidases or proteinases (STOREY, 1986) and exopeptidases (MIKOLA & MIKOLA, 1986) have been isolated from higher plants. Some of the former (e.g. papain, ficin, bromelain) have been well characterized and are currently used in the food industry (CAFFINI et al., 1988).

Most plant proteinases belong either to the "cystein" or to the "serine" group, that is, they respectively contain an active cystein or serine residue in their active centers (MCDONALD, 1985).

Papain and ficin, which are present in the latex of "papaya" (*Carica papaya* L.) and figs (*Ficus carica* L. *F. glabrata* L.), respectively, and the two kinds of bromelain that can be obtained either from stems or fruits of "pineapple" (*Ananas comosus* L.) (Merrill) are cystein proteinases, like the proteases recently isolated from fruits of *Bromelia hieronymi* Mez (PRIOLO et al., 1991).

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On the other hand, serine proteinases can be usually found in the latex of Euphorbiaceae (LYNN & CLEVETTE-RADFORD, 1988), but they often occur in other plant families, as demonstrated by the presence of this type of proteases in *Cucurbita ficifolia* Bouché (CUROTTO et al., 1989) and other members of the family Cucurbitaceae (KANEDA et al., 1986; KANEDA & TOMINAGA 1977; KANEDA & TOMINAGA, 1987).

In the present paper the isolation and purification of the proteases present in the latex obtained from fruits of *Maclura pomifera* (Raf.) Schneid. (Moraceae) and the partial characterization of the main proteolytic fraction, a serine proteinase of 70 kD, is reported. No previous chemical work about this species could be ascertained, except for the pioneer reference of TAUBER (1949).

1. Material and methods

1.1. Plant material

Nearly ripe (light green outside) fruits of *Maclura pomifera* (Raf.) Schneid. ("osage orange") collected in Villa Elisa (Argentina) in March 1990 were used in the present work.

1.2. Crude preparations

Fruits were scrupulously cleaned with tap water and a soft brush, and rinsed twice with distilled water. Superficial incisions (not deeper than 2 mm) usually provided a semifluid, milk-like latex (average: 0.5 cm^3 latex per 100 g fruits), which was received on cold 0.1 M phosphate buffer (pH 6.0). Crude preparations were obtained by centrifuging suspensions containing 3% latex for 20 min at 4 °C and 1600 g (remotion of gums and other insoluble materials).

1.3. Proteolytic activity assays

In most cases casein was the proteolytic substrate assayed: the reaction mixture contained 1.1 cm^3 of 1% casein solution, and 0.1 cm^3 enzyme solution, both in 0.1 M phosphate buffer (pH 7.0). The reaction was carried out at 37 °C and stopped by the addition of 5% trichloroacetic acid (1.8 cm^3), then test tubes were centrifuged at 4000 g for 20 min and the absorbance of supernatants was measured at 280 nm. An arbitrary enzyme unit (U_{cas}) was defined as the amount of enzyme that produces an increase of one absorbance unit per minute in the assay conditions.

When azocoll was used as substrate (O'REILLY et al., 1981) the reaction mixture contained 10 mg of azocoll and 0.5 cm^3 of a dilution of the enzyme in a

suitable buffer. The reaction was carried out at 37 °C and stopped by the addition of 3 cm^3 of cold water (4 °C), then the tubes were vigorously mixed, the contents removed by filtration through Whatman No. 1 filter paper, and the absorbance of the colored solution was measured at 520 nm. One enzyme unit ($U_{azocoll}$) was defined as the amount of enzyme that produces an increase of one absorbance unit per minute on the assay conditions.

1.4. Assays with synthetic substrates

The activity of the proteases on the following N-α-carbobenzoxy-L-amino acid p-nitrophenyl esters was tested: N-α-carbobenzoxy-L-alanine p-nitrophenyl ester, N-α-carbobenzoxy-L-asparagine p-nitrophenyl ester, N-α-carbobenzoxy-β-benzyl-L-aspartic acid p-nitrophenyl ester, N-α-carbobenzoxy-glycine p-nitrophenyl ester, N-α-carbobenzoxy-L-isoleucine p-nitrophenyl ester, N-α-carbobenzoxy-L-leucine p-nitrophenyl ester, N-α-carbobenzoxy-L-lysine p-nitrophenyl ester, N-α-carbobenzoxy-L-phenylalanine p-nitrophenyl ester, N-α-carbobenzoxy-L-tryptophan p-nitrophenyl ester, N-α-carbobenzoxy-L-tyrosine p-nitrophenyl ester, and N-α-carbobenzoxy-L-valine p-nitrophenyl ester. The reaction mixture contained 2.5 cm^3 of 0.1 M Tris-HCl buffer (pH 8.0), 1 cm^3 of substrate solution (1 mM in dioxane), and 1.0 cm^3 of enzyme solution. The reaction was carried out at 30 °C and changes in the absorbance were measured at 405 nm for 3 min. In this case one enzyme unit (U_{cbz}) was defined as the amount of enzyme that releases one micromol of p-nitrophenol per min at 30 °C and pH 8.0.

1.5. Protein and carbonhydrate content

Proteins were measured according to BRADFORD (1976), using bovine albumin as standard. In all chromatographic procedures protein concentration was estimated by measuring the absorbance at 280 nm.

Carbohydrate content was determined using the method of DUBOIS and co-workers (1956).

1.6. pH-dependence of enzyme activity

Proteolytic activity of enzyme solutions was measured on casein (range: pH 6.0 to 11.0) and on azocoll (range: pH 4.0 to 11.0) using 0.01 M sodium acetate and 0.01 M sodium salts of the following "Good" buffers: MES, MOPS, TAPS, AMPSO and CAPS (GOOD & IZAWA, 1972).

1.7. Thermal stability

In order to ascertain the effect of heat, enzyme solutions were kept at 37 °C, 45 °C, 55 °C, 60 °C, and 65 °C for 5, 10, 20, 40, 60, 90, and 120 min respectively, and then the residual caseinolytic activity was measured as indicated above.

1.8. Effect of activators and inhibitors

EDTA (5 mM), cystein (5 mM), mercuric chloride (0.1 mM), and PMSF (1 mM) were added to the crude preparation and to the main purified proteolytic fraction: after incubation at 37 °C for 10 min the residual caseinolytic activity was measured as indicated above.

1.9. Fractionation with acetone

One volume of crude preparation (4 °C) was successively treated with one, two, and three volumes of cold (−20 °C) acetone with gentle agitation. The suspensions were left to settle at −20 °C for 10 min and then centrifuged at 16 000 g for 15 min at 4 °C. All the precipitates were redissolved with 0.1 M phosphate buffer (pH 7.0) and their caseinolytic activities, and protein and carbohydrate contents were determined as indicated above.

1.10. Ion-exchange chromatography

Anionic exchange chromatography was performed on a column (1.5 × 30 cm) of DEAE-Sepharose CL−6B equilibrated with 50 mM Tris-HCl buffer (pH 8.0). After washing the column with 60 cm^3 of the same buffer, the retained proteins were eluted with 200 cm^3 of a 0.0−0.25 M sodium chloride linear gradient in the starting buffer.

The unretained fraction was diafiltrated (Amicon 8050, membrane YM 10) in order to perform buffer exchange (Tris-HCl 50 mM pH 8.0 to sodium phosphate 10 mM pH 7.5) and then was submitted to cationic exchange chromatography using a column (1.5 × 30 cm) of CM-Sepharose CL−6B equilibrated with 10 mM sodium phosphate buffer (pH 7.5). After washing the column with 60 cm^3 of the same buffer, the elution was performed with 200 cm^3 of 0.0−0.47 M sodium chloride linear gradient in the starting buffer.

1.11. SDS-Polyacrylamide gel electrophoresis

SDS–PAGE was performed following the method of LAEMMLI (1970). Current was kept constant at 20 mA during stacking and then increased to 30 mA and kept constant for 3 h. Gels were stained with Coomassie Brilliant Blue R–250 as described by HAMES (1981).

2. Results and discussion

2.1. Crude preparations

The addition of EDTA, cystein, and mercuric chloride does not modify the caseinolytic activity of crude preparations, but PMSF produces irreversible inhibition, suggesting that the proteases present in the latex of *Maclura pomifera* fruits belong to the "serine" group.

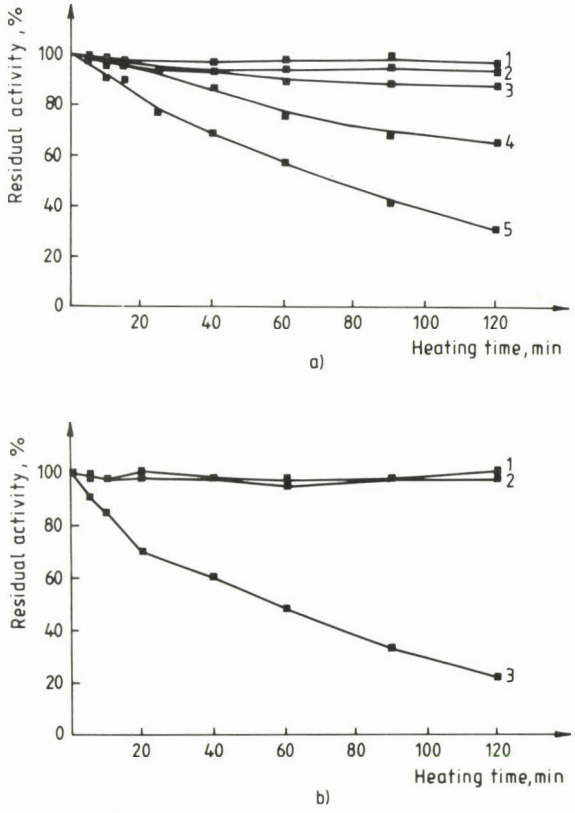

Fig. 1. Effect of different heat treatment on the stability of crude preparations (A), and fraction III (B). Data points represent the mean value of five determinations and each experiment was repeated twice.
(A) 1 = 37 °C, 2 = 45 °C, 3 = 55 °C, 4 = 60 °C, 5 = 65 °C;
(B) 1 = 37 °C, 2 = 45 °C, 3 = 60 °C

Thermal stability of crude preparations is shown in Fig. 1 (A): after 2 h at 37 °C or even at 45 °C caseinolytic activity remains practically unchanged, and is still fairly high (88%) at 55 °C; the proteases also show high stability during the first 25 min at 60 °C and 65 °C, but the residual activity notably decreases with the increasing time (65% and 30% after 2 h at 60 °C and 65 °C, respectively).

As in the case of most serine proteinases (BARRETT, 1986), maximal activity of crude preparations is reached at alkaline pH, using either casein or azocoll as substrate (Fig. 2). More than 80% of maximal activity is retained between pH 9.2 and 10.8 (casein) and between pH 8.0 and 9.9 (azocoll). Crude preparations are notably stable within the range of pH of higher activity (Fig. 3), but ionic strengths higher than 0.15 M at neutral pH promotes abrupt falling of activity (Fig. 4).

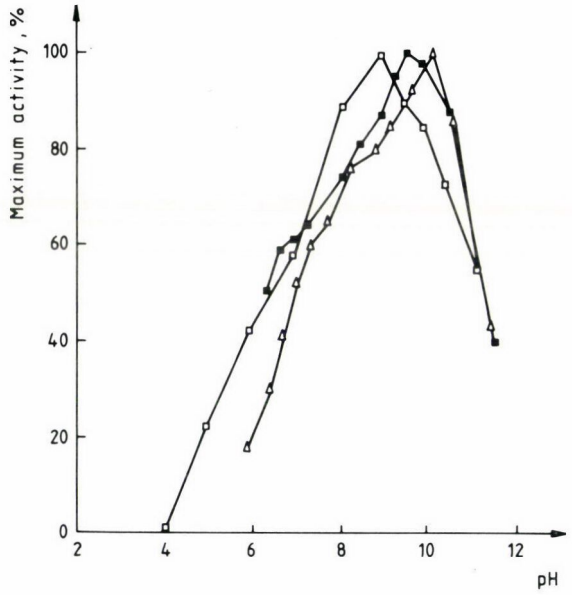

Fig. 2. Effect of pH on the activity of crude preparations and fraction III. Values consigned correspond to pH assay mixtures. Data points represent the mean value of five determinations and each experiment was repeated twice.
Δ: crude (casein);□ : crude (azocoll); ■: fraction III (casein)

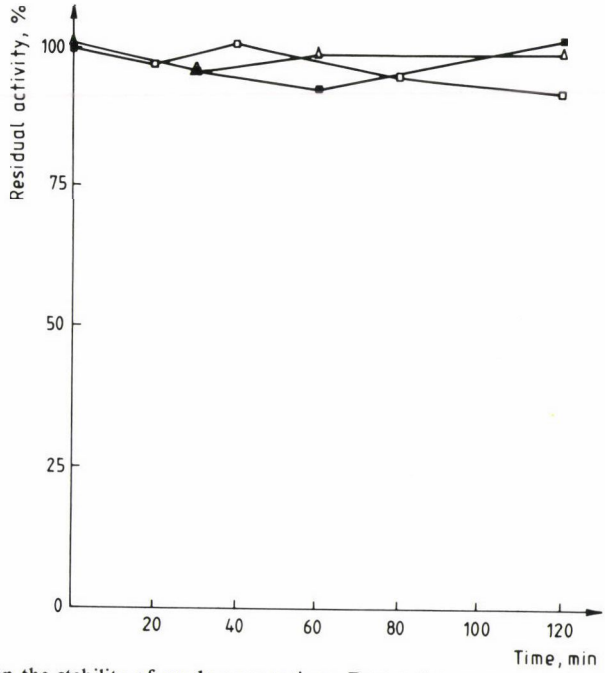

Fig. 3. Effect of pH on the stability of crude preparations. Data points represent the mean value of five determinations and each experiment was repeated twice. ■: pH = 8.0;□: pH = 8.9; Δ: pH = 10.4

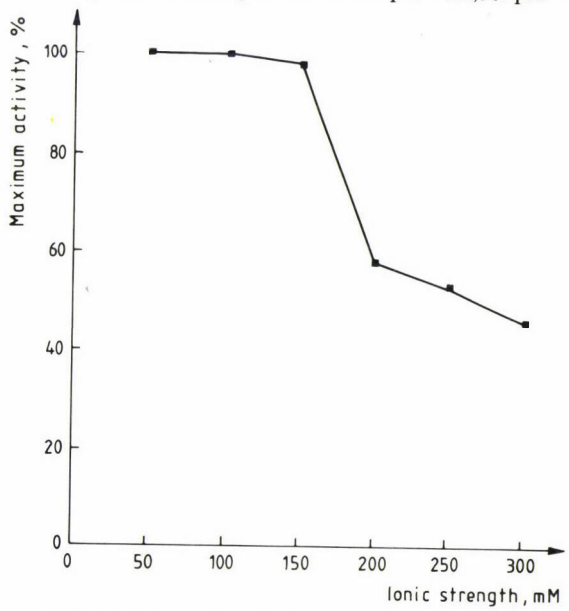

Fig. 4. Effect of ionic strength on proteolytic activity (azocoll) of crude preparations (buffer MES, pH = 6.8). Data points represent the mean value of five determinations and each experiment was repeated twice

As it has been reported for other plant proteases (PRIOLO et al., 1991), lyophyllization seems to be an appropriate way for keeping crude preparations, as decrease of enzyme activity is negligible when freeze-dried preparations are redissolved.

2.2. Purification

Crude preparations contain yellow pigments and a high content of soluble carbohydrates (carbohydrate/protein ratio = 1.6), which are mostly removed by addition of 2 volumes of cold (−20 °C) acetone: the white precipitate contains only 8% of the carbohydrates but 92% of the proteins present in the crude preparation and retains 97% of the initial activity.

The acetone precipitate is redissolved in 50 mM tris-HCl buffer (pH 8.0) and applied to a DEAE-Sepharose CL−6B column (Fig. 5): the bulk of active protein elutes with the starting buffer, while another two active fractions (I and II) leave the column when a sodium chloride linear gradient is applied (0.10 M and 0.17 M, respectively). After diafiltration, the unretained fraction is applied to a CM-Sepharose CL−6B column (Fig. 6): the main proteolytic fraction (III) elutes when the sodium chloride gradient arrives to 0.13 M and a last active fraction (IV) appears when the gradient reaches 0.18 M concentration.

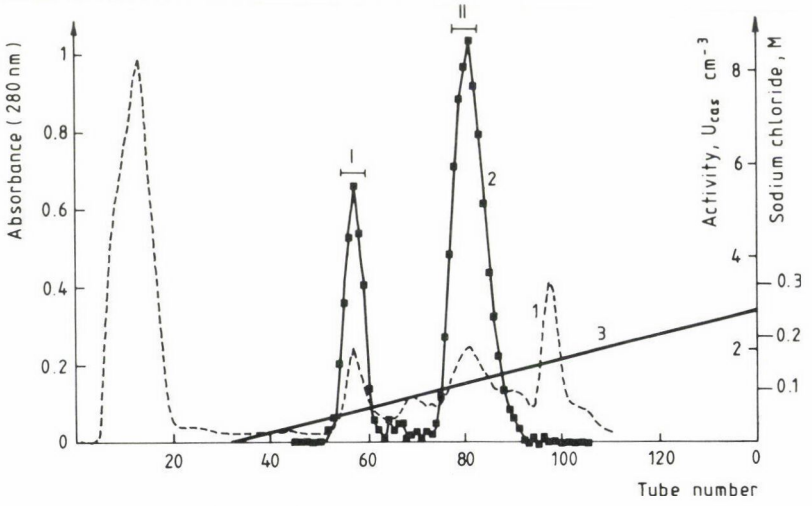

Fig. 5. Chromatography on DEAE-Sepharose CL−6B of the redissolved acetone precipitate. Starting buffer: 50 mM Tris-HCl (pH = 8.0); flow rate: 9.0 cm^3 h^{-1}; fractions of 1.8 cm^3 were collected. 1: absorbance at 280 nm; 2: caseinolytic activity; 3: sodium chloride gradient. Bars indicate the fractions that were pooled

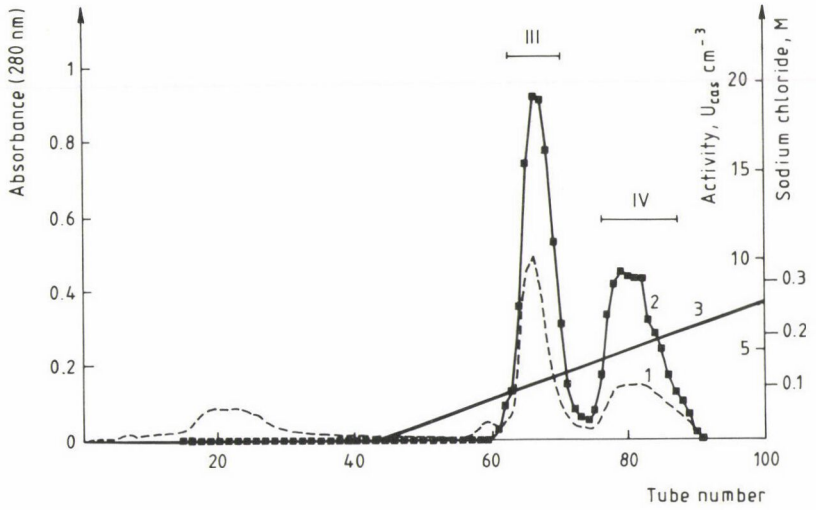

Fig. 6. Chromatography on CM-Sepharose CL-6B of the unretained fraction. Starting buffer: 10 mM sodium phosphate (pH = 7.5); flow rate: 9.0 cm^3 h^{-1}; fractions of 1.8 cm^3 were collected. 1: absorbance at
280 nm; 2: caseinolytic activity; 3: sodium chloride gradient. Bars indicate the fractions that were pooled

SDS-polyacrylamide gel electrophoresis shows (Fig. 7) that the relative molecular weights of the purified proteolytic fractions are closely related (63-71 kD) and are of the same order as those of other serine proteinases (CUROTTO et al., 1989; KANEDA & TOMINAGA, 1975; KANEDA & TOMINAGA, 1977; LYNN & CLEVETTE-RADFORD, 1988). The electrophoretic pattern also reveals that fraction II is not homogeneous, as suggested by the respective chromatogram (Fig. 5).

As it is shown on the purification scheme given in Table 1, fraction III is the main purified proteolytic fraction (yield: 17.8%). The behavior of this fraction in the presence of activator and inhibitor substances is similar to that of crude preparations, and also caseinolytic activity in dependence of pH shows a similar pattern (Fig. 2), though slightly enlarged towards lesser pH values (80% of maximal activity between pH 8.2 and 10.6). Nevertheless, thermal stability (Fig. 1, B) was smaller than in the case of crude preparations. Table 2 contains values of specific activity of fraction III on synthetic substrates: relative activity on N-α-carbobenzoxy p-nitrophenyl ester amino acids was much higher for alanine than for other amino acid derivates.

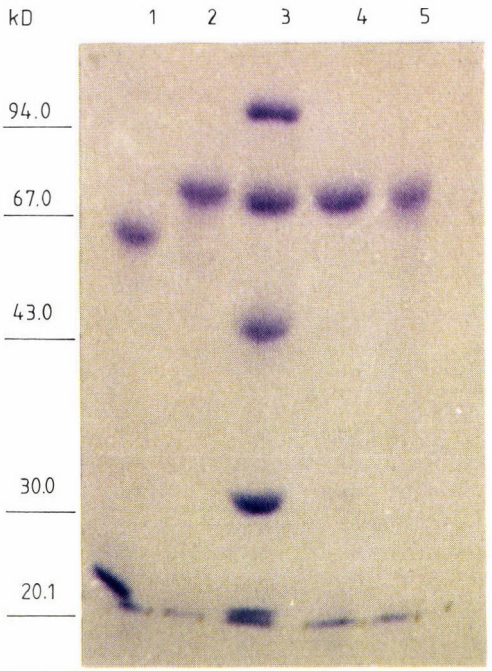

Fig. 7. SDS-Polyacrylamide gel electrophoresis of purified enzymes, according to the method of LAEMMLI (1970). 10% polyacrylamide gel was run with: 1: fraction II; 2: fraction I; 3: standard protein molecular weight markers: soybean trypsin inhibitor (M_r 20100), carbonic anhydrase (M_r 30000), ovalbumin (M_r 43000), bovine serum albumin (M_r 67000), and phosphorylase b (M_r 94000); 4: fraction III; 5: fraction IV

Table 1

Purification of proteases present in the latex of Maclura pomifera *fruits*

Step	Activity (U_{cas})	Protein (mg)	Specific activity (U_{cas} mg^{-1})	Purification (-fold)	Yield (%)
Crude	1702	19.80	86.0	1.0	100.0
A.P.	1651	18.22	90.6	1.1	97.0
I	78	0.48	163.0	1.9	4.6
II	82	0.43	190.7	2.2	4.8
III	303	1.07	283.2	3.3	17.8
IV	225	0.71	317.6	3.7	13.2

A.P.: redissolved acetone precipitate

Table 2

Activity of the main purified fraction (III) on synthetic substrates (N-α-carbobenzoxy p-nitrophenyl esters of different amino acids)

Amino acid	Specific activity (U_{cbz} mg^{-1} protein)	Relative activity (%)
Alanine	13.8	100.0
Glycine	4.3	31.2
Leucine	2.6	18.8
Phenylalanine	0.9	6.5
Valine	0.4	2.9
Tryptophan	–	–
Tyrosine	–	–
Asparagine	–	–
Aspartic acid	–	–
Isoleucine	–	–
Lysine	–	–

On the basis of the present information, a new source of plant proteases could be now available for the food industry, as well as for any other industrial processes requiring proteolytic enzymes with higher activity at alkaline pH. Because of the nature of plant material (latex secretion, instead of plant tissue extract), "crude" preparations are in fact, sufficiently pure materials that could be advantageously used in the industry by itself or, at the most, with a further acetone fractionation.

Abbreviations

AMPSO: 3-[(1,1-dimethyl-2-hydroxyethyl) amino]-2-hydroxypropane-sulfonic acid;
CAPS: 3-(cyclohexylamino)-1-propanesulfonic acid;
EDTA: ethylenediamine tetra-acetic acid (sodium salt);
MES: 2-(N-morpholino) ethanesulfonic acid;
MOPS: 3-(N-morpholino) propanesulfonic acid;
PMSF: phenylmethylsulfonyl fluoride;
TAPS: N-tris (hydroxymethyl) methyl-3-aminopropanesulfonic acid.

*

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OAK TREE DECAY INVESTIGATION BY SDS GEL ELECTROPHORESIS AND ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

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The banding pattern of oak leaf proteins resulting from sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS–PAGE) of extracts from decaying samples has been used to distinguish species within the genus Quercus. Different banding patterns in the range 70 000–10 000 D were obtained for leaf proteins from a range of varieties, some very closely related. These patterns were also checked for lines in relation to their decay which might have been caused by the fungi *Ceratocystis roborum* or *Ceratocystis piceae*. Characteristically doubled sub-units of molecular weight 12 000 D and the extent of disease were found to be related. Similar sub-units detected in the healthy oak samples may indicate the early stage of decay. In order to investigate whether the doubled bands are related to fungal contamination, ELISA method was applied using polyspecific antisera produced against the above fungi.

High ELISA values in relation to decaying samples and doubled bands have provided proof of the fungal contamination or the transfer of fungal metabolites to the leaves. However, this point will be further investigated.

Keywords: oak tree decay, SDS gel electrophoresis, ELISA method

In the last decade much research work has been spent on the investigation of forest decay. According to investigations, either acidic rains or epidemic ignited by aggressive fungi threaten health conditions of oak forests.

However, oak tree decay is found to be epidemiologic among predisposed, weak species. Predisposition may derive of unfavourable biotic and abiotic conditions like drought, hard winter, insects and fungi. Trees in poor health condition are attacked by fungi ab ovo present in oak forest. The roles of fungi *Ceratocystis roborum*, *Ceratocystis piceae* and *Armillaria mellea* were found predominant in the process.

Electrophoretic techniques that separate either individual proteins or isozymes are becoming increasingly popular for identifying crop cultivars (COOKE, 1984). Sodium dodecylsulfate polyacrylamide gel electrophoresis has successfully been

applied for grass cultivar identification (GARDINER et al., 1986). Seeds of cereals have been characterized by gel electrophoresis (COOKE et al., 1986). Plant membrane proteins were analysed by two-dimensional gel electrophoresis (HURKMAN & TANAKA, 1986). However, gel electrophoretic methods lack sensitivity when too many isoenzymes or protein fractions are present. WERRES and CASPER (1987) reported successful application of the enzyme-linked immunosorbent assay (ELISA) for the detection of fungi in strawberry roots.

1. Materials and methods

1.1. Oak leaves

The leaves of 50 oak trees, belonging to species *Quercus dalechampii*, *Q. petraea*, *Q. polycarpa*, *Q. cerris*, *Q. dalechampipetraea*, *Q. petraea-dalechampi* and *Q. dalechampii-polycarpa* (Table 1), were collected at Tetvesrét, Mátra Hills, Hungary.

1.2. Extraction of protein (BOLL, 1987)

Leaves were immediately frozen in liquid nitrogen and broken into small pieces in mortar. One hundred mg of leaf pulp were suspended in 1 cm^3 buffer mixture (0.05 mol l^{-1}), prepared from equal volumes of borate pH 8.8; Tris-HCl pH 7.5; phosphate pH 7.5; and supplemented with polyvinyl-pyrrolidon (PVP 40, Sigma St. Louis, USA) to final concentration of 1% (w/v) and 0.01 cm^3 β-mercaptoethanol.

Leaf pulp was homogenized in the buffer mixture for 5 min on ice. Insoluble material was removed by centrifugation (in Beckman type 72–21, 2000 r.p.m. for 15 min). Supernatant was further separated applying 17 000 r.p.m. (Beckman type 72–21) for 4 h. Soluble fraction was found to contain cytosol and microsomal fractions.

1.3. SDS- polyacrylamide gel electrophoresis

Molecular weights of separated fractions were determined by SDS vertical slab electrophoresis according to WEBER and OSBORN (1969). Proteins were stained with the silver nitrate method (MERRIL et al., 1982). Slab gels were evaluated with a Shimadzu Model CS–930 dual-wavelength thin-layer chromato-scanner.

Table 1

Indication of species and extent of infection of the 50 oak trees collected at Tetvesrét, Mátra Hills, Hungary

Number	Sign of tree	Species	Extent of infection[a]
1	1	Quercus dalechampii-petraea	5
2	2	Quercus dalechampii-petraea	5
3	3	Quercus dalechampii-petraea	5
4	4	dalechampii	5
5	5	dalechampii	5
6	5	dalechampii	5
7	7	dalechampii	5
8	8	polycarpa	5
9	9	dalechampii	5
10	10	dalechampii	5
11	11	dalechampii	5
12	12	dalechampii	5
13	13	dalechampii-polycarpa	5
14	14	dalechampii-petraea	5
15	15	dalechampii-petraea	5
16	16	dalechampii-petraea	5
17	17	dalechampii-petraea	5
18	18	petraea-dalechampii	5
19	21	dalechampii	5
20	22	dalechampii	5
21	23	dalechampii	5
22	24	dalechampii	4
23	26	dalechampii	5
24	27	dalechampii-petraea	5
25	28	dalechampii	4
26	29	dalechampii-polycarpa	4
27	30	dalechampii	5
28	33	dalechampii	5
29	34	dalechampii	5
30	35	cerris	5
31	36	dalechampii	5
32	37	dalechampii	5
33	38	dalechampii	5
34	39	petraea-dalechampii	5
35	40	dalechampii-petraea	5
36	41	dalechampii-petraea	5
37	42	dalechampii-polycarpa	5
38	43	dalechampii	5
39	44	dalechampii-petraea	4
40	45	petraea-dalechampii	4
41	48	dalechampii	4
42	53	dalechampii	4
43	54	dalechampii	5
44	55	petraea-dalechampii	5
45	56	dalechampii	5
46	57	dalechampii	5
47	62	petraea	4
48	64	dalechampii	4
49	9/k	unknown	–
50	H/9	unknown	–

[a] Number 5 means healthy trees. Numbers between 1 and 5 symbolize extent of infection. Number 1 indicates decaying objects

1.4. Antisera

Antisera were raised against the fungi *Ceratocystis piceae* and *Ceratocystis roborum*, respectively. The fungi were streaked onto agar plates and the cells were harvested after 3 days. Rabbits were inoculated with the 3% (w/v) solution of fungal cells sonicated for 3 × 1 min at 300 W in a Labsonic 1510 (Georg Becker Laboreinrichtungen, Wien, Austria) every two weeks during a 2-month period (SIGEL et al., 1983). The immunoglobulin fraction was purified according to STEINBUCH and AUDRAN (1969).

1.5. Indirect enzyme linked immunosorbent assay

Samples containing 1 μg of leaf extract were applied onto microtiter plates in triplicates. Three blanks containing only reagent buffer and antibody enzyme-conjugate-antiglobulin were also analyzed to determine nonspecific binding to solid phase.

Sample aliquots of 100 μl were applied to microtiter plates. The plates were incubated at 37 °C overnight to allow binding of antigen to solid phase. The plates were blocked with 100 μl PBS–BSA (3% bovine serum albumin in PBS: 0.041 mol l^{-1} NaH_2PO_4 H_2O, 0.061 mol l^{-1} Na_2HPO_4, 0.01% thimerosal, 0.9% NaCl, pH 7.0). The bound antigens were then incubated with 100 μl antisera (1:250) and subsequently allowed to bind with goat anti-rabbit-IgG-peroxidase (1:500) for 2 h each at 37 °C. Excess reagents were rinsed off 4 × with PBS–T (0.05% Tween in PBS) after each step.

Substrate (100 μl) containing 0.034% (w/v) *o*-phenylene-diamine, 30 μl hydrogenperoxide (30% v/v) in citrate phosphate buffer (0.1 mol l^{-1} citric acid; 0.2 mol l^{-1} Na_2HPO_4, pH 5.0) was added onto microtiter plates.

The chromogens were allowed to develop for 15 min at room temperature. The reaction was stopped by addition of 50 μl 1.25% aqueous sulfuric acid and absorbance was measured at 492 nm. Serological activity was expressed in % of those measured for decayed oak tree proteins. Standard error of the determination was 10%, A_{492} values of the blank were 0.15.

2. Results and discussion

Of the forest of Tetvesrét, Mátra, the banding patterns of 50 oak tree leaf extracts have been investigated by SDS–PAGE (Figs. 1, 2). According to the results, the disturbing polyphenol-content of the oak leaves has been successfully removed during the preparation of the samples. However, traces can be found in several cases, covering the fractions of molecular weight 70 000 (6, 13, 18, 24, 54).

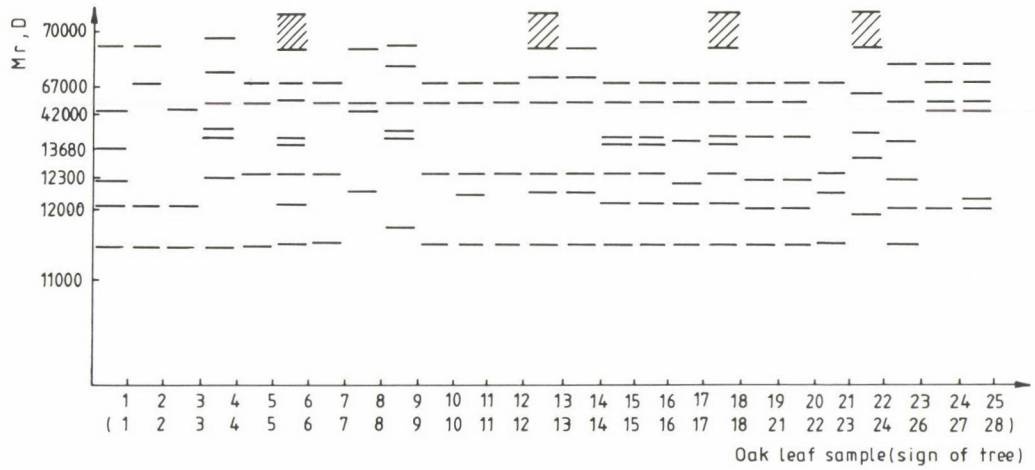

Fig. 1. Molecular weights of the oak leaf samples 1–25. The determinations were carried out by SDS vertical slab electrophoresis according to WEBER and OSBORN (1969); proteins were stained with the silver nitrate method (MERRIL et al., 1982)

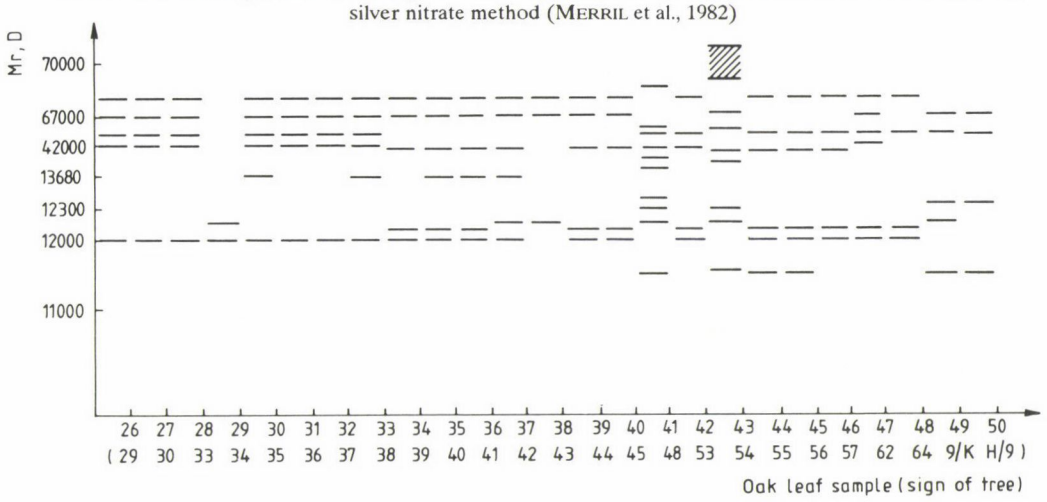

Fig. 2. Molecular weights of the oak leaf samples 26–50. The determinations were carried out by SDS vertical slab electrophoresis according to WEBER and OSBORN (1969); proteins were stained with the silver nitrate method (MERRIL et al., 1982)

The fractions of leaf proteins cover the range 70 000 – 10 000 D. Several oak leaf patterns were found to be similar (6 – 15 – 16 – 18; 39 – 40 – 41 – 42). Most samples showed heterogeneity ragarding the quantity and quality of sub-units (e.g. characteristic yellow discolourization in samples 10, 12, 28, 29, 30, 35, 40, 41, 43, 45; low number of fractions in samples 37, 40, 42; numerous subunits in samples 6, 15,

16, 18). Comparing the banding patterns and the grade of decay (numbers less than 5 indicate trees with advanced disease) the doubled sub-units of molecular weight 12 000 in the samples 44, 45, 62, 28, 53 and 64 and the progress of decay (grade 4) were found to be related. In the patterns of healthy trees (grade 5) the appearance of doubled bands (samples 39, 40, 41, 55, 56, 57) may indicate the early stage of infection. However, the decay (grade 4) was not accompanied by doubling in the case of samples 24, 29 and 48.

No data were furnished on samples 9/K and H/9. In order to investigate correlation between the decay and the doubled bands, enzyme linked immunosorbent assay was applied, using antisera produced against the fungi *Ceratocystis roborum* and *C. piceae* (Fig. 3). Most of the leaf proteins gave positive immunological reaction with the antisera.

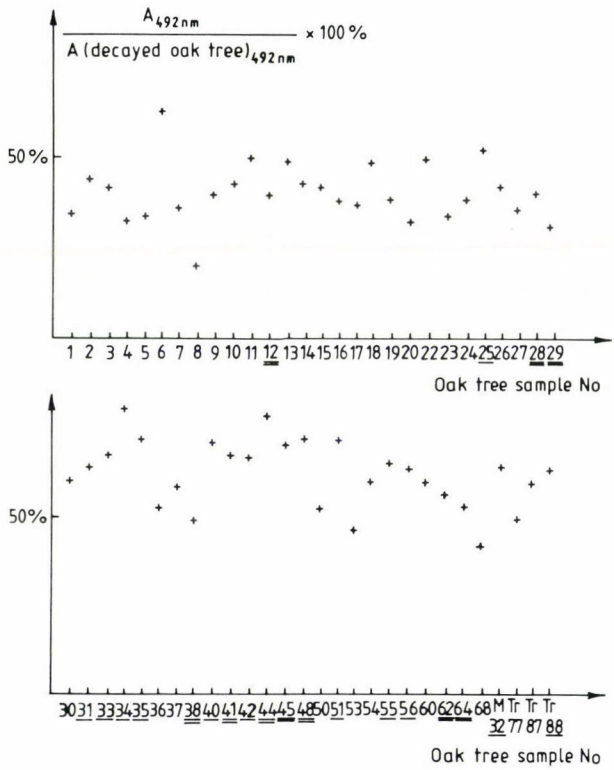

Fig. 3. Investigation of the oak leaf samples with the enzyme linked immunosorbent assay (ELISA). Serological activity of leaf proteins was expressed in percentage of decayed oak tree proteins against *Ceratocystis antisera*. =: samples containing doubled bands in the SDS–PAGE patterns; _: samples lacking doubled bands in the SDS–PAGE patterns but showing high ELISA values

High ELISA values were detected for many samples, which can be related to the progress of decay, to doubled bands and to the infection of trees in proximity of the decaying ones (samples 44, 45, 46, 62, 64). Several samples originally declared healthy, but containing doubled bands gave high ELISA values (41, 42, 55, 56, 57) which may be attributed to the early stage of decay.

3. Conclusion

In spite of the similarities of the banding patterns of oak leaf proteins, the SDS–PAGE separations resulted in great heterogeneity which can be explained by the different oak tree species. The theory – correlations between the doubled bands and the progress of decay – is supported by the results. However, strong evidence could be produced only by the continuous analysis of the same samples for several years.

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EFFECT OF EXTRUSION TEMPERATURE AND INITIAL MOISTURE CONTENT ON THE PROTEIN SOLUBILITY AND DISTRIBUTION IN FULL FAT SOYBEAN

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In the present study five extrusion die temperatures (140, 160, 170, 180 and 200 °C) at 8% initial moisture content and five initial moisture content values (8%, 12%, 14%, 16% and 18%) at 160 °C extrusion temperature were used as treatments to evaluate their effects on the protein structure of full fat soy flours, extruded in a Brabender extruder.

The increase of extrusion temperature caused a significant effect on protein extractability, while the different initial moisture content did not. The extrusion increased the insolubility of protein, which resulted in a significant decrease in solubility of albumin and globulin fractions, and an increase in the insoluble residue.

The buffer containing sodiumdodecyl sulfate (SDS) and 2-mercaptoethanol (2–ME) could disrupt a part of bondings resulted during extrusion. SDS-polyacrylamide gel electrophoresis showed changes in protein distribution during extrusion. Higher temperatures (160–200 °C) caused great changes in gel electrophoretic patterns of different soy fractions.

Keywords: full fat soy flour, extrusion temperature, initial moisture content, extractability of soy protein, SDS–PAGE, molecular weight distribution of soy protein fractions

Extrusion process is a widely used technology for soybean heat treatment for both food and feed purposes. It is well known that the trypsin inhibitors and other antinutritive factors, the enzymes (e.g. lipoxygenase) of soybean are inactivated during extrusion, the biological value of soybean is increased, the functional properties are modified.

Thermoplastic extrusion modifies the structure and texture of soy, and mostly these changes are the results of alteration occurring in the soy protein (SHEARD et al., 1984, 1986). Several studies have shown that soy proteins are denaturated during extrusion, and at low-temperature extrusion (up to 150 °C) intermolecular disulfide bonding is an important factor contributing to extrudate structure

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accompanied by changes in noncovalent bonding (HAGER, 1984). In these works mostly defatted soy products (flours, grits, concentrates, isolates) were extruded and their characteristics were determined. In an earlier work (HORVÁTH et al., 1989) the effect of extrusion temperature on functional properties, solubility, trypsin inhibitor and urease activities, and biological value of full fat soy flour were studied.

There is little information on the changes occurring in protein structure of full fat soy flour during extrusion, therefore the effects of different extrusion temperatures and different initial moisture contents on the protein structure of full fat soy beans were investigated. A solubility test was carried out to study the forces responsible for the insolubilization of soy proteins during extrusion and an electrophoretic work using sodiumdodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) was done to investigate the extracted soy fractions and their subunits as a function of changing extrusion circumstances.

1. Materials and methods

1.1. Soy samples

Soybean seeds (*Glycine max.*) were steamed (127 °C, 3 min), dehulled, passed through an air flow to remove coats, and after that extruded.

The dehulled soybean and extruded soy samples were ground in a laboratory mill to flour (particle size $\leq$ 600 μm). Extruded samples were collected, placed in plastic bags and stored at 5 °C, until analysed.

1.2. Extrusion technique

The dehulled soybeans were extruded in a Brabender laboratory extruded, type 20 DN (Brabender OHG, Duisburg, Germany) at a constant screw rate of 120 r.p.m. (compression ratio 4:1). The diameter of die was 3 mm.

Part of soybeans was extruded at different die temperatures, at constant initial moisture content (8.0 g per 100 g sample), the other part was extruded at constant temperature with different initial moisture content, adjusted prior to extrusion. The initial moisture content and temperature setting of the three heating zones are shown in Table 1.

Table 1

Initial moisture content and temperature of extrusion zones applied in the extrusion of full fat soybean flours

Sample	Initial moisture content (g per 100 g sample)	Temperature of extrusion zones (°C)		
		Zone I	Zone II	Zone III
Extruded soy flour 1	8.0	120	130	140
Extruded soy flour 2	8.0	130	150	160
Extruded soy flour 3	8.0	150	160	170
Extruded soy flour 4	8.0	160	170	180
Extruded soy flour 5	8.0	180	190	200
Extruded soy flour 6	12.0	130	150	160
Extruded soy flour 7	14.0	130	150	160
Extruded soy flour 8	16.0	130	150	160
Extruded soy flour 9	18.0	130	150	160

The experimental parameters (initial moisture content and extrusion temperatures) were chosen according to the results of PETRES and CZUKOR (1989). They found that these treatment combinations effectively destroy the antinutritive materials in soybean, whose proper inhibition is important for the further use of soybean.

1.3. Determination of moisture, protein and lipid contents

Moisture content was measured by drying the samples to constant weight at 105 °C.

Crude protein (N × 6.25) content was determined by the Kjeldahl procedure, in an automatic Kjell–Foss equipment.

Lipid content was determined by petroleum ether extraction (b.p. 70 °C) in a Soxhlet apparatus.

1.4. Extraction and fractionation of soy proteins

The fat free soy samples were fractionated according to the method of OSBORNE (1924). Soy samples were also extracted with 0.5 mol l^{-1} Na_2HPO_4 (pH = 7.8) buffer, and with the same buffer containing either 5% (w/w) sodiumdodecyl sulfate (SDS), either 1% (v/v) 2-mercaptoethanol (2–ME), or both of them by the method of HORVÁTH and CZUKOR (1988).

1.5. Sodiumdodecyl sulfate polyacrylamide gel electrophoresis (SDS – PAGE)

SDS – PAGE was performed on vertical 2 mm slab gels (10% w/v polyacrylamide) according to the method of WEBER and OSBORN (1975). Gels were stained and destained by the method of STECK and co-workers (1980). Standard proteins were: low molecular weight KIT of Pharmacia, Sweden.

2. Results and discussion

2.1. Chemical composition

Table 2 shows effects caused by different extrusion temperatures and Table 3 presents those caused by different initial moisture content of soybeans on the moisture, lipid and protein (N × 6.25) contents of extruded full fat soy flours.

Table 2

Chemical composition of soy flours extruded at different temperatures
(g per 100 g total weight, n = 3)

Sample	Moisture content		Lipid content		Protein (N × 6.25) content	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Soy before extrusion	8.0	0.04	23.5	0.04	36.7	0.2
Extruded soy 1 (140 °C)[a]	7.6	0.15	22.2	0.33	39.4	1.0
Extruded soy 2 (160 °C)[a]	7.1	0.19	23.1	0.03	37.6	0.8
Extruded soy 3 (170 °C)[a]	5.8	0.24	20.0	0.01	39.5	0.1
Extruded soy 4 (180 °C)[a]	5.1	0.09	21.1	0.11	40.2	0.7
Extruded soy 5 (200 °C)[a]	4.6	0.13	16.2	0.11	42.7	0.1

$\bar{x}$: mean value of three measurements
±s: standard deviation
[a]: extrusion temperature

Table 3

Chemical composition of extruded soy flours with different initial moisture content
(g per 100 g total weight, n = 3)

Sample	Moisture content		Lipid content		Protein (N × 6.25) content	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Soy before extrusion	8.0	0.04	23.5	0.04	36.7	0.2
Extruded soy 2 (8.0%)[a] (160 °C)[b]	7.1	0.19	23.1	0.03	37.6	0.2
Extruded soy 6 (12.0%)[a] (160 °C)[b]	7.6	0.07	23.1	0.27	36.5	0.1
Extruded soy 7 (14.0%)[a] (160 °C)[b]	8.4	0.05	22.5	0.35	36.5	0.2
Extruded soy 8 (16.0%)[a] (160 °C)[b]	8.7	0.22	22.1	0.03	36.5	0.1
Extruded soy 9 (18.0%)[a] (160 °C)[b]	9.0	0.21	21.7	0.17	38.2	0.1

$\bar{x}$: mean value of three measurements
±s: standard deviation
[a]: initial moisture content of soy samples before extrusion
[b]: extrusion temperature

The moisture content of soy samples extruded with the same initial moisture content (8.0%), decreased with the increase of extrusion temperature.

The moisture content of samples wetted prior to extrusion up to 18.0% and extruded at the same temperature (160 °C) increased as a function of the initial moisture content.

The lipid content of soy flour 5 extruded at 200 °C decreased significantly, which is in agreement with our earlier results (HORVÁTH et al., 1989). IZZO and HO (1989) reported, that ether extract of the zein sample extruded at 165 °C displayed stronger interaction than that extruded at 120 °C, possibly due to the presence of polar protein-lipid interactions, and in the sample extruded at 165 °C, a light decrease in ether-extractable material was seen. Some polar bands would probably be unaffected by ether extractions, and at higher extrusion temperatures and at higher initial moisture content some free oil was expelled from soybean during extrusion as Tables 2 and 3 show. As the lipid content of samples 3, 4, 5, 9 decreased, the protein content (N × 6.25) of the same samples increased.

2.2. Extraction and fractionation of soy proteins

Protein solubility of extruded soybean flours was examined using different extraction solutions.

The percentage of H_2O-, NaCl-, C_2H_5-OH-, and KOH-soluble protein fractions was determined according to the Osborne method and the results are given in Table 4 and Table 5.

Table 4

Extraction yield of proteins by Osborne method of soy flours extruded at different temperatures
(n = 3)

Sample	Extracted proteins (% of total protein w/w)		Percentage of fractions in total protein							
			H_2O-		NaCl-		C_2H_5OH- soluble proteins		KOH-	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Soy before extrusion	56.1	0.6	25.3	0.1	26.3	0.4	1.2	0.1	3.3	0.2
Extruded soy 1 (140 °C)[a]	36.6	0.6	16.8	0.4	17.1	0.1	1.2	0.1	1.7	0.1
Extruded soy 2 (160 °C)[a]	21.6	0.3	6.4	0.4	7.1	0.2	1.1	0.1	7.0	0.1
Extruded soy 3 (170 °C)[a]	18.7	0.1	6.3	0.3	5.8	0.2	1.0	0.1	5.6	0.1
Extruded soy 4 (180 °C)[a]	10.6	0.3	4.9	0.1	3.6	0.2	0.6	0.1	1.5	0.1
Extruded soy 5 (200 °C)[a]	7.2	0.2	3.6	0.1	2.3	0.1	0.7	0.1	0.7	0.04

$\bar{x}$: Mean value of three measurements
±s: standard deviation
[a]: extrusion temperature

Table 5

Extraction yield of proteins by Osborne method of soy flours extruded with different initial moisture content (n = 3)

Sample	Extracted proteins (% of total protein w/w)		Percentage of fractions in total protein							
			H_2O-		NaCl-		C_2H_5OH- soluble proteins		KOH-	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Soy before extrusion	56.1	0.6	25.3	0.1	26.3	0.4	1.2	0.1	3.3	0.2
Extruded soy 2 (8.0%)[a] (160 °C)[b]	21.6	0.3	6.4	0.4	7.1	0.2	1.1	0.1	7.0	0.1
Extruded soy 6 (12.0%)[a] (160 °C)[b]	11.5	0.4	5.1	0.2	3.9	0.2	0.8	0.1	1.7	0.2
Extruded soy 7 (14.0%)[a] (160 °C)[b]	16.2	0.2	7.4	0.1	5.9	0.1	0.8	0.1	2.1	0.3
Extruded soy 8 (16.0%)[a] (160 °C)[b]	12.3	0.1	4.6	0.1	4.6	0.1	0.7	0.03	2.4	0.1
Extruded soy 9 (18.0%)[a] (160 °C)[b]	9.6	0.2	3.3	0.1	3.3	0.1	0.8	0.04	2.3	0.1

$\bar{x}$: mean value of three measurements
±s: standard deviation
[a]: initial moisture content of soy samples before extrusion
[b]: extrusion temperature

The data confirmed our earlier findings (HORVÁTH et al., 1989), that increasing the extrusion temperature the proportion of H_2O- and NaCl-soluble proteins progressively decreased, denoting denaturation of albumin and globulin fractions of soybeans and reducing the percentage of extracted proteins in total protein.

Different initial moisture content of soy samples did not affect the percentage of proteins extracted from soy flours extruded at 160 °C. The increase of initial moisture content from 8.0% in all cases decreased the amount of extracted total proteins and the percentage of H_2O- and NaCl-soluble proteins, but from the extruded soy 7, wetted up to 14.0% moisture content 16.2% of proteins could be extracted and from the extruded soy 6, wetted up to 12.0% moisture content prior to extrusion 11.5% of proteins could be extracted only.

The extrusion at 160 °C resulted in a higher percent of KOH-soluble proteins, than at other extrusion temperatures. Higher initial moisture content decreases the percentage of KOH-soluble proteins at this extrusion temperature, but not proportionally. It can be seen in Tables 4 and 5 that the alcohol-soluble fractions of soybean samples are very small and are not influenced by the extrusion.

GUJSKA and KHAN (1991) also found a marked decrease in solubility of water- and salt-extractable proteins after extrusion for another legumes (navy and pinto beans) compared to the raw material. An extrusion temperature of 110 °C had a greater effect on solubility of albumin and globulin fractions of pinto than that of navy bean. When extrusion temperature increased to 150 °C, the solubility of these two fractions of navy bean decreased significantly.

Solubility of soy proteins was determined using 0.5 mol l^{-1} Na_2HPO_4 buffer containing 5% SDS, 1% 2-ME, 5% SDS+1% 2-ME, respectively. The results are presented in Table 6 and Table 7.

Table 6

Extraction yield of proteins from soy flours, extracted at different temperatures in 0.5 mol l^{-1} Na_2HPO_4 *solution* (pH = 7.8) *and in* 0.5 mol l^{-1} Na_2HPO_4 *solution* (pH = 7.8) *containing* 5% (w/w) SDS, 1% (v/v) 2-ME, *and both of them, respectively*
(n = 3)

Sample	Extracted proteins in total protein, % (w/w)							
	Extraction solutions							
	Na_2HPO_4		Na_2HPO_4+2-ME		Na_2HPO_4+SDS		Na_2HPO_4+SDS+2-ME	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Soy before extrusion	52.8	4.0	64.1	1.0	21.7	0.6	52.5	3.7
Extruded soy 1 (140 °C)[a]	44.8	4.1	49.3	2.1	32.7	0.4	50.5	1.4
Extruded soy 2 (160 °C)[a]	24.2	0.9	42.2	0.4	35.8	3.3	62.4	2.1
Extruded soy 3 (170 °C)[a]	22.2	0.3	31.5	3.6	37.1	0.6	56.7	0.9
Extruded soy 4 (180 °C)[a]	14.4	0.5	25.4	0.8	28.7	0.3	55.3	1.3
Extruded soy 5 (200 °C)[a]	7.9	0.3	10.1	0.3	15.8	0.9	42.0	0.8

$\bar{x}$: mean value of three measurements

±s: standard deviation

[a]: extrusion temperature

Table 7

Extraction yield of proteins from soy flours, extruded with different initial moisture content in 0.5 mol l^{-1} Na_2HPO_4 *solution* (pH = 7.8), *and in* 0.5 mol l^{-1} Na_2HPO_4 (pH = 7.8) *containing* 5% (w/w) SDS, 1% (v/v) 2-ME, *and both of them, respectively*

Sample	Extracted proteins in total protein, % (w/w)							
	Extraction solutions							
	Na_2HPO_4		Na_2HPO_4+2-ME		Na_2HPO_4+SDS		Na_2HPO_4+SDS+2-ME	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
Soy before extrusion	52.8	4.0	64.1	1.0	21.7	0.6	52.5	3.7
Extruded soy 2 (8.0%)[a] (160 °C)[b]	24.2	0.9	42.2	0.4	35.8	3.3	62.4	2.1
Extruded soy 6 (12.0%)[a] (160 °C)[b]	16.5	0.4	23.8	2.5	22.8	2.9	58.6	0.7
Extruded soy 7 (14.0%)[a] (160 °C)[b]	23.8	0.05	35.6	0.7	28.0	0.5	62.8	1.1
Extruded soy 8 (16.0%)[a] (160 °C)[b]	14.9	0.1	23.2	2.5	29.9	1.4	63.2	1.2
Extruded soy 9 (18%)[a] (160 °C)[b]	11.4	0.8	19.1	0.2	25.9	0.5	59.6	2.1

$\bar{x}$: mean value of three measurements

±s: standard deviation

[a]: initial moisture content of soy samples before extrusion

[b]: extrusion temperature

The increase of extrusion temperature and the increase of initial moisture content did not cause the same effect on soy protein solubility after extrusion.

Increasing the extrusion temperature the solubility in buffer and in buffer + 2-ME was decreased, but adding of 2-ME to the buffer resulted in a higher protein solubility of the same sample than in buffer alone. In soy samples extruded at 160 °C or at higher temperature the solubilizing activity of the buffer containing both SDS and 2-ME always was higher than that of buffer containing only SDS or 2-ME. It can be explained that the interactions of proteins by non-covalent and disulfide bonds are more intensive at temperature 160 °C and above. 2-ME could react with disulfide bonds only after that SDS reduced the non-covalent interactions. The buffer alone should dissolve protein molecules that remain in their native states (HAGER, 1984). From soy 1 extruded at 140 °C the buffer could dissolve 44.8% of proteins, and from soy 5, extruded at 200 °C the buffer dissolved only 7.9% of proteins. The buffered SDS in addition will dissolve denaturated, but not highly aggregated molecules, non-covalent bonds, and 2-ME reduces disulfide bonds. The

buffer containing both SDS and 2-ME could dissolve 50.5% of proteins from soy 1 (extruded at 140 °C) and 42.0% of proteins from soy 5 (extruded at 200 °C).

Increasing the initial moisture content of full fat soy flours from 8.0% up to 18.0% before extrusion at 160 °C no proportional effect of moisture content on protein solubility in different buffers was found as it can be seen in Table 7. From soy 7 wetted prior to extrusion up to 14.0% more proteins could be extracted with all types of solutions than from soy 6 wetted to 12.0% moisture content or from soy 9 wetted up to 18.0% moisture content. The buffer alone or the buffer containing either 2-ME or SDS could dissolve less protein from all extruded soy samples wetted prior to extrusion, than from soy samples extruded with original moisture content. The buffer containing both SDS and 2-ME could dissolve the same amount of proteins all soy samples extruded at 160 °C.

2.3. SDS-PAGE gel electrophoresis

The protein fractions extracted from soybean and extruded soy flours were submitted to gel electrophoresis by SDS-PAGE. Figure 1 shows the molecular weight distribution of water soluble fractions of soy samples.

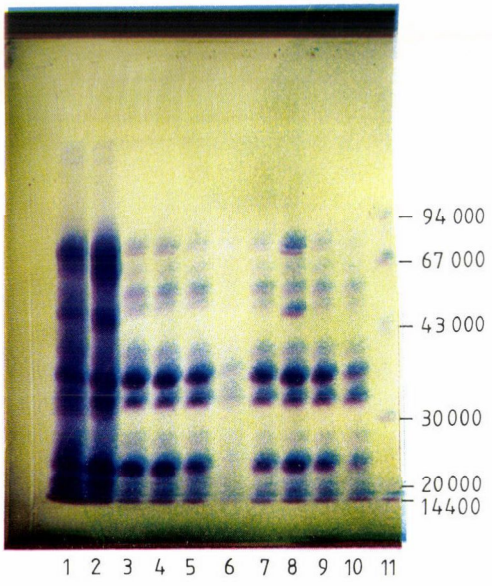

Fig. 1. SDS-PAGE patterns of water-soluble proteins of soy samples, extruded at different temperatures or with different moisture contents.
1: Unextruded soy; 2: Extruded soy 1 (140 °C); 3: Extruded soy 2 (160 °C); 4: Extruded soy 3 (170 °C); 5: Extruded soy 4 (180 °C); 6: Extruded soy 5 (200 °C); 7: Extruded soy 6 (12.0% moist., 160 °C); 8: Extruded soy 7 (14.0% moist., 160 °C); 9: Extruded soy 8 (16.0% moist., 160 °C); 10: Extruded soy 9 (18.0% moist., 160 °C); 11: Standard mol. wt. Kit

High molecular weight subunits over 94 000 D were detected in water soluble proteins of unextruded soybean and extruded soy 1. Increasing the extrusion temperature (lanes 2–6) some subunits between 30 000 and 90 000 D disappeared or were extracted in smaller quantity. From soy samples extruded at 160 °C with different moisture content (lanes 7–10) fractions with the same molecular weight distribution were detected, and it can be seen, that the most protein was extracted from the sample 6 (lane 8) as the intensity of gel dye shows, supporting the data in Table 5.

Figure 2 shows the molecular weight distribution of NaCl-soluble protein fractions of soy samples.

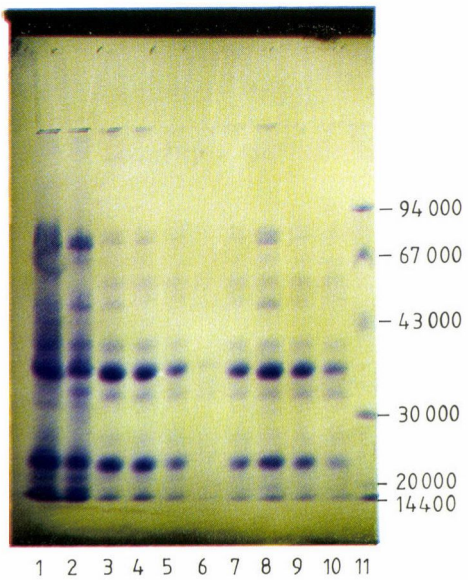

Fig. 2. SDS–PAGE patterns of NaCl-soluble proteins of soy samples, extruded at different temperatures or with different moisture contents.
1: Unextruded soy; 2: Extruded soy 1 (140 °C); 3: Extruded soy 2 (160 °C); 4: Extruded soy 3 (170 °C); 5: Extruded soy 4 (180 °C); 6: Extruded soy 5 (200 °C); 7 Extruded soy 6 (12.0% moist., 160 °C); 8: Extruded soy 7 (14.0% moist., 160 °C); 9: Extruded soy 8 (16.0% moist., 160 °C); 10: Extruded soy 9 (18.0% moist., 160 °C); 11: Standard mol. wt. Kit

The molecular weight distributions of NaCl-soluble proteins are nearly the same as those of H_2O-soluble proteins. Increasing the extrusion temperature (lanes 2–6) some subunits above 30 000 D disappear or their intensity became lower, while the different initial moisture content (12.0–18.0%) did not affect significantly the subunit composition. The NaCl-soluble proteins of unextruded soy and extruded soy

samples 1, 2, 3, 6, 7 contained very high molecular weight subunit, that could not enter into the gel.

The concentrations of C_2H_5OH-soluble protein fractions of all soy samples were very small, as it can be seen in Tables 4 and 5, so their SDS–PAGE pattern intensity was also very small. (Patterns not shown.) For all samples the C_2H_5OH-soluble proteins had subunits less than 36 000 D, and most of them were 22 000 and 14 000 D.

Figure 3 shows the molecular weight distribution of KOH-soluble soy proteins.

It can be seen that increasing the extrusion temperature up to 170 °C (lanes 1–4), the intensity of protein bands above 94 000 D was also increasing, and the more protein was extracted, the higher intensity of other subunits was detected.

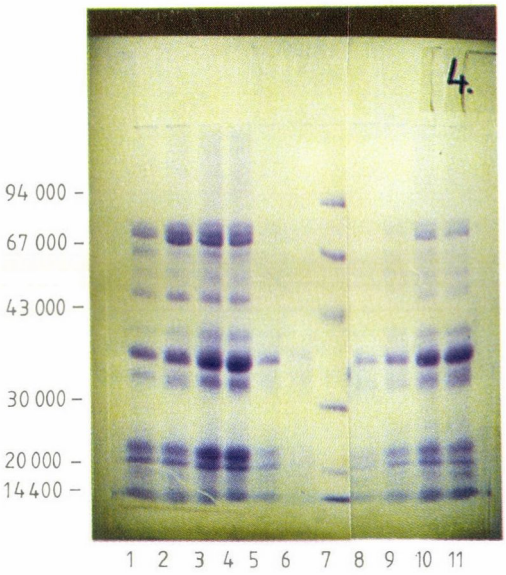

Fig. 3. SDS–PAGE patterns of KOH-soluble proteins of soy samples, extruded at different temperatures or with different moisture contents.
1: Unextruded soy; 2: Extruded soy 1 (140 °C); 3: Extruded soy 2 (160 °C); 4: Extruded soy 3 (170 °C); 5: Extruded soy 4 (180 °C); 6: Extruded soy 5 (200 °C); 7: Standard mol. wt. Kit; 8: Extruded soy 6 (12.0% moist., 160 °C); 9: Extruded soy 7 (14.0% moist., 160 °C); 10: Extruded soy 8 (16.0% moist., 160 °C); 11: Extruded soy 9 (18.0% moist., 160 °C)

From soy flours extruded at 180 °C and 200 °C only very small quality of KOH-soluble proteins could be extracted (lanes 5 and 6). Changing the moisture content of soy samples before extrusion only the intensity of protein bands was modified, but their molecular weight distribution remained the same (lanes 8–11).

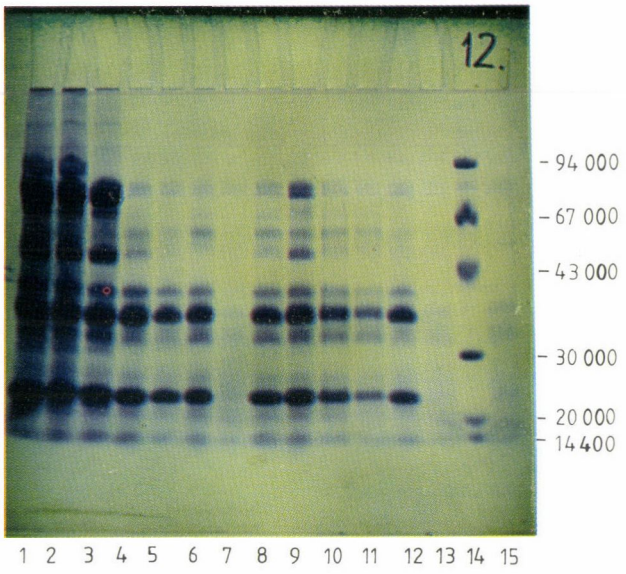

Fig. 4. SDS-PAGE patterns of proteins extracted by 0.5 mol l^{-1} Na_2HPO_4 buffer (pH = 7.8) from extruded soy samples, extruded at different temperatures or with different moisture contents.
1, 2: Unextruded soy; 3: Extruded soy 1 (140 °C); 4: Extruded soy 2 (160 °C); 5, 12: Extruded soy 3 (170 °C); 6: Extruded soy 4 (180 °C); 7, 13, 15: Extruded soy 5 (200 °C); 8: Extruded soy 6 (12.0% moist., 160 °C); 9: Extruded soy 7 (14.0% moist., 160 °C); 10: Extruded soy 8 (16.0% moist., 160 °C); 11: Extruded soy 8 (18.0% moist., 160 °C); 14: Standard mol wt. Kit

The protein fractions of soy samples extracted with 0.5 mol l^{-1} Na_2HPO_4 buffer (pH = 7.8) are presented in Fig. 4.

As the extrusion temperature increased (lanes 3–7) the intensity and quantity of protein bands had changed. Some subunits, most of them above 43 000 D, disappeared. The higher extrusion temperature resulted in less protein bands with lower intensity. The SDS-PAGE patterns of proteins extracted from soy samples extruded with different moisture content at 160 °C were similar, only the intensity of protein bands changed in accordance with the quantity of extracted protein fractions.

The molecular weight distribution profiles of soy protein fractions, extracted with 0.5 mol l^{-1} Na_2HPO_4 (pH = 7.8) buffer containing 2-ME are presented in Fig. 5 (lanes 1–10).

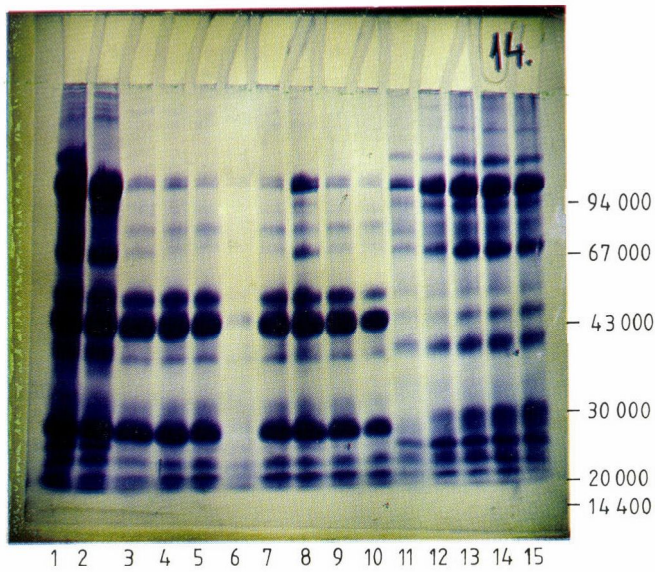

Fig. 5. SDS-PAGE patterns of proteins extracted by 0.5 mol l^{-1} Na_2HPO_4 buffer (pH = 7.8) containing either 2-ME (lanes 1-10) or SDS (lanes 11-15) from extruded soy samples, extruded at different temperatures or with different moisture contents.

1, 11: Unextruded soy; 2, 12: Extruded soy 1 (140 °C); 3, 13: Extruded soy 2 (160 °C); 4, 14: Extruded soy 3 (170 °C); 5, 15: Extruded soy 4 (180 °C); 6: Extruded soy 5 (200 °C); 7: Extruded soy 6 (12.0% moist., 160 °C); 8: Extruded soy 7 (14.0% moist., 160 °C); 9: Extruded soy 8 (16.0% moist., 160 °C); 10: Extruded soy 9 (18.0% moist., 160 °C)

The addition of 2-ME to the buffer caused more changes in the SDS-PAGE patterns of protein fractions. Some new bands were noted above 93 000 D and below 24 000 D. Increasing the extrusion temperature the intensity of protein bands decreased except for subunits of 24 000 D and 36 000 D (lanes 2-5). It can be seen that from soybean extruded at 200 °C only very small quantity of protein could be extracted (lane 6). Different initial moisture content did not cause significant changes in the SDS-PAGE patterns of these protein fractions (lanes 7-10).

The molecular weight distribution profiles of soy protein fractions, extracted with 0.5 mol l^{-1} Na_2HPO_4 buffer (pH = 7.8) containing SDS are presented in Fig. 5 (lanes 11-15) and in Fig. 6 (lanes 11-15).

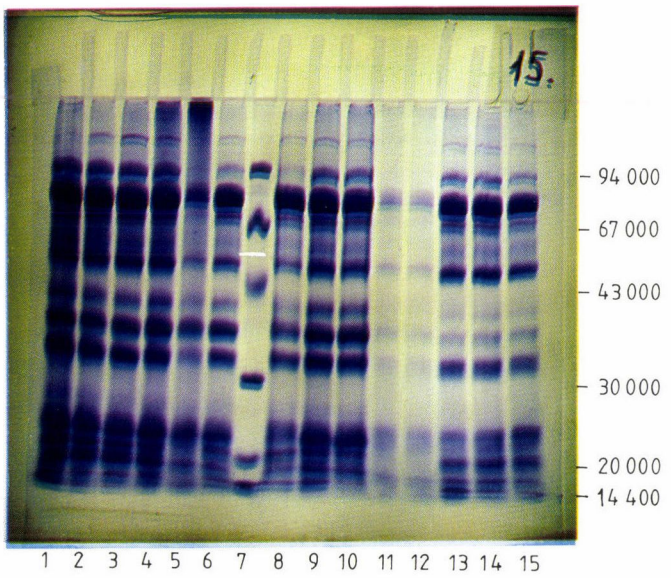

Fig. 6. SDS-PAGE patterns of proteins extracted by 0.5 mol l^{-1} Na_2HPO_4 buffer (pH = 7.8) containing either SDS + 2-ME (lanes 1-6, 8-10) or SDS (lanes 11-15) from extruded soy samples, extruded at different temperatures or with different moisture contents.
1: Unextruded soy; 2: Extruded soy 1 (140 °C); 3: Extruded soy 2 (160 °C); 4: Extruded soy 4 (180 °C); 5, 11: Extruded soy 5 (200 °C); 6, 12: Extruded soy 6 (12.0% moist., 160 °C); 7: Standard mol. wt. Kit; 8, 13: Extruded soy 7 (14.0% moist., 160 °C); 9, 14: Extruded soy 8 (16.0% moist., 160 °C); 10, 15: Extruded soy 9 (18.0% moist., 160 °C)

The SDS-PAGE patterns of all protein fractions extracted by buffer, containing SDS are very different from the SDS-PAGE patterns of proteins of the same soy sample extracted with the same buffer, containing either 2-ME or nothing. In the protein fractions extracted by the buffer, containing SDS two major subunits with molecular weight 24 000 and 36 000 D were noted with fainter intensity. The molecular weight distribution of these protein fractions are the same, the intensity of protein bands are depending on the concentration of fractions. The highest intensity of all bands can be seen at soy protein samples extracted from soy extruded at 160, 170 and 180 °C (Fig. 5, lanes 13-15, Fig. 6, lanes 13-15). From unextruded soy and extruded soy samples 1, 5 and 6 less proteins were extracted.

Figure 6 (lanes 1-6, 8-10) shows the SDS-PAGE patterns of soy proteins, extracted with 0.5 mol l^{-1} Na_2HPO_4 buffer (pH = 7.8) containing both SDS and 2-ME. These protein fractions have the highest number of protein bands, independently from the extrusion circumstances. Increasing the extrusion temperature up to 180 °C some new bands above 94 000 D appeared (lanes 2-4). In the protein fraction extracted from soy extruded at 200 °C some protein bands

between 43 000 and 94 000 D disappeared or became fainter, and subunits with molecular weight above 100 000 D appeared (lane 5). The different moisture content of soy did not cause any significant changes in the molecular weight distribution of protein fractions at 160 °C extrusion.

3. Conclusions

The protein solubility and distribution data, the protein molecular weight profiles showed that the high-temperature extrusion caused significant changes in the protein structures of full fat soy flour. The increase of extrusion temperature significantly decreased the amount of proteins extracted by the Osborne method, while the initial moisture content in the range investigated did affect neither the extractability, nor the distribution of proteins.

Increasing the extrusion temperature the quantity and proportion of water- and salt-soluble proteins decreased most of all, showing their denaturation. The buffer containing either SDS or 2-ME, or both of them could disrupt part of bondings, forming during extrusion. It supported the hypothesis, that in soy protein structure during extrusion changes could take place as a result of hydrophobic interactions, hydrogen bonding, and intermolecular disulfide bridges. In the given experiments 40-50% of soy proteins could not be extracted from extruded samples, so it can be supposed that the dehulling by steaming and the extrusion process may produce other changes, like new covalent interactions, peptide bonds, and these might cause the insolubilization of soy proteins.

SDS-PAGE showed changes in protein molecular weight distribution. Mostly the increase of extrusion temperature affected the molecular weight profiles of protein fractions, and the moisture content of soy samples did not have great effect. The most protein fractions could be characterized by the predominance of some subunits typical for soy proteins. All protein fractions had a characteristic profile, and in different protein fractions the changes were also different. It can be seen in the figures that protein bands showed not only quantitative, but qualitative changes as well.

In summary, the increase of extrusion temperature from 140 °C to 200 °C caused great changes in soy protein structure, while the increase of initial moisture content before extrusion from 8.0% up to 18.0% did not cause great effects.

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ABSTRACTS

of papers presented at the
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PROFESSIONAL COOPERATION BETWEEN MEDICAL FOOD MICROBIOLOGISTS AND FOOD PROCESSING SPECIALISTS: THE INDISPENSABLE CONDITION FOR A SUBSTANTIALLY IMPROVED MANAGEMENT OF LOSS OF THE MICROBIOLOGICAL INTEGRITY OF FOOD[a]

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Foodborne infections and intoxinations continue be a source of human suffering and immense financial burden. Between 20 and 40% of the food produced worldwide deteriorates before it can be ingested due to microbial degradation. On the other hand, an impressive amount of microbiological knowledge as applied to maintenance of the integrity of food has been collected, reviewed and taught since the 1930's. This striking failure of control in spite of available knowledge points to adherence to an unsuccessful management strategy.

Indeed, whereas the early pioneers of medical microbiology from 1920 onwards advocated and practised efficacious prevention scenarios, later generations adopted the fictitious retrospective monitoring approach, borrowed from assurance of chemical food safety where it allows effective consumer protection. For microbiological-ecological reasons confusing of inspection with intervention will constitute, and indeed has created an inexpugnable obstacle to ensuring microbiological integrity of foods and meals.

[a] Address presented upon the awarding of the Honory Degree of Doctor of Agricultural Sciences.

Akadémiai Kiadó, Budapest

The now immensely popular hazard analysis/control of critical point-approach (HACCP) of BAUMAN (1974) is consequently no novelty but rather a reintroduction of a once classical public health strategy. *Avant la lettre*, DACK (1956) has recommended to extend control to the entire food and catered meal production, distribution, storage and culinary preparation chain. In the 1980's Lord PLUMB has elaborated this sound principle to his "farm-to-fork" – scenario, more generically denominated Longitudinally Integrated Assurance of Microbiological Safety, Quality and Acceptability, condensed in the mnemonic LISA.

Adherence to LISA includes (1) ecological studies, as initiated by the Cambridge and Utrecht Schools 1935-1950, to identify and quantify events leading to microbial contamination and colonization of foods; (2) designing technological measures of intervention of intrinsic and extrinsic nature leading to efficacious management of microbial deterioration *sensu latu*; (3) validation of developed technologies by holistic quantitative risk analyses, where required followed by rectification and repeated risk estimation; (4) codification of verified Good Practices and methods of examination allowing to substantiate that prescribed practice guidelines have indeed guided practice, as an essential element of performance criteria.

Abundant survey data indicate that not a single raw food originating from the present severely contaminated environment can be ingested without risk or can be preserved at conventional chill temperatures for more than a few days at most. Consequently raw foods of animal as well as vegetable origin have to be processed-for-safety *sensu* WILSON (1933) – HOBBS (1963) before being distributed to the food industry. PASTEUR's principle of heat inactivation of micro-organisms, named after him, is of course the classical example that allowed turning raw milk into a safe, wholesome and reasonably stable commodity. Spontaneous, and more recently directed "fermentation" of raw foods as a result of predominance of lactic acid bacteria is an other example of long standing. Transradiation of raw foods with gamma rays or electron beams, introduced in the 1940's could constitute a substantial advance as soon as perceived risk feelings were quelled by an intelligent reassurance approach. Pending such an essential operation, as well as afterwards, an attractive alternative may be decontamination of fresh meat and poultry by immediate-post-slaughter treatment with lactic acid. The ever increasing risk of ingestion of salad vegetables contaminated with enteric pathogens, pro- as well as eukaryotes, can be successfully managed by application of a three-stage processing for safety, including prerinsing, decontamination and final rinsing. Intrinsic mechanisms are equally attractive: the addition of antibiotics and bacteriocins produced by lactic acid bacteria may not only preserve, but in addition control the growth of pertinint pathogens in particular commodities presenting serious microbiological hazards.

Beyond a shine of doubt, even the most well-trained professional microbiologist cannot hope to achieve the goal of preserving the microbial integrity of foods along these lines without the assistance of professional food processing specialists. Their participation in these efforts constitutes an indispensable part of ensuring that the food is not only microbiologically sound, but also, and perhaps above all, not becoming devoid of more of its nutritive value and sensory quality than is absolutely unavoidable.

In a balanced treatment of the subject it should not be concealed that one of the segments of the food branch where the LISA approach has, so far, mostly failed is smaller scale catering. Nonetheless also in this instance the solution is within reach. It has to rely on the well-established WILSON triad (1935) of intervention. The catering industry – outside as well as inside the hotellerie – should be (1) provided exclusively with adequately decontaminated raw foods of animal as well as vegetable (*vide supra*) origin, in an attempt to markedly reduce the infectious pressure on food preparation lines; (2) equipped with machinery that is easily cleanable and apt to disinfection; (3) supplied with concise, crystal-clear instructions with respect to hygiene and temperature control, laid down in Manuals summarizing practices, arrived at by procedure seeking consensus amongst acknowledged experts (4) employing line – and management – staff that is (a) properly educated – and examined! – in basic food microbiology; (b) encouraged and trained to exercise consistent self-inspection, for which purpose ample reliable measuring devices should be made available at a reasonable price, allowing to manage the notorious "double failure" menacing catering: lack of hygiene ("sanitation") and temperature abuse.

Here, once again close cooperation between food microbiologist and processing specialist is essential to assuring safety and quality of the product as finally ingested: pathogens not exceeding the level set by holistic risk analysis and saprophytes limited to cfu numbers of the specific spoilage association corresponding to metabolite levels below the threshold of organoleptic detectability, The societal impact of this development can hardly be overrated. The challenge in terms of research, academic education and interdisciplinary development is indeed fascinating. Academic and applied food scientific life in Hungary owes it to its nestor and pioneer Professor Károly VAS, to meet this and allied defiances in the same vein as he did: making his foreign colleagues admire the Hungarian academic setting wherein he was privileged to function despite often all too difficult circumstances.

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STUDIES ON INTERACTIONS OF ANTIMICROBIAL FACTORS

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Effects of combination treatment were favourite subjects of Prof. K. VAS, outstanding authority of the Hungarian food microbiology until his untimely death in 1981. Lack of predictability of combined effects hampers, however, the design and implementation of rational combination processes. Only during the recent years, parallel with the astonishing development of computerization – even in the microbiological research – became the predictive microbiological modelling as one of the most intensively investigated areas of food microbiology. The lecture devoted to the memory of the late Professor VAS describes combined effects of reduced pH and water activity on the growth of untreated and irradiated *Listeria monocytogenes*. Mathematical models describing the effects of hydrogen-ion concentrations and salt concentrations on the apparent growth rate of the test organism at various temperatures show synergistic interactions between salt and hydrogen-ion concentrations, which were enhanced in case of the irradiated inoculum. The excess of the interactions was considerably influenced by the nutrients of media used for growth of the stressed cells.

MICROBIOLOGY OF FERMENTED SAUSAGES

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Since fermented sausages are not heat-treated items, their microbial flora differs basically from that of the other meat products. This gives the difference in sensory value, but is also responsible for the need of more precise processing technology in order to assure microbial safety. Fermented sausages may be classified according to several criteria, the most important being their level of acidity. This acidity plays namely the decisive role in microflora composition also in terms of pathogenic micro-organisms. Low acid fermented sausages are traditionally produced in Hungary and have an a_w-value below 0.90 in general. This low value is practically the only factor assuring safety and good shelf-life. High acid fermented sausage, a relatively new item in Hungarian meat industry, is produced by the help of starter cultures and addition of carbohydrates, These sausages have a pH-value below 5.3 that in combination with lowered a_w-value inhibits the growth of undesired bacteria. In the paper the changes in microflora under the influence of different factors will be dealt with in more details, as well as examples will be given for special application of hazard analysis.

THE APPLICATION OF THE NIR-NIT TECHNOLOGY IN THE FOOD SCIENCE AND ANALYSIS

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In 1972 at one of his trip in the USA Dr. Károly VAS, director of the Central Food Research Institute (CFRI) paid a visit to the Instrumentation Laboratory of the USDA Agricultural Research Center in Beltsville. There he got acquainted with a new technology using the near infrared spectral region of the electromagnetic radiation for multicomponent analysis. Recognizing the importance and the perspectives of this technology, Dr. Károly VAS opened me the opportunity to study it in Beltsville at Dr. Karl H. Norris' Laboratory from 1973 to 1974. Following this, the CFRI has sent several other researchers to Beltville to study this technology for food composition analysis. So in 1978 Hungary joined in this activity both in research and development as well as in industrial production and application. In cooperation

with respective research institutes of the different industry branches, the CFRI achieved good results in determining relationship between near infrared transmission or reflection data and composition of stone fruits, vegetables, sunflower seed, bran, pastry products, cocoa powder, wine, lupine etc. In 1989, the University of Horticulture and Food Industry purchased a sophisticated NIR-NIT instrument with the help of which research work began also here. Results were achieved in determining the gamma irradiation effect on paprika powder and egg powder as well as in the field of qualitative analysis applied for raw meat, milk powder and coffee mixtures. A wide range of international cooperation greatly contributed to our achievements. The results using NIR-NIT technology provided us with such depths of knowledge and details that have so far never been thought of.

THE IUFoST AND ITS HUNGARIAN RELATIONS

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The International Union of Food Science and Technology (IUFoST) is now more than 20 years old and has over 40 adhering bodies representing well over 100 000 food scientists and technologist around the world. Among these, the Union is best known for its Congresses and Symposia. The 9th World Congress will be held in July–August 1995 in Budapest, hosted by the Hungarian Scientific Society for Food Industry (MÉTE). IUFoST represents organizations of the world's food scientists and technologists. Since its foundation in 1970 Hungary is member of the Union thanks to the late Prof. Károly VAS, the first Hungarian Delegate in the organisation. He was member of the Executive Committee of IUFoST and for long time responsible for selecting sites and locations of Congresses and Symposia organized by the Union. Since his death in 1981 there are many activities of the Union in which Hungarian scientists are involved. IUFoST officers and Executive Committee members are frequently visiting Hungary, thus establishing regular contact with MÉTE and the National Committee of Food Science and Technology, which is the governing body of MÉTE. We are planning and organizing the World Congress in 1995 in good cooperation and hope to be successful.

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BOOK REVIEWS

Unsaturated fatty acids
Nutritional and physiological significance

Chapman & Hall, London, Glasgow, New York, Tokyo, Melbourne, Madras, 1992, 211 pages

This book is "The Report of the British Nutritional Foundation's Task Force".

In the recent years the role of fats in human nutrition as well as in animal feeding has been the subject of several epidemiological and experimental studies. The importance of the quality of fats and oils was first published in 1929 by Burr and Burr. Since then innumerable studies have been made, many papers and books were published and a series of recommendations have been formulated in the field of the role of fats and oils in human nutrition as well as in animal husbandry. These studies resulted in many new discoveries but these were not free from concentrations, therefore it was necessary to review objectively the present state of knowledge of unsaturated fatty acids.

This mission was performed by the Task Force invited by the Council of the British Nutrition Foundation. The Chairman, Dr A. Garton FRS, the members and corresponding members as well as the observers were recruited from different countries of the world and all of them were well known representatives of their special scientific field.

The book starts by outlining the structure and chemical characteristics of various fatty acids (Chapter 1). This is followed by introducing the sources of unsaturated fatty acids in the diet and with a short comprehensive overlook at the effects of various technological procedures on the chemical composition of fats and oils (Chapter 2). Chapter 3 is devoted to the surveys on fat consumption in different populations. In the next Chapters (4–8) the mechanisms of the digestion, absorption, transport, metabolism and functions of unsaturated fatty acids are summarized. An interesting problem forms the topic of the next Chapter, namely the role of fatty acids in early development in the fetal life.

The Chapters 10–19 concern the health problems related to the quantity and composition of the fat consumed. Coronary heart disease, changes in plasma lipids, thrombogenesis and fibrinolysis, blood pressure, cardiac arrhythmias, cancer, diabetes, skin disease, immune disorders are discussed.

The Chapters 20 and 21 deal with practical problems. What kinds of supplementary sources of unsaturated fatty acids – fats, fatty acid concentrates – can be used and how to label foods to inform the consumers in relation to unsaturated fatty acids and cholesterol?

In Chapter 22 recommendations for intakes of unsaturated fatty acids are given for adults, infants, pregnant and lactating women living in UK and in other countries.

At the end the book short conclusions and suggestions for future progress are given to each Chapter. A glossary for explanation of special terms and more than 770 references (with title) are also given.

Each of the 211 pages of this book provides very important concise information on the above mentioned topics with many tables, well selected chemical formulas and biochemical pathways.

Personally I feel myself lucky to have the opportunity to introduce this book and I would like to recommend it for nutritionists, dietetitians, medical doctors, university students, colleagues working in food industry, and for everybody interested in nutrition. I think this book is easily understandable by

everyone with basic knowledge in science so I can encourage even those readers who are not familiar with the recent results in nutritional evaluation of fats.

I am quite sure that this book first of all is a valuable tool for the experts in nutrition because one can find a critically compiled scientific review on unsaturated fatty acids with clear conclusions, recommendations and good ideas for future research.

Ö. GAÁL

Advances in baking technology

B. S. KAMEL and C. E. STAUFFER (Eds)

Blackie Academic and Professional, 1992, 408 pages

This book excels not only in the particular execution but in the actuality of the discussed matter from the books dealing with certain domains in-detail and synthetizing the knowledge of them. The author staff, consisting of professionally well known and in the international scientific life highly esteemed experts, is in itself remarkable.

Authors treat of new technologies e.g. freezing of doughs or procedures known long ago as rye breadmaking, its new technologies having renaissance nowadays.

Outstanding chapters are those that deal with the up-to-date dough processing, additives and their mechanism of action, the technologically significant role of special fats and fat replacers.

Discussion of dietary fibers gives a compendium for the nutrition biologist, the baking industry technologist, and for those carrying out examinations, as well.

The rheological investigation of the various doughs and baked products was always a matter of primary importance in the baking industry. Here also new equipments, methods and results are acquainted with.

The part of the book dealing with enzymes as dough improvers, contributes to getting acquainted with the fundamental principles or recent technologies.

The use of microwave technology was outlined in the 1970's. The keen interest in it was strongly increased by the applicability of the equipments manufactured by mass production. This work traits of applicability of microwaves exceeding the baking process.

A number of versions of the lately all over the world wide-spread extrusion processing are included with moderate informations.

Chapter of sensory evaluation deals with problems of methodology and possibilities of solving them. Of course, comparison in international scale is in this case the most difficult as taste and demands can never be standardized.

The book is designed for baking industry specialists being engaged in technical, economical and technological developing as well as in teaching these subjects and also for those fulfilling everyday duties of production. Redaction and style is of usual level of scientific publications but simple and concise.

L. SZALAI

The technology of vitamins in food

P. B. OTTAWAY (Ed.)

Blackie A. & P., London, Glasgow, 1993, 270 pages

The book written by 9 authors deals with the role of vitamins in human nutrition in 9 chapters as an important aspect of nutrition and food science. Vitamins are reviewed giving a detailed overview on their structures, stability, natural presence and all biochemical, chemical and technological characters that are indispensable for food science and technology.

The first chapter entitled "Biological functions of vitamins" (by J. MARKS) is not simply an overall statement which embraces each and every biological functions of the vitamins (vitamins act as either coenzymes or prohormones) but involves certain other vital functions too. For convenience two separate chemical categories are given, first the four oil soluble members of the group and then the water soluble ones. The first group includes several compounds which are often grouped together under the term vitamin B-complex (or vitamin B-group). The B-complex vitamins are very important for they play a vital role in enzyme reactions which are necessary for carbohydrate, fat and protein metabolism.

The second chapter deals with "Natural occurrence of vitamins in food" (by H. CRAWLEY). This chapter provides an overview of the nature and concentrations of vitamins naturally occurring in foodstuffs commonly consumed by man. Tables showing vitamin contents of common foodstuffs have been compiled from a number of food tables and published articles from different geographical regions to represent "typical" values for the foods specified.

The third chapter is entitled "Nutritional aspects of vitamins" (by D. H. SHRIMPTON). Due to its importance this chapter discusses all aspects of vitamins, especially in relation to their presence or use in different foods. The optimum level of vitamins and the diet to achieve it are also discussed.

The fourth chapter deals with the "Industrial production" (by M. J. O'LEARY). Topics discussed include some excellent reviews of the chemistry, synthetic routes and technology of vitamin production. It shows the new techniques in biotechnology that gives boost to the search for improved processes for vitamin production.

The fifth chapter is entitled "Stability of vitamins in food" (by P. B. OTTAWAY). This chapter investigates the most important factors that may cause the loss of vitamins and discusses vitamin losses in the food due to the different technological processings and storage. It deals with shelf life of food products and the degradation rate of vitamins.

"Vitamin fortification of food (specific applications)" (by A. O'BRIEN, D. ROBERTON) is the title of the sixth chapter. Due to the importance of vitamin fortification this chapter discusses all aspects of vitamin addition to foods, i.e. the consequences of addition of up to thirteen vitamins as a single ingredient are as follows: inventory levels are reduced; quality control, production and assay procedures are simplified giving marked savings in labour costs and time. It deals with specific applications of vitamins in beverages, cereal products, dairy products and confectionery.

Chapter seven deals with "Vitamins as food additives" (by J. N. COUNSELL). Of the recognized vitamins, five have had some applications in food technology as possible food additives. These are ascorbic acid and its salts and ester, the carotenoids (provitamin A), riboflavin (vitamin B_2), niacin, dl-α-tocopherol (vitamin E). As their molecular structures and physical, chemical and biological functions are all quite different they are used in different fields of the food industry. This chapter shows them as food additives used in the industry and their effects on the quality of food.

The eighth chapter bears the title "Vitamin analysis in foods" (by I. D. LUMLEY). Perhaps it is the most comprehensive chapter in this book, because the determination of vitamins in foodstuffs causes significant problems for analysts. This chapter is to provide an overview of methods from the biological, microbiological assays, some physicochemical methods to the recently used methods; gas liquid chromatography (GLC) and high performance liquid chromatography (HPLC) applied for determination of vitamins in foodstuffs. It is remarkable that this chapter mentions 119 references.

"Food fortification" is the last (9th) chapter (by D. P. RICHARDSON). This chapter deals with general policies for nutrient additions, leglistation concerning addition of nutrients to foods, claims for nutrients and labelling of fortified foods, restrictive regulations and policies on health claims.

As last Appendix 1 shows: the chemical and physical characteristics of vitamins (structural formula, principial commercial forms, standardization, solubility, melting point, absorption spectrum). Appendix 2 contains the recommended nutrient reference values for food labelling purposes.

The references enlisted at the end of each chapter consist mainly of relevant primary literature of the last years. The book contains 409 references.

This book is highly recommended to researchers in food science, to food technologists as well as to all who are engaged in this field.

É. SZÁNTÓ-NÉMETH

First Hungarian representative nutrition survey (1985–1988) Results
Vol. 1.

GY. BIRÓ (Ed.)

Medical Officer Head Office, Institute of Health Promotion, Budapest, 1992, 249 pages

Volume 1 of the "First Hungarian Representative Nutrition Survey 1985–1988" (published by G. Bíró) came out at the end of 1992.

Because of the importance of the population level survey, the book may be of interest for both Hungarian and foreign experts, this interest is met by the book's being bilingual: Hungarian and English.

The 249 pages of volume 1 of the compilation *elaborate nutritional data* of 16 641 persons over 14 years of age, taken in 114 settlements and grouped according to residence, age and sex. The examined population group is representative on a 0.2% level in regard of the population of the country and of the different counties, respectively. Beside the already mentioned parameters, characteristics of occupation, income and education of the examined sample group are also dealt with.

The nutrition survey was performed by personal interviews, applying questionnaires about 2×24 hours' meals, covering menus of a working day and a Sunday. According to the calculated nutrient intake data, averages of working days, of Sundays and weighted averages calculated from the two, were recorded.

Beside the interviews, blood pressure measurements, targeted case history taking, anthropometric measurements (body mass; body height; upper arm circumference; skinfold – in 4 points of the body, by the use of Lange caliper) were performed. In the case of volunteers – about 30% of the examined persons – biochemical laboratory tests were made, too, in regard to parameters indicating increased risk of cardiovascular diseases and with regard to parameters suitable for the evaluation of nutrient supply

and liver function. Results of anthropometric measurements and laboratory tests will be presented in volume 2.

Furthermore, the survey covered applied meal preparing procedures, consumption of alcohol, smoking, physical activity, nutritional habits, living conditions, nutritional habits in childhood, cooking knowledge and diseases which may be related to nutrition. The applied methods are presented in the first chapters of the book.

The comprehensive, complex nutritional-epidemiological survey was coordinated by the National Institute of Food Hygiene and Nutrition, in data collection and in the elaboration of the examination materials, about 800 health professionals – mainly from the field of public health – took part. The list of the participants is given on pages 9–10 of the book.

The volume presents an enormous mass of valuable data in well arranged, easy to survey form. With full knowledge of morbidity and mortality indices, indicating the extremely bad health status of the population (cardiovascular diseases and malignant tumors cover two-thirds of total mortality) the published data are startling, but not at all unexpected.

After the presentation of energy intake data, intake data of different macro- and micro-nutritients are shown. Among macro-nutrients, intakes of total protein; animal- and plant-proteins; total fat; saturated-, mono- and polyunsaturated fatty acids; added sugar; dietary fibres can be studied. We get informed about the share of energy-providing nutrients in the total energy intake, expressed in percentage. In the case of all nutrients, beside the averages and standard deviations of each group, also percentage distributions of intake levels are given, thus the reader gets good informations about the details, too. Except for the oldests, protein intake is exaggerated with the dominance of proteins of animal origin (66% in men, 63% in women). Plus energy comes primarily from fats: fat energy exceeds 40% of the total daily energy intake. Fat intake over 30% presents a risk factor in itself. The ratio of polyunsaturated : saturated fatty acids (P : S) is highly unfavourable, instead of the recommended 0.6–0.8, it is only 0.24.

Cholesterol intake is very high, in men 531 ± 271 mg, in women 418 ± 208 mg, exceeding considerably the recommended quantity of 300 mg.

Sugar intake is excessive: 12.9 and 15.3 energy %, respectively, contrary to the desirable 10%; at the same time, complex carbohydrates represent only 28 energy %, considerably less, than the recommended level.

In half of the examined sample group, the intake of dietary fibre is inadequate.

Similarly to the macro-nutrients: intake data of the following vitamins were elaborated, retinol, carotene, tocopherols, thiamine, riboflavin, pyridoxine, niacin, cobalamines, ascorbic acid.

Marginal deficiency in regard to different vitamines may be detected in a part of the population.

Ascorbic acid supply is in the whole, inadequate. Intakes are below 60 mg/day in 58% of men and in 55% of women. Retinol-, thiamine-, riboflavin- and tocopherol intakes are deficient, too.

In case of macro- and micro-elements, intakes of sodium, potassium, calcium, magnesium, copper and zinc and the sodium/potassium ratio are shown in the tables, detailed similarly to the former data. Average iron intake is sufficient in men, iron deficiency can be observed primarily in women of reproductive age. Calcium intake is insufficient, magnesium intake is low.

Sodium intake is more than threefold of the acceptable value. Potassium supply is inadequate, first of all in women. Na/K ratio is 2.7 ± 0.7 in men, 2.5 ± 0.7 in women, contrary to the acceptable, less than 1 value.

The book presents facts to support the connection – already obvious to experts – between the nutrition of the population and the morbidity and mortality indices, which are among the worst in international comparison. Thousands of data, presented from several aspects, cannot be reported in a single review, but the book, as essential work of reference, offers indispensable help in all fields of

nutrition science and hygiene and is useful even for professionals of agricultural and food production. That is why we look forward to the – as early as possible – publication of the further volumes.

M. BARNA

(The book may be purchased in the National Institute of Food Hygiene and Nutrition, 1097, Budapest, Gyáli út 3/a)

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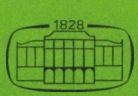

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MODELING OF EXPANSION AND WATER SOLUBILITY INDEX OF WHEAT STARCH DURING EXTRUSION COOKING

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Wheat starch was processed in a Baker–Perkins MPF-50D co-rotating twin-screw extruder, at moisture contents of 25% and 30%, screw speeds of 200 r.p.m. and 300 r.p.m., feed rate of 30 kg h^{-1}, and barrel temperature settings of 100, 120, 140 and 160 °C. The water solubility index and water absorption index of starch samples obtained along the extruder channel were investigated. The changes in the functional properties of the starch during extrusion cooking were found to be dependent on the changes in the molecular structure of the starch. The regression analysis showed that WSI is an exponential function of the degree of gelatinization, while the extrudate expansion is a more complex function of both starch gelatinization and degradation. The temperature and the moisture content also affect the expansion both directly and indirectly, through effects on viscosity. An expression for extrudate expansion was developed in terms of the degree of gelatinization, degree of degradation, temperature, and moisture content.

Keywords: starch, extrusion cooking, expansion, water solubility, gelatinization, water absorption, WSI, WAI

During extrusion cooking starch undergoes several significant structural changes such as gelatinization and degradation, resulting in changes in its functional properties. Water absorption and water solubility are two of the key functional characteristics defining the interaction of the extruded starches with water. Although there has been considerable work on the effects of extrusion conditions on water absorption index (WAI) and water solubility index (WSI) (ANDERSON et al., 1969; COLONNA et al., 1984; DAVIDSON et al., 1984; PATON & SPRATT, 1984; KIRBY et al., 1989), the relationship between WSI (or WAI) and extrusion conditions is not based on a fundamental understanding of the mechanism of the molecular processes occurring in the extruder.

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Extrudate expansion is an important characteristic of extruded foods such as expanded snacks and ready-to-eat cereals. Although the effects of extrusion conditions on the extrudate expansion have been widely investigated (LAUNAY & LISCH; 1983; CHINNASWAMY & HANNA, 1988; FALCONE & PHILIPS, 1988), a quantitative relationship between extrudate expansion and extrusion conditions has still not been adequately developed. LAUNAY and LISCH (1983) and KIRBY and co-workers (1989) pointed out that expansion is linked to the rheological properties of the melt. ALVAREZ-MARTINEZ and co-workers (1988), based on the viscosity model developed by HARPER and co-workers (1971), derived an expression that relates extrudate expansion with moisture content, temperature, and shear strain. Although it is clear that the functional properties of extruded products are affected by their structural properties, a clear explanation of these relationships have not been reported in the literature.

The purpose of our research program is to develop a detailed understanding of extrusion cooking of starch on a molecular level. Mathematical expressions for starch gelatinization and starch degradation during extrusion cooking have been reported earlier (CAI & DIOSADY, 1993; CAI et al., 1993). In the present paper, the changes in WSI and WAI in starch during extrusion are presented. Both WSI and specific volume were related quantitatively to the structural properties of the starch.

1. Materials and methods

Whetstar-4, a commercial wheat starch Ogilvie Mills Ltd, was processed by a Baker–Perkins MPF–50D co-rotating twin-screw extruder. The details on the equipment were reported earlier (CAI & DIOSADY, 1993). The extruder was operated with a constant feed rate of 30 kg h^{-1}. Screw speeds of 200 r.p.m. or 300 r.p.m. were used. Moisture contents were set at 25% or 30%. Barrel temperature settings were 100, 120, 140 and 160 °C.

The horizontal split design of the Baker–Perkins extruder allowed the collection of samples inside of the extruder channel with a "dead-stop" run. When the extruder was running at steady state as indicated by the steady values of the screw torque and die pressure, the extruder was suddenly stopped by cutting the feed supply, the screw rotation and the electrical heating. The barrel was cooled by the refrigeration system to ambient temperature and dismantled very quickly. Five gram samples were then taken at ~2.5 cm intervals along the extruder channel for detail analyses.

The degree of gelatinization was defined as the weight ratio of gelatinized starch to the total weight of the sample. The determination of the degree of gelatinization was based on the method described by BIRCH and PRIESTLEY (1973), which measures the blue iodine complex by the amylose released during

gelatinization. A 0.04 g sample was dispersed in 50 cm^3 0.060 mol l^{-1} KOH solution and then gently agitated for 15 min. The slurry was centrifuged and 1 cm^3 aliquots of the supernatant were removed, mixed with 9 cm^3 0.00667 mol l^{-1} HCl. Then 0.1 cm^3 of iodine reagent (1 g iodide and 4 g potassium iodide per 100 cm^3 water) was added, and after mixing the absorbance α_1 was read at 600 nm in the spectrophotometer against a reagent blank. The estimation was repeated using 50 cm^3 0.4 mol l^{-1} KOH solution, and 9 cm^3 0.0445 mol l^{-1} HCl to obtain the absorbance α_2. The degree of gelatinization was calculated by the ratio of the two absorbances α_1 and α_2 obtained. The reported results are averages of three replicated analyses (CAI & DIOSADY, 1993).

Water solubility index (WSI) and water absorption index (WAI) were measured using the method reported by ANDERSON and co-workers (1969). WAI was defined as the weight of gel obtained per weight of solids (the total solids in the original sample were corrected for the loss of solubles in the supernatant) (DAVIDSON et al., 1984); and WSI was defined as the dried water-soluble fraction as a percentage of the dry sample.

Expansion ratio (diameter ratio, *Dp/Dd*) of the extrudate was calculated by dividing the diameter of extrudate, *Dp*, by that of the die, *Dd*. Each reported value was an average of ten readings. Specific volume, V_s, of the extrudate was determined by weighing about 20 cm^3 of extrudate and measuring its volumetric displacement with fine glass beads. The measurement was repeated three times for each sample.

2. Results and discussion

1.2. WSI and WAI

WSI of the samples obtained along the extruder channel as well as the extrudates were measured. The relations between WSI and starch gelatinization (Fig. 1), were determined by correlating all the data of WSI to the data of starch gelatinization. Regression analysis showed that the data fit the following exponential equation:

$$WSI = 4.876\ e^{2.05f} \qquad (1)$$

where f is degree of gelatinization, with the correlation coefficient $R^2 = 0.932$. A correlation with gelatinization was expected. Raw starch granules are insoluble in cold water due to the hydrogen bonding between the molecules. During gelatinization starch granules were collapsed by the breakdown of the hydrogen bonds and water molecules attached themselves to the exposed hydroxyl groups much more easily. This resulted in an increased solubility. This relationship between WSI and starch gelatinization is very helpful in understanding the changes of WSI during extrusion processing.

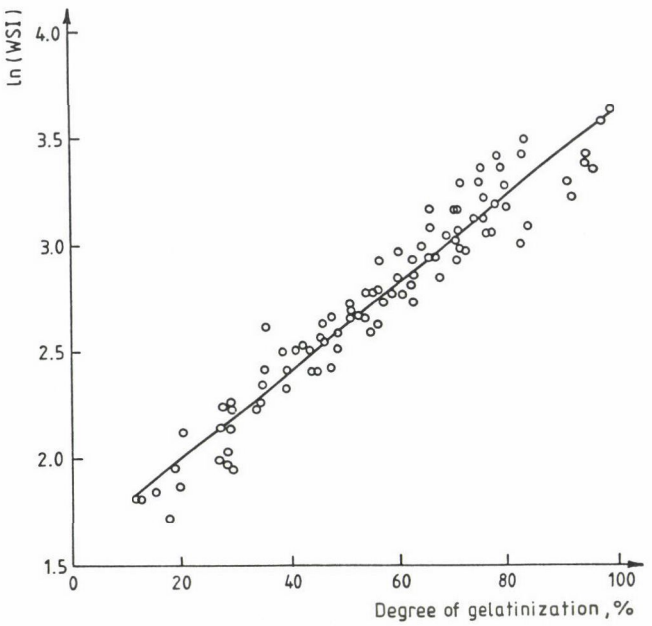

Fig. 1. Correlation between WSI and degree of gelatinization

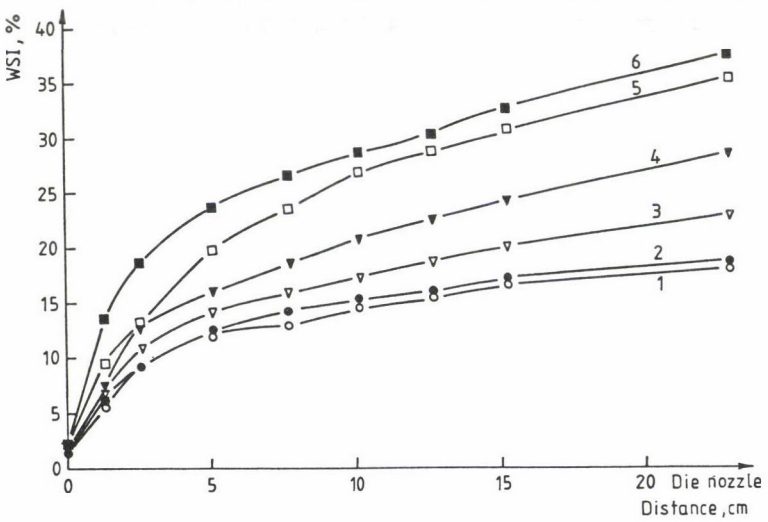

Fig. 2. WSI along the extruder channel starting from beginning of the cooking zone. Moisture content: 25%. 1: 100 °C, 200 r.p.m.; 2: 100 °C, 300 r.p.m.; 3: 120 °C, 300 r.p.m.; 4: 140 °C, 200 r.p.m.; 5: 160 °C, 200 r.p.m.; 6: 160 °C, 300 r.p.m.

The results showed that the changes in WSI during extrusion cooking occurred only in the cooking zone, whose length was mainly determined by the geometrical parameters of the extruder (CAI & DIOSADY, 1993). Figure 2 illustrates the typical changes in WSI in the cooking zone. WSI changed rapidly at the beginning of this zone then more gradually till the die. This trend was the result of the dependence of WSI on starch gelatinization in the extruder. CAI and DIOSADY (1993) reported that starch gelatinization only takes place in the cooking zone. At the beginning starch gelatinization occurred rapidly and was described by a second order reaction. After this initial period the rate slowed down, and followed a first order rate law.

The gelatinization process during extrusion can be described by the following equation (CAI & DIOSADY, 1993):

$$\ln(1-f) = -k_0 \exp\left[-(\Delta E_0 - \beta\tau)\,(RT)\right] t \qquad (2)$$

where t is the residence time, counted from the beginning of the cooking zone, ΔE_0 the activation energy, T the temperature, R the ideal gas constant, k_0 the preexponential factor, τ the shear stress, and β the activation volume. Substituting Eq. (2) into Eq. (1), we obtained a more detailed expression for WSI:

$$WSI = 4.876 \exp\left\{2.05\left[1-\exp\left(-k_0 \exp\left(-(\Delta E_0 - \beta\tau)/(RT)\right) t\right)\right]\right\} \qquad (3)$$

Equation (3) relates the value of WSI to the residence time, and the mechanical and thermal energy input. WSI increases with barrel temperature due to the higher thermal energy input into the system. Similarly a higher shear stress results in increased WSI, due to the increased mechanical energy input. The effects of the energy inputs on WSI are illustrated in Fig. 3. A higher moisture content decreased WSI because the increased moisture content lowered the viscosity, which, in turn, resulted in a lower mechanical energy input. Although it caused shorter residence time, t, higher screw speed resulted in a higher WSI because of the increased shear rate and then the increased shear stress.

The change in WAI during extrusion cooking was also investigated by examining the samples obtained along the extruder channel. The changes in WAI followed the trend for WSI. WAI changed only in the cooking zone, very rapidly at the beginning and then more gradually all the way to the die (e.g. Fig. 4). The change of WAI of the extrudates was not significant, between 5.5 and 6.5 for the extrusion conditions used. WAI increased with barrel temperature, but no apparent effects of screw speed and moisture content were observed. PATON and SPRATT (1984) reported similar results for a single-screw extruder.

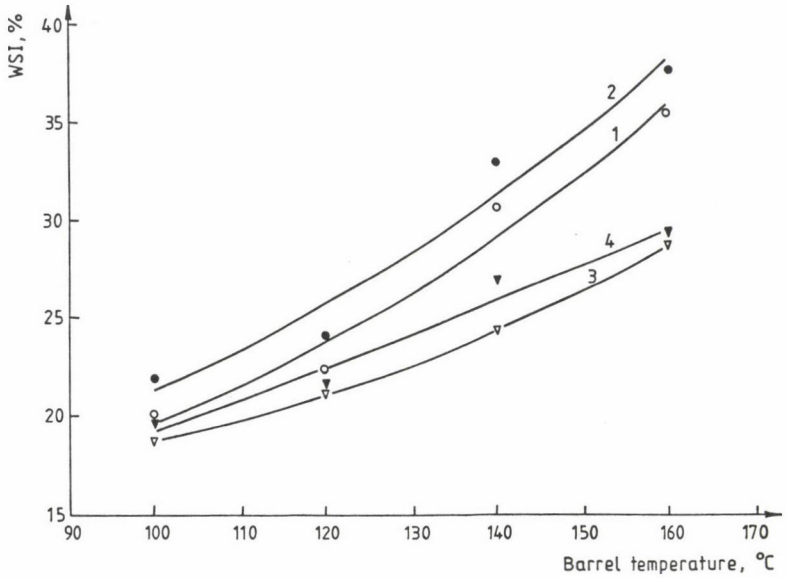

Fig. 3. The effects for extrusion conditions on WSI of the extrudate. 1: screw speed = 200 r.p.m.; moisture content = 25%; 2: 300 r.p.m., 25%; 3: 200 r.p.m., 30%; 4: 300 r.p.m., 30%

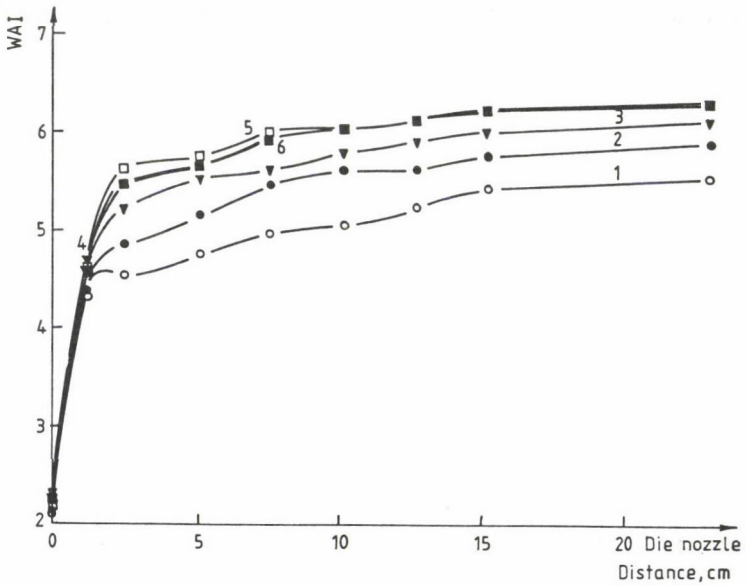

Fig. 4. WAI along the extruder channel starting from the beginning of the cooking zone. The values for 140 °C/300 r.p.m. effectively overlap those for 160 °C/200 r.p.m. Moisture content: 30%. 1: 100 °C, 200 r.p.m.; 2: 100 °C, 300 r.p.m.; 3: 120 °C, 200 r.p.m.; 4: 140 °C, 300 r.p.m.; 5: 160 °C, 300 r.p.m.; 6: 160 °C, 200 r.p.m.

2.2. *Extrudate expansion*

Measurements of expansion ratio (diameter ratio, *Dp/Dd*) and specific volume (V_s) are summarized in Table 1. These two measurements were not in agreement. For some of the extrusion runs the differences in expansion ratios were very small, but the differences in specific volumes were very significant. This indicates that there were differences in the longitudinal expansion and that the expansion ratio, which only describes the cross-sectional expansion, is not a good criterion for describing extrudate expansion. The overall expansion is better described by changes in specific volume or bulk density.

Table 1

Values of the expansion measurements

Conditions: Temperature (°C)	Screw speed r.p.m.	H_2O (%)	Die pressure (bar)	Expansion ratio *Dp/Dd*[a]	Specific volume ($cm^3\ g^{-1}$)
100	200	25	62.06	4.10	5.34
100	300	25	36.55	4.15	6.53
120	200	25	55.17	4.15	6.31
120	300	25	31.72	4.22	7.24
140	200	25	42.07	4.15	6.52
140	300	25	24.14	4.17	7.97
160	200	25	26.90	4.12	7.70
160	300	25	22.76	4.23	8.11
100	200	30	37.93	3.83	4.42
100	200	30	34.48	3.89	4.56
120	200	30	33.10	3.92	4.78
120	300	30	27.58	3.91	5.23
140	200	30	27.58	3.89	5.73
140	300	30	20.68	3.84	6.25
160	200	30	22.06	3.92	6.33
160	300	30	16.55	3.97	6.65

[a] *Dp/Dd*: diameter ratio of the product to the die

Figure 5 shows the effects of temperature and the moisture content on the specific volume of the extrudate. Higher barrel temperatures resulted in increased expansion in the temperature range tested, because temperature affected both the flow properties and the vapour pressure. The viscosity of the melted starch decreased exponentially with increasing temperature, allowing the melt to expand more easily. Meanwhile, higher temperatures increased the vapour pressure, enhancing the puffing of the material.

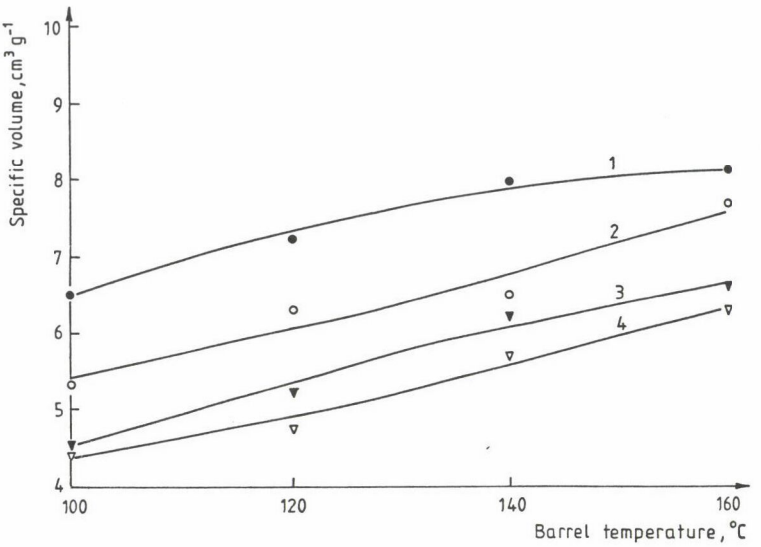

Fig. 5. The effects for extrusion conditions on the specific volume of the extrudate. 1: 300 r.p.m., 25%; 2: 200 r.p.m., 25%; 3: 300 r.p.m., 30%; 4: 200 r.p.m., 30%

Higher moisture content resulted in lower expansion. This trend agrees with the literature, but the reasons for it are not clear. PARK (1976) reported that high moisture resulted in larger pore sizes and thicker walls of the extruded corn starch. Although HAYTER and co-workers (1986), working with corn grits, found the same trend, in their work the pore size actually decreased. Therefore, the pore size is probably not the key variable. The shrinkage of the extrudate after expansion was significant only when the moisture content was very high. The major effect of moisture on the expansion should be due to its effect on the structural properties. A lower moisture content caused a higher shear stress, which resulted in a higher extent of gelatinization (CAI & DIOSADY, 1993), and this then lead to a greater expansion.

Figure 5 also shows that shear rate had a positive effect on the expansion. For all of the operating conditions, a higher screw speed resulted in a shorter heat treatment time, but caused a higher expansion most probably due to the higher shear rate.

The specific volumes were found to have a negative correlation with the die pressure at a given moisture content (Table 1). The lower the die pressure, the higher the specific volume. KIRBY and co-workers (1989) reported similar results and indicated that the die pressure could be used as an indication of the melt viscosity, which was an important factor in extrudate expansion. However, the melt viscosity was also an indicator of the structural characteristics of the starch melt. Starch gelatinization and starch degradation play a very important part in the viscosity

changes of the starch melt and they are critical factors in the expansion. This could be also seen from the effect of moisture content on the specific volume. We found that constant temperature and screw speed higher moisture content caused a lower viscosity and thus lower die pressure, but this did not result in higher expansion since the higher moisture content caused a lower degree of gelatinization (Table 1), (CAI & DIOSADY, 1993).

Temperature and moisture content not only affected the structural properties of the melted starch, but also directly affected the expansion. The material having suitable structural properties for expansion will not expand if its temperature drops to below 100 °C just before the die nozzle. Moisture content affects the formation of steam bubbles in the extrudate and the shrinkage of the extrudate after expansion.

From the discussion above, we can see that the two major factors affecting the extrudate expansion are the structural properties of starch and the temperature and moisture content of the material just before expansion. These two factors are reflected by the viscosity of the melted starch at the die. Therefore, by modifying the viscosity equation:

$$\eta = \exp(a + bM) \exp\frac{E_y}{RT} \gamma^{n-1} \tag{4}$$

developed by HARPER et al. (1971), ALVAREZ-MARTINEZ et al. (1988) derived an expression for extrudate expansion:

$$Y = \exp(a + bM) \exp \frac{E_y}{RT} \gamma^f{}_a \gamma^g{}_{ms} \tag{5}$$

where Y is expansion, η apparent viscosity, M moisture content, E_y thermal activation energy, γ shear rate, $\gamma^f{}_a$ the total strain in the die, $\gamma^g{}_{ms}$ the total strain in the metering section, and a, b, f, and g are constants obtained by regression analysis of Eq. (5). They found a correlation coefficient of $R^2 = 0.891$ when they fitted the data of the extrudate specific volume. Although the correlation was based on the empirical relationship between expansion and viscosity, this model actually included the effects of the structural properties and the extrusion conditions, since the viscosity is determined by these two factors.

The present work expanded the fundamental basis of this model. The temperature and the moisture content affect the expansion directly. Since the shear strain only affected the structural properties of the melted starch, for describing the expansion, the structural property parameters were used instead of the shear strain. The degree of gelatinization was shown to be an important structural property for expansion. Starch degradation also affected the expansion, since it affected the viscosity (DAVIDSON et al., 1984, and DIOSADY et al., 1985). Therefore, the effects of the degradation must be also included in a comprehensive model.

We are unaware of reports which quantitatively relate extrudate expansion with gelatinization and degradation of starch. Like ALVAREZ-MARTINEZ and co-workers (1988), we used viscosity to link expansion and structural properties. Both authors (REMSEN & CLARK, 1978; DOLAN & STEFFE, 1989), who took into account of the effect of the extent of gelatinization on the viscosity of starch, suggested that the viscosity is an exponential function of the fraction of gelatinized starch:

$$\eta = f(e^f) \tag{6}$$

Based on the power law relationship between molecular weight and viscosity for polymers (VINOGRADOV & MALKIN, 1980), the relationship between the viscosity and the extent of degradation becomes:

$$\eta = f'(\chi^\lambda) \tag{7}$$

where χ is the degree of degradation and λ is a constant. Therefore, according to these relations, we modified Eq. (5) into the form below, Eq. (8), by replacing the shear strain term in Eq. (5) with the degree of gelatinization and degree of degradation:

$$V_s = k_v \chi^\lambda \exp(\varepsilon f) \exp(\xi M) \exp(\zeta/T) \tag{8}$$

where V_s = specific volume, k_v, ε, ζ, ξ and λ are fitted constants. The parameters $k_v = 3.639\ cm^3g^{-1}$, $\varepsilon = 1.267$, $\lambda = -0.227$, $\xi = -1.393$, and $\zeta = -182.5$ K were found using regression analysis based on the specific volume data and the starch gelatinization and degradation data reported by CAI and DIOSADY (1993) and CAI and co-workers (1993), respectively. The correlation coefficient was calculated to be $R^2 = 0.931$ with the mean square of error $s^2 = 0.0944$. This expression relates expansion to the structural properties and provides a more fundamental understanding of the expansion. The degree of starch gelatinization can be calculated using Eq. (2) and the degree of starch degradation can be determined by Eq. (9), (CAI et al., 1993).

$$\chi = 1 - f(1 - e^{-k\tau t + B}) \tag{9}$$

where k and B are constants.

The expansion model and the WSI model illustrate how the functional properties of the extrudates are determined by the changes in the structural properties of the starch. The thorough understanding of the dependence of the functional properties on the structural properties will make it possible to use the extrusion system parameters, such as residence time, shear stress, temperature, and moisture content to predict the properties of the final products.

3. Conclusions

The results indicate that changes in WSI and WAI during extrusion cooking only take place in the cooking zone. These changes occur rapidly at the beginning of the cooking zone and then gradually to the die. The changes in the functional properties of the starch during extrusion cooking are dependent on the modification of the molecular structure of the starch. The regression analysis showed that WSI is an exponential function of the degree of gelatinization, while the extrudate expansion is a more complex function of both starch gelatinization and degradation. The temperature and the moisture content also affect the expansion, both directly and indirectly, through their effects on viscosity. An expression for extrudate expansion was developed in terms of the degree of gelatinization, degree of degradation, temperature, and moisture content.

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PROTEOLYTIC ENZYME ACTIVITY OF *S. CEREVISIAE* BAKER'S YEAST AND *S. CARLSBERGENSIS* BREWER'S YEAST

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Investigation of the soluble proteinases from *S. cerevisiae* and *S. carlsbergensis* showed that the pH-enzyme activity profiles are significantly different. In baker's yeast proteinase B activity, for brewer's yeast carboxypeptidase Y activity seemed to be characteristic. SDS-PAGE separation of IEF fractions resulted in different protein prints for the investigated yeasts. Despite the serological similarity of the protein fractions representing proteinase B activity their SDS-PAGE molecular weight spectra are different. Soluble proteinases could be activated by adding surface active agents to sonicated samples however not to the plasmolysed ones. The cell wall disintegration method had also influence on the pH optimum of the soluble proteinase sample. Temperature optimum depends both on pH and cell wall disintegration method for both yeasts.

Cell wall bound proteolytic enzyme activity represents a remarkable level for *S. cerevisiae* and was dominant in case of *S. carlsbergensis*. Temperature optimum and heat resistance of cell wall bound proteinases were different from those of the soluble ones.

Keywords: yeast, protease activity soluble, cell wall bound

Baker's yeast proteinases had been reported by DERNBY (1917) who characterized the proteolytic enzymes by their pH-optimum. The rapid development in protein chemistry, analytical chemistry and separation techniques resulted in a drastic increase in the knowledge of proteinases. New synthetic substrates and production of auxotrophic mutants enabled the detection of several specific minor proteinases (EMTER & WOLF, 1984; WOLF 1982; ACHSTETTER et al., 1983).

Main part of the proteolytic activity, about 90% of it is located in the vacuole (WIEMKEN, 1969; WORTH, 1982; WOLF & EHMANN, 1983). EMTER and WOLF (1984) described proteinase D in the cytosol.

KUSUNOSE and co-workers (1980) however reported that greater part of the proteolytic activity is cell wall bound. Similar results were found by CHEN and MILLER (1968), ACHSTETTER and co-workers (1984) who described a minor proteolytic activity, protease P which is also a non-soluble enzyme.

Proteinase activity of the yeast cell is influenced by substrate composition, pH, temperature, C- or N- starvation and insufficient aeration (RIEWAY & TRÖGER, 1978). HALÁSZ and co-workers (1991) investigated activity changes in the exponential growth phase and transient growth phase at different glucose concentrations and aeration levels. Compared with the stage before inoculation, de novo synthesis was observed for proteinases A and B in the exponential phase of growth while carboxypeptidase Y remained unchanged. Consequently the increase in protease activity can be attributed to higher biocatalyst concentration and activation. Changes in activity related to hemoglobin substrate in the transient phase followed by the stationary growth phase can be explained by the change in the active form of antigen at 0.1% glucose, while at 0.5% glucose content the synthesis of protease B plays an important role as well.

Our investigation on baker's yeast showed, that highest proteinase activity could be detected at the transition from intensive aerob fermentation stage to respiration phase, followed by diminished enzyme activity which was constant during the stationary growth phase (HALÁSZ & LÁSZTITY, 1991).

The aim of our present work was to characterize and compare proteinase activity of *S. cerevisiae* baker's yeast and *S. carlsbergensis* brewer's yeast.

1. Materials and methods

1.1. Investigated yeast strains

Saccharomyces cerevisiae baker's yeast, a production strain of the Hungarian Yeast and Distillery Factory Budafok.

S. carlsbergensis brewer's yeast, a production strain of the Hungarian Brewery in Böcs.

1.2. Propagation of the yeast

The inoculum was grown in shaken culture, harvested by centrifugation and resuspended in mineral at a concentration of 0.5%. Batch fermentation was done in glass column fermenters (500 cm^3 working volume) at 32 °C and pH was maintained at 4.5. Cells were harvested in the stationary growth phase, washed with sterile water and used immediately or freeze dried for further examinations.

Investigated sugar concentrations: 0.1 and 0.5%.

Investigated aeration levels: 200, 400 and 600 l h^{-1}.

Composition of the medium: $MgSO_4$ $7H_2O$, 0.1 g l^{-1}; KH_2PO_4 0.14 g l^{-1}; Na_2HPO_4 $12H_2O$, 0.8 g l^{-1}; NaCl 1 g l^{-1}; $(NH_4)_2$ SO_4, 4 g l^{-1}.

Yeast extract (DIFCO) 5.0 g l^{-1}, pH = 4.5. Medium was sterilized at 121 °C for 20 min.

Carbon source was in case of baker's yeast glucose, for brewer's yeast maltose.

1.3. Preparation of crude enzyme extract

1.3.1. Preparation of crude enzyme extract by sonification. Fresh yeast was resuspended in 40 cm^3 distilled water, dispensed in an ice bath and sonicated three times with the Labsonic type 1510 sonicater (3 min at 300 W). Half cm^3 of Triton 100 was added to the sample and this was transferred to shaker for 1 h at 32 °C. The cell-free extract was separated by centrifugation.

1.3.2. Preparation of crude enzyme extract by plasmolysis. Fresh yeast cell mass (10 g dry material) was mixed with 10 cm^3 chloroform and shaken for 50 min at room temperature. Then 100 cm^3 phosphate buffer pH = 7.0 was added and shaken for 18 h. Cell-free extract was separated by centrifugation.

1.4. Determination of soluble proteinase enzyme activity

1.4.1. Endoproteinase B activity. Forty-eight hours acetate (pH = 5.0) activated enzyme solution was used on Azocoll substrate, pH = 5.0 in a stoptest according to SAHEKI and HOLZER (1975) modified by SCHWENCKE (1981).

1.4.2. Endoproteinase A activity. Forty-eight h activated acetate (pH = 5.0) enzyme solution was used on pH = 3.0 hemoglobin substrate according to HATA and co-workers (1967).

1.4.3. Carboxy-peptidase Y activity. One hour Triton activated enzyme solution was used on pH = 5.0 hemoglobin substrate.

1.4.4. Determination of soluble proteinase enzyme activity. Proteolytic enzyme activity was determined on hemoglobin. Three cm^3 portions of substrate solution were filled into 25 cm^3 Erlenmayer flasks which were shaken in water bath at 40 °C. One cm^3 of cell-free extract was added to each flask. The reaction was stopped after 60 min by adding 10 cm^3 of 0.3 mol^{-1} of TCA. After centrifugation at 3000 r.p.m. for 15 min, to 5 cm^3 of the supernatant 10 cm^3 Merck Folin reagent (1:2 Folin reagent to distilled water) was added.

The absorbance was measured at 660 nm against blank. Proteolytic enzyme activity was calculated as follows:

$$U = \frac{\text{Absorbance of the sample at 660 nm} \times k \times 16}{\text{solids content of the sample (g)}}$$

1.5. Determination of cell wall bound enzyme activity

Fresh yeast cells (4 g dry material) were broken by sonification as described in *1.3.1* except addition of Triton. Cell wall fraction was collected by centrifugation, resuspended in 50 cm^3 H_2O + 1% Triton and used for determination of cell wall bound proteolytic enzyme activity in case of baker's yeast, and pure H_2O in case of brewer's yeast.

1.6. Surface active agents

For protease activation two surface active agents were used, Triton X100 and Tween 80.

1.7. Separation of yeast protein by IEF technique

Flat-bed analytical and preparative isoelectric focusing was performed according to the Pharmacia Manual, using Ampholine pH 3.5 – 10, Agarose IEF for the analytical and Sephadex IEF for the preparative gel. All chemicals were purchased from the Pharmacia Fine Chemicals, Uppsala, Sweden. To remove the salts, the yeast supernatant was passed through a 1000 cutoff ultrafiltration membrane prior to the separations. After analytical focusing the gel was stained for proteins using Coomassie Brilliant Blue. After preparative focusing the gel was cut into 28 strips. The fractions were eluted using distilled water, the pH and the activities of each fraction were determined. The Ampholine content was removed using 1000 cutoff Millipore ultrafiltration membrane.

1.8. SDS-PAGE characterization of the IEF yeast protein fractions

Proteins were resolved by denaturing sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 5.0% stacking, 10% resolving gels) by the method of SAMBROOK and co-workers (1985). Gels were silver stained.

2. Results

2.1. The effect of pH on soluble proteinase activity of S. cerevisiae *baker's yeast*

Crude proteinase solution prepared by sonification showed the highest activity at pH = 5.0. Enzyme solution prepared by plasmolysis showed a significantly different pH-activity profile. In this case there was no difference among proteinase activity values at pH = 5.0 and pH = 7.0. Proteolytic activity of plasmolytic enzyme solution was significantly higher than those of sonificated extract both at pH = 3.5 and pH = 7.0 (Tables 1 and 2).

Table 1

The effect of pH on soluble proteinase activity of S. cerevisiae *baker's yeast (sonicated sample)*

pH =	2.0	3.0	4.0	5.0	6.0	7.0
Hb activity	$\bar{x} = 0.36$ $\pm s = 0.02$ $PA_G = 2.92 \times 10^{-2}$	$\bar{x} = 0.02$ $\pm s = 0.01$ $PA_G = 0.16 \times 10^{-2}$	$\bar{x} = 0.06$ $\pm s = 0.02$ $PA_G = 0.4 \times 10^{-2}$	$\bar{x} = 0.60$ $\pm s = 0.03$ $PA_G = 4.86 \times 10^{-2}$	$\bar{x} = 0.07$ $\pm s = 0.05$ $PA_G = 0.57 \times 10^{-2}$	$\bar{x} = 0.03$ $\pm s = 0.02$ $PA_G = 0.24 \times 10^{-2}$

Significance test

pH	7	6	5	4	3
2	***	***	***	***	***
3	0	0	**	0	
4	0	0	**		
5	**	**			
6	0				

Table 2

The effect of pH on soluble proteinase activity of S. cerevisiae *baker's yeast (plasmolysed sample)*
(incubation time: 60 min)

pH = 3.5	$\bar{x} = 0.08$ $\pm s = 0.02$ $PA_G = 2.6 \times 10^{-3}$
pH = 5.0	$\bar{x} = 0.70$ $\pm s = 0.19$ $PA_G = 2.3 \times 10^{-2}$
pH = 7.0	$\bar{x} = 0.68$ $\pm s = 0.19$ $PA_G = 2.24 \times 10^{-2}$

Significance test

pH	7.0	5.0
3.5	**	***
5.0	0	

Results indicate that plasmolysis destroys not only cell wall membrane, but has also an effect on the proteinases.

2.2. The effect of pH on soluble proteinase activity of S. carlsbergensis *brewer's yeast*

As Fig. 1 shows there was a strong dependence on pH-value for *S. carlsbergensis*, too. At pH = 3.5 proteolytic activity was almost zero, pH optimum was at pH = 5.0. Prolongation of activation time from 1 to 24 h resulted in an increase in enzyme activity at pH = 5.0 and pH = 7.0 as well.

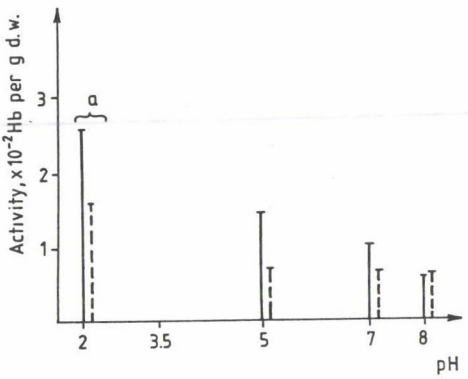

Fig. 1. The effect of pH on the proteolytic enzyme activity of *S. carlsbergensis* (ultrasonicated sample). ----- : 1 h activated sample; —— : 24 h activated sample; x = 10^{-3} Hb g^{-1}.
[a] Value of activity: $\times 10^{-3}$ Hb g^{-1}

2.3. Activation of soluble proteinases by surface active agents or salt addition

Table 3

The effect of surface agents on the proteinase activity of baker's yeast (ultrasonicated sample)
(incubation time: 60 min; pH = 5.0)

Control	$\bar{x}$ = 0.15 ±s = 0.05 $PA_G = 1.23 \times 10^{-2}$
1% Tween	$\bar{x}$ = 0.58 ±s = 0.07 $PA_G = 4.77 \times 10^{-2}$
1% Triton	$\bar{x}$ = 0.64 ±s = 0.07 $PA_G = 5.27 \times 10^{-2}$

Effect of detergents F = 93.48*** (very highly significant at 99.9% probability level)

As Tables 3 and 4 show addition of surface active agents (Triton, Tween, SDS) resulted in an increase of soluble proteinase activity for baker's yeast, in case of sonicated proteinase solutions. SDS and Tween resulted in activation however

addition of Triton + SDS caused a decrease of brewer's yeast soluble proteinase activity (Fig. 2).

Table 4

The effect of surface active agents on the proteinase activity of baker's yeast (plasmolysed sample)
(incubation time 60 min; pH = 7)

Control	$\bar{x} = 0.10$ $\pm s = 0.05$ $PA_G = 8.24 \times 10^{-3}$
1% Tween 80	$\bar{x} = 0.40$ $\pm s = 0.08$ $PA_G = 3.29 \times 10^{-2}$
1% Triton X-100	$\bar{x} = 0.36$ $\pm s = 0.03$ $PA_G = 2.97 \times 10^{-2}$
Effect of detergents	$F = 37.87^{***}$

***: very highly significant at 99.9% probability level

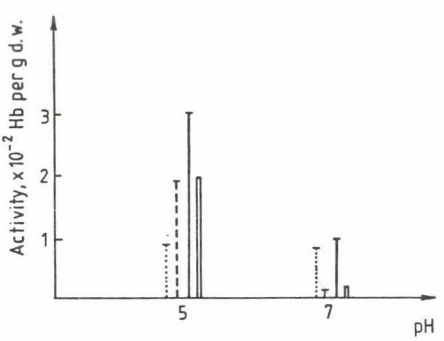

Fig. 2. The effect of surface active agents on the proteolytic enzyme activity of *S. carlsbergensis* (ultrasonicated sample). --- : 1% Triton; —: 1% SDS; ═══: 1% SDS + 1% Triton X-100; ····· : control

However, plasmolysed yeast extract could not be further activated by surface active agents, moreover Triton caused a decrease in proteolytic enzyme activity (Fig. 3).

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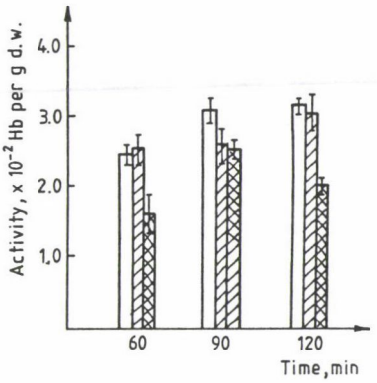

Fig. 3. The effect of surface active agents on the proteolytic enzyme activity of *S. carlsbergensis* (plasmolysed sample). ⌐¬ : control; ▨ : 1% Triton 80; ▩ : 1% Triton X-100

2.4. The effect of cell wall disintegration method and pH on temperature optimum of baker's yeast soluble proteinase

Our results show that both cell wall disintegration methods (ultrasonication or plasmolysis) and substrate pH affect the temperature optimum of the soluble proteinase. As Figs 4 and 5 demonstrate the ultrasonicated sample had higher enzyme activity than the plasmolysed one. In both cases enzyme activity was higher at pH 5.0 than at pH = 2.

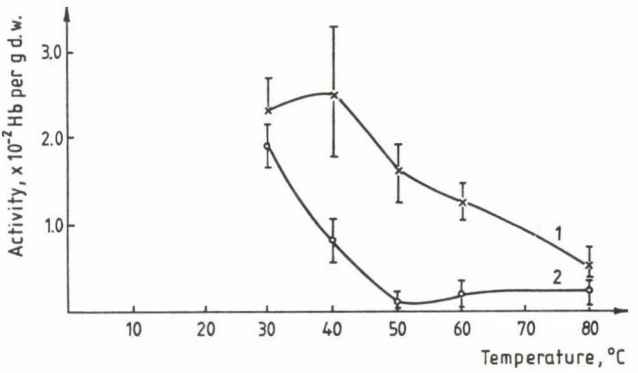

Fig. 4. The effect of substrate temperature and pH on the protease activity of baker's yeast (ultrasonicated sample). 1: pH = 5.0; 2: pH = 2.0; the vertical bars represent standard deviations

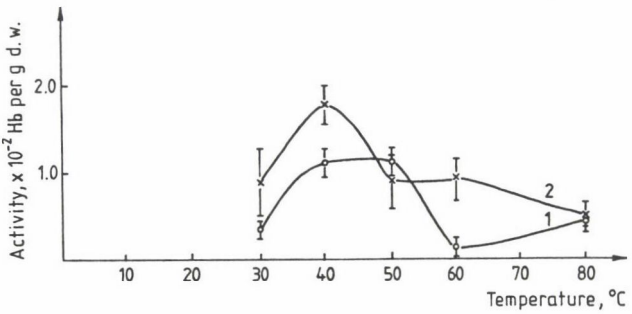

Fig. 5. The effect of substrate temperature and pH on the protease activity of baker's yeast (plasmolysed sample). 1: pH = 2.0; 2: pH = 5.0

At pH = 5.0 temperature optimum seemed to be independent of the cell wall disintegration method, however the plasmolysed sample had a more distinct optimum at 40 °C while the sonicated sample showed an almost equal activity at 30 °C. At pH = 2.0 there was a more significant difference between the two cell extracts. The sonicated one had almost no activity at 50 °C, while the plasmolysed one showed higher activity in temperature range of 40–50 °C.

2.5. The effect of cell wall disintegration method and pH on the temperature optimum of brewer's yeast soluble proteinase

Table 5

The effect of temperature and pH on the proteolytic enzyme activity of brewer's yeast

T (°C)	Sonicated sample pH = 2.0	pH = 5.0
30	5.44×10^{-3}	2.2×10^{-2}
40	5.44×10^{-3}	4.53×10^{-2}
50	0	2.97×10^{-2}
T (°C)	**Plasmolysed sample pH = 2.0**	**pH = 5.0**
30	5.00×10^{-3}	4.2×10^{-2}
40	2.50×10^{-2}	5.6×10^{-2}
50	2.50×10^{-2}	2.2×10^{-2}

The cell wall disintegration method and pH had significant influence on the temperature optimum of soluble protease. The plasmolysed enzyme extract showed

higher activity at optimal conditions than the sonicated one, especially at lower pH. Both extracts had higher proteolytic activity at pH = 5.0 with temperature optimum at 40 °C (Table 5).

At lower pH the sonicated sample had no detectable activity at 50 °C while the plasmolysed one showed the same values at 40 °C and 50 °C.

2.6. Comparison of baker's yeast and brewer's yeast soluble proteinases by protein separation techniques and serological activity

Crude enzyme extracts of the investigated yeast strains prepared by sonification, were separated by preparative IEF technique. The isoelectric points of the 28 yeast protein fractions were found to cover the pH range 3.7–9.14 in both cases. Most of the baker's yeast soluble protein content (76 per cent) was found in the fractions 1–15 with pI values in the range 3.70–6.19 (Fig. 6). Fractions 9–14 covered the pI range 4.46–5.62 contained the greatest part of protease B activity. Carboxypeptidase Y activity was mainly located in the fractions 15–21 with pI values 6.06–6.91 (peak II). In peak III protease A and carboxypeptidase Y could have been detected.

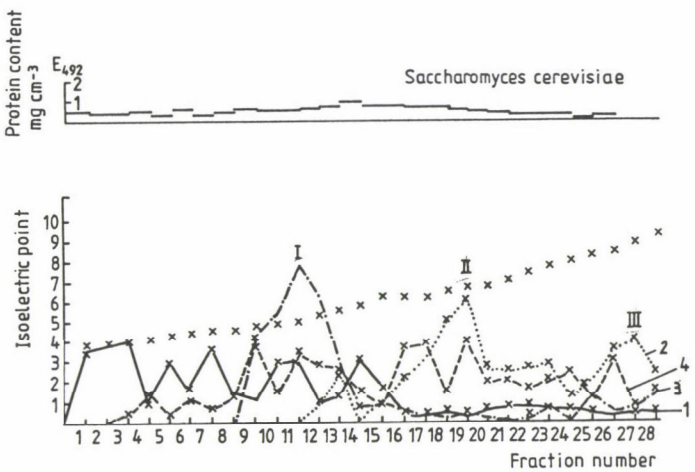

Fig. 6. Activities of *S. cerevisiae* components separated by preparative isoelectric focusing (IEF). 1: Folin protein, mg cm^{-3}; 2: Protease Y, 10^{-5} Hb mg $protein^{(-1)}$; 3: Proteinase B, 10^{-1} Δ Ext $min^{(-1)}$; 4: Proteinase A, 10^{-5} Δ mg $protein^{(-1)}$. Protein content determined by Lowry

The IEF spectrum of brewer's yeast was significantly different from that of baker's yeast. As Fig. 7 shows in case of brewer's yeast proteinase B activity was detected in all protein fractions, with a maximum peak in fractions 9–11. Proteinase B activity of the investigated yeasts was significantly different, that of baker's yeast

had higher values by two magnitudes. At the same time carboxypeptidase activity of brewer's yeast, located in IEF fractions 16–22 was several times higher than in baker's yeast (10^{-3} Hb mg^{-1} prot., and 10^{-5} Hb mg^{-1} prot., resp.).

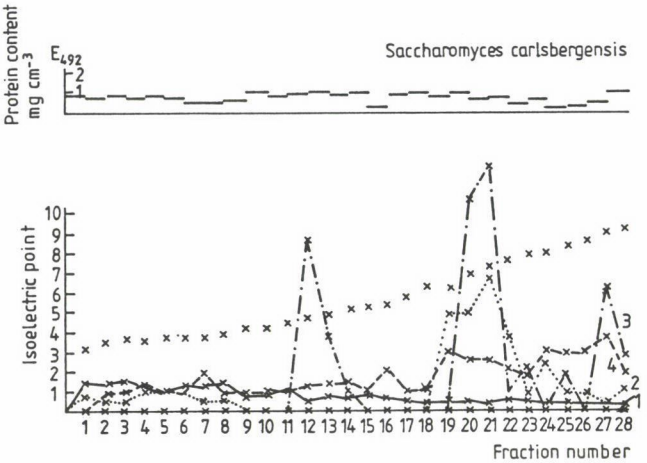

Fig. 7. Activities of *S. carlsbergensis* component separated by preparative isoelectro focusing (IEF). 1: Folin protein mg cm^{-3}; 2: Protease Y, 10^{-3} Hb mg^{-1} protein; 3: proteinase, B 10^{-3} Δ Ext. min^{-1} mg^{-1} protein; 4: Proteinase A, 10^{-5} A mg^{-1} protein. Protein content determined by Lowry

SDS-PAGE separation of the IEF protein fractions showed very different protein prints for *S. cerevisiae* and *S. carlsbergensis* (Figs 8 and 9). In case of baker's yeast the whole spectrum contains a protein fraction whose molmass is higher than 60 000 Da. In *S. carlsbergensis* even the highest molecular weight subfractions are below 60 000 Da and the dominant fractions are below 50 000 Da.

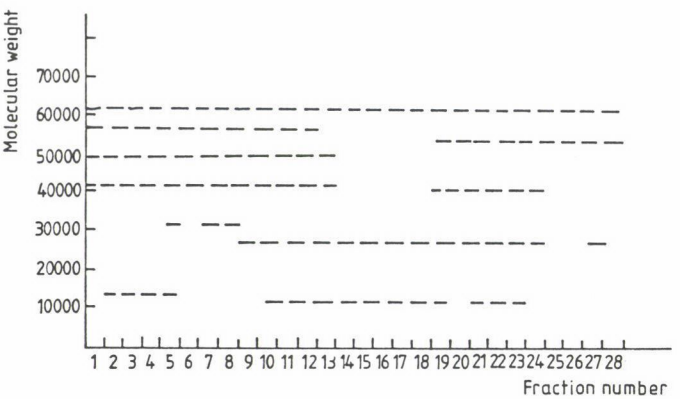

Fig. 8. Molecular weights of the fractions of *S. cerevisiae* separated by preparative isoelectric focusing (SDS vertical slab elfo)

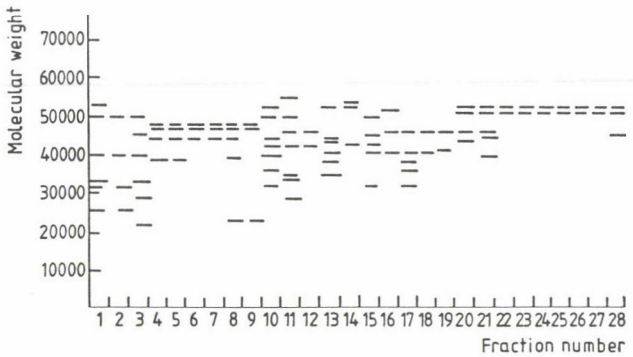

Fig. 9. Molecular weights of the fractions of *S. carlsbergensis* separated by preparative isoelectric focusing (SDS vertical slab elfo)

Proteinase B activities of brewer's and baker's yeast resp. belong to different molecular weights. The absence of 40 000–55 000 Da subfractions seemed to be characteristic for baker's yeast proteinase B, whereas in case of brewer's yeast this activity is more or less present in all IEF fractions. Carboxypeptidase of baker's yeast has no protein subfractions in the range of 40 000–55 000 Da, at the same time carboxypeptidase activity of brewer's yeast has dominant subfractions of 40 000 Da – 45 000 Da and those of 50 000 Da are absent.

Despite the differences in the enzyme profile and SDS-PAGE protein print the two yeast extracts show serological similarity. Antisera developed against IEF peak I (proteinase B) of *S. cerevisiae* was also active against *S. carlsbergensis* protein fractions especially in the fraction range of 1–16, but seemed to be nonreactive against brewer's yeast carboxypeptidase Y fractions.

2.7. Cell wall bound proteolytic enzyme activity of baker's yeast and brewer's yeast

Cells were disrupted by sonification, after separation of soluble proteinases baker's yeast cell debris were resuspended in Triton containing water while brewer's yeast in simple water and shaken for 60 min. Resolved enzyme activity was determined at 30°, 40° and 50 °C pH 3.5, pH 5.0 and pH 7.9. Enzyme activities are summarized in Table 6 as % of soluble proteinase activity measured at 40 °C and pH = 5.0 (optimal conditions).

As data show (Table 6) the cell wall-bound proteolytic enzyme activity represents a considerable amount for both investigated yeasts.

Table 6

Cell wall bound proteolytic enzyme activity of baker's and brewer's yeasts

	Enzyme activity (10^{-2} Hb per g dry matter)								
	Temperature (°C)								
	30			40			50		
	pH								
	3.5	5.0	7.9	3.5	5.0	7.9	3.5	5.0	7.9
Baker's yeast	0	1.41	0.47	0	2.0	0.61	0.17	1.76	0
Brewer's yeast	0	4.53	0.59	0	4.04	0	0	3.84	0.24

Soluble protease activity at 40 °C and pH = 5.0
baker's yeast: 4.63×10^{-2}
brewer's yeast: 3.25×10^{-2}

In case of baker's yeast this activity is 46% of the soluble enzyme at optimal conditions. As protein content of the cell debris is much lower, the specific enzyme activity is higher than for soluble proteases. Temperature and pH optima of the cell wall bound protease were the same as for the soluble enzyme of baker's yeast.

Cell wall bound proteolytic enzyme activity of brewer's yeast seemed to be different from the soluble one in respect of temperature optimum and temperature stability (Table 6). The highest measured activity was 139% of the soluble enzyme. Specific proteolytic activity was higher than those of the soluble enzyme.

2.8. Heat stability of soluble proteinases from baker's yeast and brewer's yeast

In the crude enzyme extract prepared by sonification all soluble proteinases and their specific proteinase inhibitors are present. However, the activation of proteinases results in a decrease in inhibitor activity.

As Fig. 4 and Fig. 5 show, temperatures higher than 40 °C caused a significant decrease in proteolytic activity.

Heat treatment of baker's yeast enzyme solution at 60°, 70° and 75 °C for 10, 20 and 30 min resulted in a significant lowering of the proteolytic enzyme activity at each investigated heating time (Tables 7a, b) in comparison to the control value (40 °C). It is interesting, that at 60 °C the enzyme inactivation did not change when treatment was extended to 20 or 30 min.

Table 7a

Effect of heat treatment on the enzyme activity of baker's yeast soluble proteinases

Temperature (°C)	Time (min) 10	20	30	Control (T = 40 °C pH = 5.0)	
60	$\bar{x} = 0.20$	$\bar{x} = 0.22$	$\bar{x} = 0.31$	$\bar{x} = 0.42$	F = 6.85
	$\pm s = 0.11$	$\pm s = 0.09$	$\pm s = 0.05$	$\pm s = 0.08$	
	$PA_G = 1.01 \times 10^{-2}$	$PA_G = 1.12 \times 10^{-2}$	$PA_G = 1.54 \times 10^{-2}$	$PA_G = 2.1 \times 10^{-2}$	
70	$\bar{x} = 0.03$	$\bar{x} = 0.09$	$\bar{x} = 0.01$	$\bar{x} = 0.37$	F = 69.76
	$\pm s = 0.03$	$\pm s = 0.04$	$\pm s = 0.02$	$\pm s = 0.07$	
	$PA_G = 0.14 \times 10^{-2}$	$PA_G = 0.45 \times 10^{-2}$	$PA_G = 0.04 \times 10^{-2}$	$PA_G = 1.49 \times 10^{-2}$	
75	$\bar{x} = 0.04$	$\bar{x} = 0.04$	$\bar{x} = 0.0$	$\bar{x} = 0.24$	F = 27.09
	$\pm s = 0.01$	$\pm s = 0.03$	$\pm s = 0.0$	$\pm s = 0.09$	
	$PA_G = 0.22 \times 10^{-2}$	$PA_G = 0.22 \times 10^{-2}$	$PA_G = 0.0$	$PA_G = 1.2 \times 10^{-2}$	
	F = 11.54	F = 12.85	F = 149.62		

Table 7b

Significance test

	60 °C			70 °C			75 °C		
	control	30'	20'	control	30'	20'	control	30'	20'
10'	*	ø	ø	**	ø	ø	*	ø	ø
20'	*	ø		**	*		*	ø	
30'	ø			**			*		

	10'		20'		30'	
	75 °C	70 °C	75 °C	70 °C	75 °C	70 °C
60 °C	*	*	*	*	**	**
70 °C	ø		ø		ø	

ø: not significant;
*: significant at P = 95% probability level;
**: highly significant at P = 99% probability level

Heat treatment at 70 °C resulted in lower activities in each case than those at 60 °C. Proteolytic activity of the 20 min sample was higher than that of 10 min at 70 °C. This phenomenon might be a result of a more rapid inactivation of enzyme inhibitor (I_y) than that of proteases.

Heat stability of brewer's yeast soluble proteinases was low (1.74×10^{-3}) even 10 min at 60 °C resulted in some decrease in enzyme activity. Longer treatments (20–30 min) or higher temperatures (70°–75 °C) caused a complete inactivation of the proteases.

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ISOLATION AND CHARACTERIZATION OF RIBULOSE-1,5-BISPHOSPHATE-CARBOXYLASE FROM NETTLE *(URTICA DIOICA* L.)

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An original laboratory method for the extraction and purification of ribulose-1,5-bisphosphate-carboxylase (EC 4.1.1.39) from nettle (*Urtica dioica* L.) is described. The method is based on the fractionated precipitation with ammonium sulfate, followed by a preparative isoelectrofocusing. The main molecular features of the purified native enzyme are given, together with its heat stability and solubility as a function of pH and ionic strength.

Keywords: ribulose-1,5-bisphosphate-carboxylase, *Urtica dioica* L., functional properties

During the last 30 years significant development has been made in the study of wet green crop fractionation (WGCF) to produce leaf proteins for animal feeding and human consumption (KOEGEL et al., 1976; BRAY & HUMPHRIES, 1978; TELEK & GRAHAM, 1983; SINGH, 1984; TASAKI, 1986). More recently, the attention has been focused on the extraction of ribulose-1,5-bisphosphate-carboxylase (RuBPcase, EC 4.1.1.39), for its high potential in human diet (FANTOZZI, 1985; FANTOZZI & SENSIDONI, 1989; DALEV, 1989). This enzyme, commonly known as Fraction-1 protein, accounts for almost half of the soluble protein present in all photosynthetic tissues and is located in the stroma of the chloroplasts. It catalyses the first step in the fixation of CO_2 during photosynthesis. The RuBPcase, though of chloroplastic origin, is not associated with pigments or lipids and has been isolated, also in crystalline form, from the leaf extracts of various plants (BAHR et al., 1977; FANTOZZI, 1987). The nutritional value of RuBPcase is high when compared to other proteins or FAO standards (ERSHOFF et al., 1978; HUGHES et al., 1980; HARMUTH-HOENE & DIEHI, 1985). This consideration, together with the fact that the enzyme is one of the most abundant proteins in the world, makes this protein of interest for human nutrition, especially for therapeutic purposes (CASOTTO et al., 1989).

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The industrial extraction of RuBPcase by WGCF technology requires the physicochemical characterization of the protein from each raw material, in order to optimize the operating conditions and to predict its physical behaviour when used in foods or beverages.

On this basis, an original laboratory method for the extraction and purification of RuBPcase has been set up starting from nettle (*Urtica dioica* L.). The protein fraction has been characterized for molecular weight, subunit composition, isoelectric point, heat stability and solubility as a function of pH and ionic strength. Several trials were conducted. Here we report the typical results of laboratory purification method and the characterization of the final product.

1. Materials and methods

1.1. Materials

The nettle (*Urtica dioica* L.) plants were harvested at the preflowering stage. RuBPcase extracted from leaves of spinach (*Spinaces oleracea* L.) and alfalfa (*Medicago sativa* L.), together with commercially available preparations, were used as reference enzyme preparations to optimize the enzyme assay.

Spinach RuBPcase (EC 4.1.1.39), phosphoglyceromutase (EC 2.7.5.3.), enolase (EC 4.2.1.11), pyruvate kinase (EC 2.7.1.40) and lactate dehydrogenase (EC 1.1.1.28) were purchased from Sigma Chemical Co., St Louis, Mo. All other chemicals were of the highest quality commercially available.

1.2. Extraction of RuBPcase

Twenty-five g portions of freshly harvested, washed leaves were homogenized in a Waring blender with 50 cm^3 of 20 mmol l^{-1} Tris-Cl buffer, pH 7.8, in the presence of 20 mmol l^{-1} $Na_2S_2O_5$, 10 mmol l^{-1} diethyldithiocarbamic acid and 0.1 mmol l^{-1} EDTA. The homogenate was filtered through cheese-cloth and saturated ammonium sulfate solution (pH 7.5) was added to the filtrate to give 30% saturation. After 30 min at 4 °C the suspension was centrifuged at 30 000 × G for 30 min. The supernatant was brought to 50% ammonium sulfate saturation, pH 7.5, and centrifuged at 30 000 × G for 1 h at 4 °C. The supernatant was discarded and the pellet was dissolved in the minimum volume of 100 mmol l^{-1} Tris-Cl buffer, pH 7.5.

1.3. Preparative isoelectrofocusing

The protein solution was dialyzed overnight at 4 °C against 50 mmol l^{-1} Tris-Cl buffer, pH 7.5. Solution aliquots of 50 cm^3, containing 2% (w/v) ampholine (pH

interval 3–10) with a protein content of 2 mg cm^{-3} were applied to a Rotofor Cell (Biorad) and the electrofocusing was achieved in 4–5 h at 4 °C using 12 W constant power; the initial conditions were 400 V and 30 mA. After the preparative electrofocusing was completed, 20 fractions (2.5 cm^3 each) were collected. Each fraction was brought to a final concentration of 100 mmol l^{-1} Tris-Cl buffer, pH 7.5, to solubilize the partially precipitated proteins.

1.4. Protein determination

The protein content of the various fractions was determined by the colorimetric method of BRADFORD (1976) using bovine serum albumin as reference protein.

1.5. Enzyme assay

The enzyme assay used in the present work is an extension of the multienzyme method of LORIMER and PIERCE (1989) for the determination of 3-phosphoglyceric acid (3-PGA). The RuBPcase activity was assayed by the amount of 3-PGA produced per minute, measured by excess of phosphoglyceromutase, enolase, pyruvate kinase and lactate dehydrogenase as ancillary enzymes. This method proved to be linear for ribulose-1,5-bisphosphate (RuBP) concentrations up to 0.5 mmol l^{-1} and, when tested with commercial spinach preparations (0.05–0.09 U mg^{-1}), its sensivity well agreed with that of the multienzyme method of RACKER (1962).

The enzyme assay was carried out at 37 °C. The final concentrations in the assay mixture were 100 mmol l^{-1} Tris-Cl buffer pH 7.5, 10 mmol l^{-1} MgCl, 5 mmol l^{-1} reduced gluthathion, 100 mmol l^{-1} KCl, 3 mmol l^{-1} ADP, 1 mmol l^{-1} 2,3-diphosphoglyceric acid, 0.2 mmol l^{-1} NADH, 50 mmol l^{-1} $NaHCO_3$ between 5 and 10 units of each of the ancillary enzymes and the reported amounts of the RuBPcase preparations. After 15 min pre-incubation the reaction was started by adding 10 μl of 50 mmol l^{-1} RuBP solution.

The activity was calculated from the decrease in absorbance at 340 nm, using a molar extinction coefficient of 6300 mol^{-1} cm^{-1} and taking into account the stoichiometry RuBP carboxylated: NADH reduced of 1:2. The enzyme activity unit (U) is defined as the amount of enzyme which causes the carboxylation of 1 μmol of RuBP per minute under the condition of the assay. Data are reported as mean ±SEM for three experiments; in all cases SEM falls within symbol size.

1.6. SDS PAGE electrophoresis

Polyacrylamide gel electrophoresis in denaturing conditions was performed according to LAEMMLI (1970) using LKB molecular mass markers ranging between 12.3 and 78.0 kDa.

1.7. Analytical isoelectrofocusing

Biorad model 111 mini IEF cell and precoated Servalyte plates, pH range 3–10, were used.

1.8. Ionic strength determination

The ionic strength values were determined by a conductivity meter Metrohom E 382 using ammonium sulfate solution for calibration.

1.9. Solubility as a function of ionic strength

Six aliquots of 300 μl of RuBPcase preparations (4.6 mg cm^{-3} of soluble protein content and 0.48 U cm^{-3} of enzyme activity) were brought to 50% saturation of ammonium sulfate. The precipitates recovered by centrifugation at 30 000 × G were separately redissolved in 300 μl aliquots of 20 mmol l^{-1} Tris-Cl buffer, pH 7.5, containing ammonium sulfate at different ionic strength (μ) values. The samples so treated were dialyzed overnight at 4 °C against 300 volumes of the same buffer. The enzymatic activity, the protein content and the ionic strength were measured in the supernatant of each sample obtained after centrifugation at 30 000 × G.

1.10. Solubility as a function of pH

Eight aliquots of 300 μl of RuBPcase preparations (4.6 mg cm^{-3} protein content and 0.48 U cm^{-3} of enzyme activity) were brought to 50% saturation of ammonium sulfate. The precipitates recovered after centrifugation at 30 000 × G, were separately redissolved in 300 μl aliquots of 100 mmol l^{-1} Tris-acetate buffers for the pH range 5.5–7.4 and in 100 mmol l^{-1} Tris-Cl buffers for the range 7.0–7.8 and dialyzed overnight at 4 °C against 300 volumes of the same buffer. After centrifugation at 30 000 × G, the supernatant of each sample was characterized for protein content and enzyme activity.

1.11. Heat stability

Nine aliquots of 300 μl of RuBPcase preparations (4.6 mg cm^{-3} of protein content and 0.48 U cm^{-3} of enzyme activity) were held at various temperatures for 10 min, rapidly chilled, centrifuged at 30 000 × G and the supernatant tested for protein content and enzyme activity.

2. Results and discussion

2.1. Extraction and purification of RuBPcase

The coupled enzyme assay method offers several advantages but it is affected by severe limitations which deserve a short discussion. Since the analytical signal is the absorbance variations at 340 nm, any substance which absorbs at this wavelength could interfere. This limitation applies particularly to the assay of activity in crude extract, where natural pigment and NADH oxidizing enzyme systems are frequently present. In this case the method could be used after appropriate correction. Figure 1 reports the comparison of the time course of the enzyme assay of RuBPcase in crude extracts of nettle and of alfalfa leaves. It shows that, while in alfalfa leaf extract the enzyme assay is affected by unspecific NADH oxidation and therefore its use is only possible after appropriate correction, in nettle leaf extract the method can be directly applied since no unspecific decrement of absorbance at 340 nm occurs. So this assay procedure was used for the evalution of active RuBPcase in the crude extract and in the various steps of the purification procedure. The fraction precipitated between 30% and 50% ammonium sulfate saturation retained 55% of the protein and 78% of the RuBPcase activity with a purification factor of 1.44.

The ammonium sulfate precipitated RuBPcase was further fractionated by preparative isoelectrofocusing. As expected, some precipitation occurred in the 20 sections of the apparatus, due to the low solubility of proteins near their pI. This precipitation could be avoided by high salt concentration and/or the addition of urea or detergents. Neither of these procedures can be used in the preparative isoelectrofocusing procedure because of technical limitations (salt concentration) and of irreversible denaturation of RuBPcase (urea, detergents). When the salt concentration of the 20 fractions collected from the preparative isoelectrofocusing was brought to 100 mmol l^{-1} Tris-Cl buffer, pH 7.5, the results reported in Fig. 2 were obtained. The maximum of protein content and of activity was recovered in fraction 6 which had a planned pH of 4.9 and a measured pH of 4.6. The discrepancy between the programmed pH and the measured pH has been attributed to the buffering power of the soluble proteins. The preparative isoelectrofocusing step resulted in a 10% protein recovered and a 13% activity recovery, with a purification

factor of 1.25. The low yield in purified RuBPcase is due to the well-known instability of the RuBPcase purified from many different sources at pH values close to pI. In

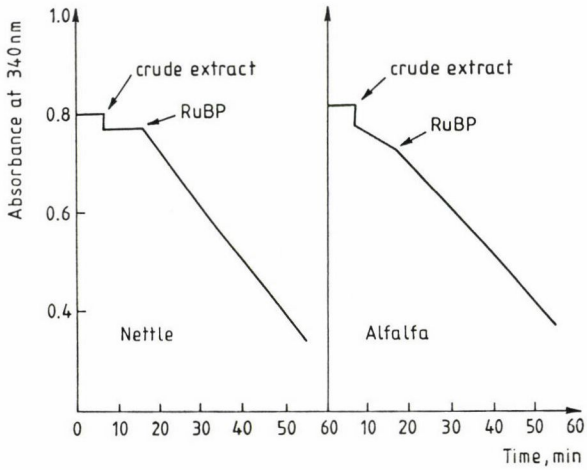

Fig. 1. Time course of A_{340nm} in the RuBPcase assay using crude extracts of nettle and alfalfa leaves. The activity of both extracts was assayed according to the procedure reported under section 1.5. using 5 µl each. A cuvette, where NADH was omitted, was used as a blank

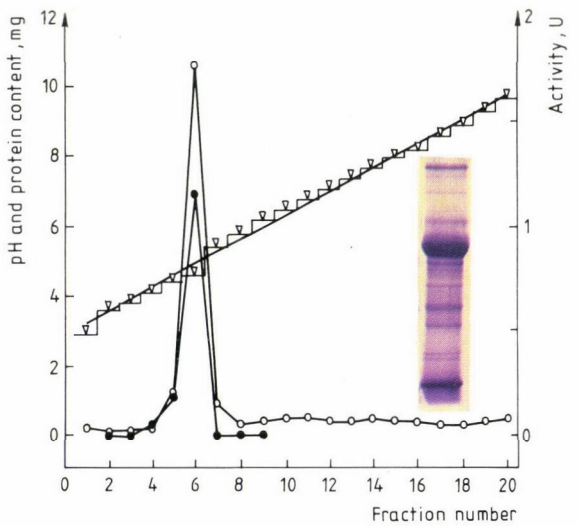

Fig. 2. Elution profile of the preparative isoelectrofocusing (Rotofor Cell). The 50 cm^3 sample contains 2 mg cm^{-3} proteins and 2% pH 3–10 ampholine concentration. The focusing was carried out at 4 °C and 12 W constant power. Proteins (o) were determined according to the Bradford method. The enzyme activity (●) was assayed as described under section 1.5. Five µl of each fraction were used in the assay. Measured pH (∇). The inset shows the SDS PAGE electrophoresis of fraction 6

our case, we can speculate that even if during the purification procedure the enzyme was kept at its pI for a short time, the irreversible denaturation occurred to some extent. Since our main aim was the investigation of the native active enzyme properties, fraction 6 was collected and used for further studies.

2.2. RuBPcase molecular features

By the combined use of purified enzyme and enzyme assay, we studied three main molecular features of the nettle RuBPcase (i.e. molecular weight, subunit composition and isoionic point). The SDS PAGE electrophoresis of the protein of fraction 6 is reported in Fig. 2. It shows two main bands as could be expected by the polymeric nature of the RuBPcase, which is known to be composed of multiple replica of two subunits of different molecular mass. The result of Fig. 2 is a clear evidence that, even if partially denatured, in fraction 6 the RuBPcase is the most represented protein.

The molecular masses of the two subunits were determined by SDS PAGE electrophoresis using commercial reference proteins of known molecular mass. The result is shown in Fig. 3. The linear regression of the log of molecular masses versus mobility of each band was calculated and the molecular masses of the two RuBPcase subunits were determined: they are 55 and 14 kDa for the large and the small subunit, respectively.

The isoionic point of the native, active nettle RuBPcase was measured by the analytical isoelectrofocusing procedure using reference proteins of known isoionic point. Figure 4 shows the linear regression related to the isoelectrofocusing result, from which a pI of 5.3 has been calculated for the nettle RuBPcase.

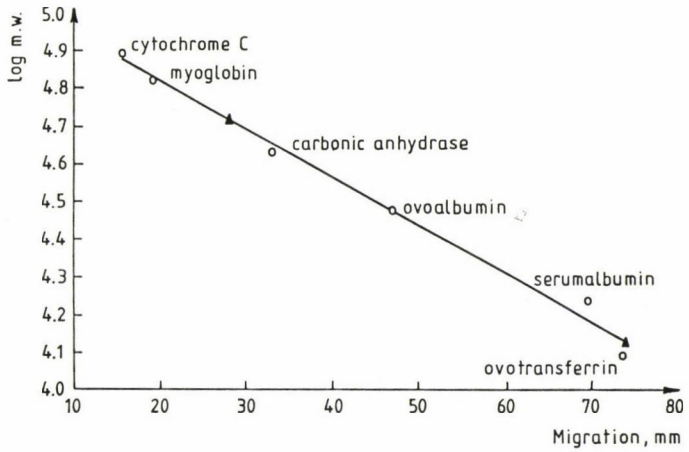

Fig. 3. SDS PAGE electrophoretic migration vs log molecular weight for nettle RuBPcase and markers proteins. RuBPcase subunits (▲), marker proteins (o)

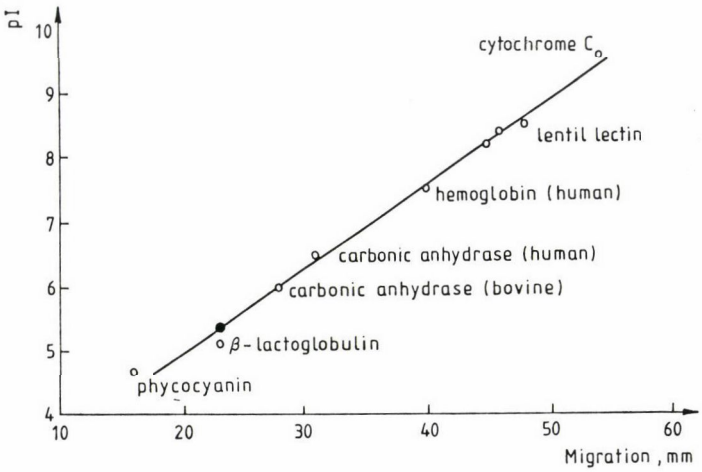

Fig. 4. Determination of RuBPcase pI (●) by analytical isoelectrofocusing. Marker proteins (o)

2.3. Functional properties (solubility and heat stability)

The commercial value of food-grade proteins is strictly related to their functional properties, which greatly affect the physical behaviour in food and/or beverages (GALOPPINI & FIORENTINI, 1985; GALOPPINI et al., 1989). As a general rule, the proteins extracted and purified without being severely denatured generally show good functional properties, which are adversely affected by strong chemical and physical treatments.

Among the physico-chemical characteristics of proteins, the solubility profile, determined as a function of pH and/or ionic strength, is one of the best protein functionality indices and reflects the intrinsic lability of the protein and how severely it has been denatured during the extraction process.

Figure 5 reports the solubility of nettle RuBPcase as a function of ionic strength. Values are expressed both in terms of soluble protein and enzyme activity. The RuBPcase, poorly soluble in very diluted salt solutions (μ 0.08), shows its maximum solubility at μ 0.11; the solubility abruptly decreases at higher salt concentration, being completely insoluble at values of μ higher than 4.

As far as the solubility of RuBPcase as a function of pH is concerned, Fig. 6 shows that the maximum solubility ranges between pH 6.5 and 7.5 in both Tris-acetate and Tris-Cl buffer. At these pH values, the solubilized protein is about 100% of the initial value.

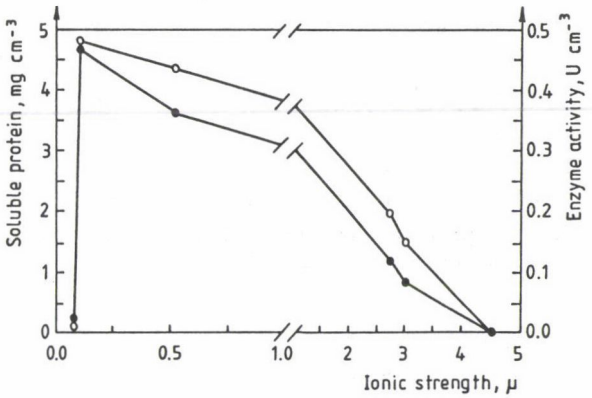

Fig. 5. Solubility of nettle RuBPcase as a function of ionic strength at pH 7.5. The graph shows the activity (●) and the soluble protein content (o) in samples where increasing amounts of ammonium sulfate were added to obtain the reported values of μ. Activity assays were carried out using 100 μl of samples. Details of the experiment are reported under section 1.9

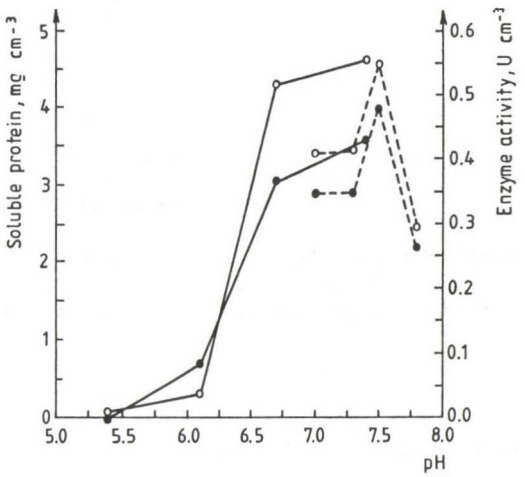

Fig. 6. Solubility of nettle RuBPcase as function of pH at μ = 0.11. The activity (●) and soluble protein content (o) at different values of pH are shown. Continuous line refers to 100 mmol l^{-1} Tris-acetate buffer, dashed line to 100 mmol l^{-1} Tris-Cl buffer. Activity was determined using 30 μl of sample solution. Details of the experiment are reported under section 1.10

In summary, the solubility profiles as a function of pH and ionic strength evidence the intrinsic lability of RuBPcase molecule, which however, when correctly extracted, can be partially redissolved in aqueous solution at low ionic strength, such as some common mineral waters and soft drinks. Considering its potential in medicine, the solubility levels showed by nettle RuBPcase in mild conditions also facilitates its therapeutic use (CASOTTO et al., 1989).

Figure 7 reports the heat stability of nettle RuBPcase. Values are expressed both in terms of soluble protein and enzyme activity. The protein is almost completely stable for 10 min at temperatures as high as 60–65 °C; over this limit a complete irreversible denaturation-preciptation of the protein occurs, with a corresponding loss of enzyme activity. The relatively high heat stability of nettle RuBPcase is technologically of great interest, considering the temperatures (50–60 °C for 5–10 min) proposed by some authors for the pilot plant production of RuBPcase from nettle (PEDONE et al., 1992).

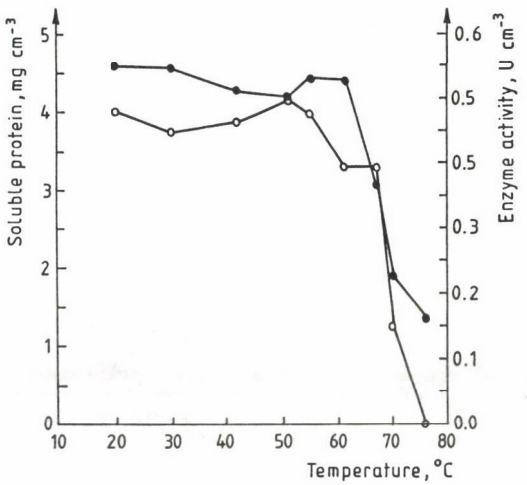

Fig. 7. Heat sensitivity of nettle RuBPcase. Aliquots of Rotofor fraction 6 were held for 10 min at the indicated temperatures, in 100 mmol l^{-1} Tris-Cl buffer, pH 7.5, rapidly chilled and centrifuged at 30 000 × G. In the supernatants, enzyme activity (o) and protein content (•) were determined

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ABSTRACTS

OF PAPERS PRESENTED AT THE "LIPPAY JÁNOS" SCIENTIFIC SESSION ORGANIZED BY

THE UNIVERSITY OF HORTICULTURE AND FOOD INDUSTRY

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SECTION OF FOOD SCIENCE

REDUCED RESISTANCE OF BACTERIAL SPORES SURVIVING IRRADIATION

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Some properties of surviving fraction of irradiated spores of *B. cereus* T, *B. subtilis, B. stearothermophilus, C. botulinum* Type A, B strains were studied. The suspension (0.14 mol l^{-1} NaCl) in some cases was bubbled with oxygen or nitrogen gas during irradiation in a Cs–137 or Co–60 source at 0 °C. In other cases the irradiation was carried out in air at 20–22 °C, either in suspension (in distilled water) or in dried form. The dose range was 0–12 kGy. The salt sensitivity was determined in recovery medium (0–8% NaCl), using plate count method. The heat sensitivity of irradiated spores was determined by MPN method. The F_0 value was measured. The distribution of development stage of spores was estimated in the function of salt concentration and irradiation dose by microscopy.

The irradiation reduced the surviving fraction and increased the salt and heat sensitivity of the spores. The CFU count of *B. cereus* at 5.2% NaCl was reduced

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nearly by 70% at 1 kGy dose, and at higher salt concentration the development stopped. The survival curves for radiation- and salt-resistance of *C. botulinum* strains 33A and 62A were different. The recovery of irradiated spores is smaller at 4% NaCl than at lower concentrations. The survival curves of strain 33A with and without added NaCl are parallel, while those of strain 62A are divergent. Survival curves for strain 51B in presence of 1 and 2% NaCl diverged from that of the control. The irradiated spores of Bacillus strains are more sensitive to heat than the unirradiated ones. In this case even a smaller F_0 value is effective. The rate of dormant spores was reduced in the function of dose and salt concentration. The outgrowth of spores was inhibited. The irradiation increased the salt and heat sensitivity of the spores.

EFFECTS OF GAMMA IRRADIATION, pH-REDUCTION AND a_w-REDUCTION ON THE SHELF-LIFE OF CHILLED "TENDERLOIN ROLLS"

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Experimental batches of refrigerated, vacuum-packaged, ready-to-fry, minced meat product, "tenderloin rolls" were preserved by combinations of reduction of pH from 6.1 to 5.6 by ascorbic acid, reduction of the water activity from a_w = 0.975 to 0.962 by sodium-lactate, and/or a radiation dose of 2 kGy. Storage of the untreated and irradiated samples at +2 °C for 4 weeks was followed by a one-week incubation at +10 °C. Total plate counts, counts of lactobacilli, the Enterobacteriaceae and sulphite-reducing clostridia were estimated at weekly intervals. pH-changes during storage were also followed. Comparative estimations of sensory qualities, thiamine contents, and TBA-values were also performed. The results demonstrated the possibility of significantly extending the shelf-life of the chilled product – without hampering the microbiological safety – by the sensorically acceptable radiation dose in combination with slight reduction of the pH and the water activity.

STUDIES OF THE GROWTH OF *LACTOBACILLUS BREVIS* AND *LACTOBACILLUS PLANTARUM* AS A FUNCTION OF pH, a_w AND TEMPERATURE

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The growth of the test organisms were studied in MRS medium with various pH-levels (3.0; 4.0 and 4.5; adjusted with hydrochloric acid), a_w = 0.94; 0.96 and 0.98, adjusted with glycerol and redox potentials (rH = 22 and 13, adjusted with thioglycollate) at 20, 25, and 30 °C, respectively.

From the growth data estimated as viable cell counts, one can conclude that the minimal pH of the growth of *Lb. brevis* was between pH = 3.0 and pH = 3.5 at a_w = 0.94 and rH value of 22, while at a_w = 0.98, the minimal pH of growth appeared to be lower than pH = 3.0. At reduced redox potential, the test organism showed an increased sensitivity against reduced pH and reduced incubation temperature.

Although it grew faster at pH 4.5 and a_w = 0.94 than *Lb. brevis*, the *Lb. plantarum* was more efficiently inhibited by a decreased pH, and the minimal pH for its growth seemed to be between pH 4.0 and 4.5. The reduced rH, however, decreased the growth of *Lb. plantarum* less than that of *Lb. brevis* under similar conditions.

ANTIMICROBIOLOGICAL EFFECT OF SPICES

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The antimicrobiological effect of horse-radish leaves, dill, garlic, onion and black pepper has been investigated.

Horse-radish leaves were added to beetroot salad and dill to sliced vegetable marrow, both filled in 370 cm^3 glasses, 10 g green spices and hot, salty and slightly acidic juice were added.

Onion, garlic and black pepper were used to flavour egg-plant – vegetable puree.

Different sets of samples were prepared to investigate the microbiological stability and organoleptic quality:

- without spices, not pasteurized
- with spices, not pasteurized
- with spices, pasteurized.

No microbiological growth has been observed in unpasteurized beetroot salad with horse-radish leaves and in unpasteurized vegetable marrow, in contrast to samples, unpasteurized and without spices.

In case of vegetable puree spices could not prevent, only delay microbial growth. *Acetinobacter*, *B. cereus, P. syringae* and *Aeromonas* species were isolated. Extracts of garlic and onion inhibited the growth of these microbes inoculated in culture-medium.

Spices improved the microbiological stability, taste and flavour of each product, but a combined preservative effect (low pH value, hot filling or low temperature storage) is essential to preserve quality.

EXAMINATION OF LACTIC ACID BACTERIA OCCURRING IN BEER

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Beer spoilage lactobacilli are nutritionally fastidious microorganisms, showing requirements for exogenous sources of vitamins, purines, pyrimidines and several amino acids.

There are two types of beer spoilage lactic acid bacteria:

- heterofermentative rods, belonging to a few species such as *Lactobacillus brevis* and *Lactobacillus buchneri*
- "tetracocci" or "beer-sarcina", such as *Pediococcus damnosus* and sometimes *Pediococcus pentasaceus*.

The effect of pH between 3.5 and 6.5 was investigated on the most frequently occurring lactobacilli causing spoilage of beer.

The characteristics of the thermal death were also determined for the industrial practice.

THE ROLE OF DETECTION OF DIAMINOPIMELIC ACIDS IN BACTERIUM IDENTIFICATION

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In most bacteria the main components of the cells are roughly similar, namely DNA, cytoplasma, plasma membrane and cell wall. However, the fine structures of these components show significant differences. The variation of the components is not equally distributed among bacteria. Each type of variation is always found among the same group of bacteria which means that these chemical types are taxon specific. It could be shown that these chemical differences in the bacterial cells are suitable for classification and identification. Gram positive bacteria show large differences in their cell walls, not only in the composition of the peptide side chain but also in the cross linkage of these chains. These differences are taxon specific and good criteria for the differentation of Gram positive and Actinomycetales bacteria. Diaminopimelic acid has never been found in proteins. Its occurrence in whole cell hydrolysates means that this molecule originates from peptidoglycan. Different methods are used for the study of DAP of the cell wall. The purpose of our experiments was to compare these methods, and based on the results to select the best one for our laboratory. Additional aim was to create short identification schemes for different bacterium genera based on the presence or absence of LL-DAP, DD-DAP or meso-DAP.

COMPARATIVE STUDY OF MATHEMATICAL MODELS DESCRIBING THE HEAT DESTRUCTION OF VEGETATIVE MICROORGANISMS

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In describing the thermal destruction of microorganisms, the most commonly used mathematical model is based on the analogy of the first order reactions. To calculate the number of the surviving cells is possible by integrating the differential equation describing the thermal death-rate. The only problem is the value of the

death-rate coefficient, because it depends on the temperature, disinfectant concentration, pH, water activity, redox-potential and other environmental factors.

Although the single effects of the environmental factors affecting the thermal death-rate are known, there has been no mathematical model for describing these effects, except for the temperature.

In our experiments the heat destruction of 8 microbes was studied in a wide range of the environmental factors and mathematical models were constructed to describe the connection between these factors and the thermal death-rate.

As a result of the mathematical-statistical evaluation it can be established that the models constructed on the base of reaction kinetics give higher correlation coefficients than the empirical models.

DETERMINATION OF VITAMIN B_1 IN FOODSTUFFS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND POST-COLUMN DERIVATIZATION

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Conventional high-performance liquid chromatography (HPLC) methods with pre-column derivatization of thiamine to thiochrome are time-consuming and susceptible to failure. On the basis of methods described in the literature a new method was developed to determine thiamine in foodstuffs by HPLC and post-column derivatization, and the HPLC parameters of this method were optimized.

The present method was tested in case of 20 different foodstuffs. The analytical results were well reproducible and in the same order of magnitude as the results of the pre-column derivatization methods employed so far. Recovery rates for vitamin B_1 were between 98 and 100%. The method was hardly susceptible to failure and perfectly suitable for routine determinations. By inclusion of a second fluorescence detector and integrator, vitamin B_2 could be simultaneously determined at 467 nm excitation and 525 nm emission wavelengths as well (retention time ca. 7 min).

ENZYMIC ANALYSIS OF FRUCTANS

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Enzymic analytical methods were elaborated and used for the study of the fructan content of *Helianthus tuberosus* treated with titanium ascorbate. The free glucose and fructose contents of the extracts of various parts of the plants were determined with the hexokinase+glucose-6-phosphate dehydrogenase test (Boehringer, Mannheim), and parallel measurements were performed with Yellow Spring YSI 27 enzymic analyser for glucose. The efficiency of hydrolysis of inulin with sulfuric acid, with NOVOZYM 230 inulinase and its separated endoinulinase and exoinulinase fractions, and with immobilized forms of the preparation were compared. The concentrations of reducing sugars in the extracts and hydrolyzates were determined by Somogyi-Nelson method, and the glucose fraction was examined with YSI 27 equipment.

Our results showed that the inulin content and the mean length of inulin molecules in the plants treated with titanium ascorbate did not differ significantly from those of the untreated control plants. Since the parameters of the individual plants exhibited high deviations from the mean values, the determination of fine biological effects of titanium-ascorbate on *Helianthus tuberosus* needs further experiments using more plants in replications.

INVESTIGATION OF ESSENTIAL OILS BY GC-MS MEASUREMENTS

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Gas chromatography-mass spectrometry is a powerful technique for identification and quantitative measurement of essential oils. Since the early 1970s, numerous reports have revealed newly discovered essential oil constituents. The GC retention index coupled with the MS fragmentation pattern is recognized as the most conclusive method for identifying unknown components positively. The MS spectra of the unknown constituents were compared to those of the authentic compounds, whenever it was possible.

Identification of terpenes and sesquiterpenes has been one of the most difficult tasks in essential oil analysis, but the modern high resolution capillary columns can separate many compounds effectively that give similar or almost identical MS fragmentation pattern.

In our work this excellent tool was used for the investigation of essential oils of *Majorana hortensis* (marjoram) that is important both as culinary and as medicinal herb.

The oil samples were prepared by the traditional steam distillation. The collected distillates were dried and injected into an HP 5891 A GC–MS system applied with a 50 m × 0.25 mm i.d. Ultra-2 capillary column. The identification was carried out by means of the Wiley mass-spectra library. The quantity of six components was determined by internal standard method in SIM and SCAN mode as well.

MEASUREMENT OF THE VOLATILE COMPONENTS OF PEPPER (*PIPER NIGRUM*) BY INTERNAL STANDARD METHOD

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A gas-chromatographic method with mass-spectrometric detection was elaborated for the measurement of volatile components in black and white pepper.

By the addition of carvone as an internal standard the recognition of the components and their quantitative data became highly accurate. According to the results the different type of samples, as well as the fresh and stored ones can be distinguished. The method enables us to identify the different cultivars and the adulteration.

IMPEDANCE MEASUREMENT FOR DETECTION OF RADIATION TREATMENT OF FOODS

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Experiments with a Hungarian potato variety "Kisvárdai Rózsa" confirmed that the impedance ratio Z_{50}/Z_5 suggested by Japanese workers significantly depends on the radiation dose applied to inhibit sprouting. However, the sensitivity and statistical reliability of this impedance ratio for identification of a radiation treatment was relatively poor. The ratio of phase angles was also tested at several frequencies and the ratio $\varphi_{15k}/\varphi_{80}$ provided statistically higher probability (r^2-value), and at least three times greater sensitivity as a response to the absorbed radiation dose than the previously recommended parameter.

THE DETECTION OF IRRADIATION OF FOODSTUFFS I. THERMOLUMINESCENCE METHOD

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Irradiation of some powdered food industrial products (e.g. spices) improves hygienic quality and extends storability. Physical methods (e.g. thermoluminescence, TL) are the most promising ones for the detection of irradiation.

The effect of ionizing gamma-radiation on milk protein powder (75% protein content) was investigated with TL method as a function of absorbed radiation dose, concentration of additional selenium trace element (up to 5 ppm) and storage time. Furthermore, the effect of absorbed dose was also studied for spices in whole and separated sample measurements.

It was found that in case of the investigated samples TL method was suitable for the detection of gamma-irradiation both qualitatively and quantitatively.

THE DETECTION OF IRRADIATION OF FOODSTUFFS II. ELECTRON SPIN RESONANCE METHOD

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Irradiation of some powdered food industrial products can be well identified with physical methods (e.g. electron spin resonance, ESR spectroscopy).

Paramagnetic centres and free radicals generated by ionizing gamma-radiation are detectable with ESR method.

In this work the effect of gamma-radiation of different doses was investigated in milk protein powder samples as a function of radiation dose, added selenium trace element content and storage time. Relations of selenium trace element to nutrition and food sciences were briefly summarized.

It was found that ESR method was also suitable for tracting radiation-induced responses in case of protein-containing food ingredients after longer storage.

INVESTIGATION OF SOLUBILITY AND EMULSIFYING PROPERTIES OF SOME PROTEIN ADDITIVES AS A FUNCTION OF pH AND TEMPERATURES

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Functional properties of two milk and two soy protein concentrates were studied using response surface methodology. The solubility and emulsifying properties were investigated in model systems, with the pH of solutions ranging from pH 4.0 to 7.0, and the incubation temperatures from 4 to 60 °C.

Both temperature and pH affected the protein solubility and emulsifying activity. Milk proteins had higher protein solubility, emulsifying activity and stability as the soy proteins. The emulsifying activity of milk proteins was relatively high at pH 4.0 and had a minimum value at pH 4.5-5.0, and increased with pH in the range of pH 5.5-7.0. The influence of pH was temperature dependent. The emulsifying activity of soy proteins was very low in the range of pH 4.0-5.0 and increased almost linearly with the increasing pH and temperature. Quadratic models were used to create the 3-dimensional response surface.

INVESTIGATION OF HOMOGENEITY OF MIXED FEEDS BY ELECTRICAL CONDUCTIVITY

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Homogeneity is one of the quality indicators of mixed feeds. Quick and simple methods are required for its measuring.

Most frequently it can be concluded from the NaCl concentration. The NaCl concentration can be measured by electrical conductivity, too.

The aim of our investigations is to decide if this method can be applied for such complex systems as the compound feeds.

The electrical conductivity of raw materials and compound feeds; the reproducibility of the results; and the connection between the composition and their electrical conductivity were studied.

Finally the findings were evaluated by mathematical methods.

COLOUR DEVELOPMENT OF SIX APPLE CULTIVARS, MEASURED BY ULTRASCAN SPECTROCOLORIMETER

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The paper reports the application of colorimetry in the quality control of apples.

The effect of time of picking on surface colour development of six different apple cultivars was studied.

The measurement was carried out by means of Ultrascan spectrocolorimeter, in the range 375 nm – 1100 nm.

The CIELAB system was the best for describing the ripening of apples and the effect of storage on changes of surface colour. The value of the colour difference ΔE^* increases in stage of the optimal picking ripeness abruptly, consequently, by the measurement of the surface colour, the optimal gathering time can be determined.

LIPID PEROXIDATIVE CHANGES OF EDIBLE OILS

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Changes of lipid peroxidation in fat-rich foods and fats cause quality deterioration of different degree depending on the intensity and the time constants of food technology. Degradation products of the peroxidation exert some detrimental changes in the living organism, too. However, the exact measure of free radicals and the oxidative intermediers leading to damage in the organism is uncertain. Therefore introduction of sensitive methods is necessary in order to check both the degradation products in foods and the in vivo injuries of the organism.

In former model experiments the characteristics of lipid peroxidation were determined in different oils after storing. The measurement of the non-specific "chemiluminescence intensity" proved to be a sensitive index of lipid peroxidation. In the present study peroxidative parameters, such as the conjugated diene and malondialdehyde content and the chemiluminescence intensity were measured in a technological model-experiment of edible oils. The oils were fried at 200 °C twice for 10 and 5 min, and they were also measured 10 weeks later. According to the results, chemiluminescence intensity proved to be a more sensitive method than the specific measurements, but the assay of conjugated diene and malondialdehyde concentration was useful for characterising the early lipid degradation of oils. The results suggest that complex information gained by different methods is necessary to evaluate the peroxidation changes occurring both in fats and in living tissues correctly.

ANALYSIS OF ANTINUTRITIVE FACTORS IN SPECIAL BREAD

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Some food products contain biologically active, antinutritive factors. The authors examined the phytic acid (phytate) content of cereals, determination of trypsin inhibitor activity of soya flour, and of a special bread was carried out. The

model samples contained wheat meal, and soya flour in various proportion (max. 6%), and two varietes of bread preparation: direct and leavened were analysed.

Various methods were tested for detection of change in phytate content and trypsin inhibitor activity.

In various technological processes, leavening reduced the phytate content. There was not any difference in the trypsin inhibitor activity between the direct and leavened process-made bread.

CAROTENOIDS OF SEA BUCKTHORN CULTIVATED IN HUNGARY

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The sea buckthorn is an euryoecic, feral plant, which is widespread in Asia and has also been domesticated in Europe and in Hungary for some years. Its yellow berries possess favourable physiological properties.

In our Institute a juice with high vitamin and mineral content, joined with prosperous biological effect, is made from sea buckthorn berries.

Our research work – dealing with the analysis of carotenoids – is in connection with the utilization of the pomace, a by-product of the juice manufacturing. The aim of our investigations was to adapt, on one hand a rapid spectrophotometric method, suitable for the determination of the total carotenoid content, and on the other hand a high performance liquid chromatographic (HPLC) method for the separation and identification of the individual carotenoid compounds of the pomace.

It was established that,

- the total pigment content of the pomace is higher than that of the juice
- the drying procedure causes significant pigment degradation even if it is made under mild conditions.

For simplification of the complex carotenoid composition a pre-separation of the extract by thin-layer chromatography was made. Subsequently, by HPLC analysis of the collected fractions, 31 peaks were separated. On the basis of systematic evaluation of spectra, related to different retention times within each chromatographic peak, about 50 compounds were distinguished. Most of them were carotenoids but some degradation products of chlorophylls were also present. Among carotenoid pigments we detected carotenes, xanthophylls and carotenoid esters simultaneously.

INVESTIGATION OF CHANGES IN MEAT PROTEINS DURING FROZEN STORAGE

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Effect of freezing, frozen storage and thawing on proteins was investigated in case of pork L. dorsi muscle.

The fresh pork was sliced (1 cm thickness), wrapped and frozen (2 different speeds), samples were taken during storage (after 48 hours, 2, 4, 6 months) and changes in proteins were investigated by DSC and PAGE.

DSC-thermograms show 4 peaks corresponding to myosin (1st peak) and actin (4th peak). The 2nd peak reflects the sarcoplasmic proteins and connective tissue contribution. Peak 3 is not defined.

When measuring the specific enthalpies (ratio between each partial enthalpy to the whole enthalpy) of the DSC-thermograms, it can be observed that the enthalpy ascribed to myosin decreases during frozen storage, while the enthalpy corresponding to actin is not affected.

At the beginning of our experiments PAGE was supposed to be a proper method for the investigation of protein denaturation. Our results show no significant changes in the chromatograms during the whole period of frozen storage.

NEW RESULTS IN THE ISOLATION OF SOY PROTEINS

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Isolation of 11 S and 7 S protein fraction and its content was determined from soy products such as defatted soy meal, soy concentrate and soy isolate.

The procedure for preparing 11 S and 7 S globulin was described by IWABUCHI and YAMAUCHI (1987). The present study was undertaken to obtain the 11 S and 7 S fractions from soybean products and to study the effect of a reductant (2-ME) on protein fractionation.

The soluble protein was extracted with TRIS-HCL buffer (pH 8.0, 0.03 mol l^{-1}) and the main globular protein fractions were separated by isoelectric precipitation at pH 6.4 and pH 4.8.

Our results show, that the quantity of 7 S protein in the soybean products is higher than that of 11 S protein. The reductant increased the solubility and extractibility of protein fractions.

ELEMENT SPECIATION USING COMBINED TECHNIQUES

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The methods used in analytical chemistry for the determination of element concentration from different samples (AAS, SP, ICP-AES, etc.) are not able for element speciation, however, the need for such determination in food and environmental control is growing. The combination of techniques (ion exchange, extraction, GC, HPLC, etc.) gives a possibility for speciation toxic and non-toxic elements such as arsenic, chromium, selenium, mercury. The sampling and sample preparation prior to determination should be important especially at speciation analysis, because there is no way to correct the mistakes at the analysis.

SECTION OF FOOD TECHNOLOGY

THE IMPORTANCE OF LIGHT BARRIER BEHAVIOUR OF PACKAGING MATERIALS IN THE ASSURANCE OF PACKED FOOD QUALITY

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To follow the quality changes of foods it is important to know the rate and mechanism of reactions which are responsible for the changes.

Quality changes are most frequently caused by oxygen uptake, that is by oxidation. The rate of oxygen uptake is influenced by the absorption property of foods and the gas permeability of packaging materials. Most oxidation reactions are accelerated, catalyzed by the presence of heavy metals and/or light. Therefore, it is especially important to know the light protectiveness of packaging materials.

Light may promote quality changes in indirect and direct ways. As an effect, various molecule transformations, for example degradation may occur, which may result the deterioration of vitamins, colouring agents and probably of essential amino acids, too. However, it may also be stated that the effect of radiation (light) depends on the food matrix and therefore a general rule cannot be drawn.

NEW VEGETABLE OIL RESOURCES

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In the past, besides traditionally cultivated oil seeds an increased interest has been shown in the utilization, cultivation of newer varieties, especially those which contain special substances.

The aim of this intention is to recover protein, carbohydrate, fiber and fat resources being unknown until now, and to find and cultivate plants providing biomass.

According to these targets, this recovery activity concerns oil plants as well, and as a result, the study of the utilization of various new plants is presently in progress.

Among them, some are cultivated also in Hungary, but the number of cultivated varieties might be widened on the basis of foreign experiences.

The most useful plants in this field: *Oenothera biennis* L., *Ribes* L., *Sinapis alba* L., *Borago officinalis* L., *Crambe abyssinica* Hochst. Ex. Fries., *Limnanthes* spp., *Lunaria annua* L., *Lesquerella fendreli* Gray, and *L. globosa* Desv., *Dimorphoteca pluvialis* L., *Euphorbia lagascae* Sprengel.

APPLICATION OF RESPONSE SURFACE ANALYSIS IN FOOD TECHNOLOGY RESEARCH

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Response surface analysis (RSA) is a set of mathematical statistical methods involving the design of the experiment, determination of the mathematical model and determination of the optimal set of the experimental factors. Food technology as a generally multivariable process is one of the research fields where RSA could successfully be used.

A three-variable, three-level fractional design with three replicates at the center point and a two-variable, five-level fractional design with five replicates at the center point were used for the investigation of the effects of extrusion cooking parameters (independent variables) on heat-labile antinutritional factors (dependent variables, response) in soybean and for some technofunctional properties in corn. From the data mathematical models were calculated by regression and variance analyses and then three-dimensional figures were plotted (responses vs parameters) to visualize the optimal conditions. Results found in this study are valid for the experimental circumstance used.

EFFECT OF SOME PROTEIN ADDITIVES ON THE FUNCTIONAL PROPERTIES OF MEAT PRODUCTS

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Protein additives are widely used in the food industry to stabilize different food emulsions.

The knowledge of physicochemical and emulsifying properties of protein additives has a great importance. Functional properties of protein additives, such as milk and soy protein concentrates, can be used to define how these proteins can be added to foods and how they can replace more expensive proteins, for example meat proteins.

The object of this study was to investigate functional properties of two milk and two soy protein products in relation to their water holding and fat binding capacity, solubility, emulsifying stability and capacity in a model system. A meat emulsion was used as a model system. Ten, 15, 20, 25% of meat protein was changed systematically by milk and soy proteins. The results of composition modifications were evaluated by measuring consistency and cooking loss.

The collected data show that 10 or 15% of meat protein can be replaced by protein additives without any decrease in the quality of product.

STUDIES ON TECHNOFUNCTIONAL PROPERTIES OF ADHESIVES OF NATURAL ORIGIN

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The packaging of the goods influences their value to a great extent. One of the provisions of the Hungarian product's export is the appropriate label. Among packaging materials the adhesive has small interest, as if the quality of the adhesive and the sticking is good, the glue is an invisible element of the product. There is no standard, so adhesives are qualified by the modelling of the conditions of the labeling (initial adhesive capacity, adhesive strength, penetration, etc.). Beside the empirical methods exact ones were sought to evaluate adhesives in an objective manner. Measuring the ultimate tensile force which is required for the discontinuity of the

bond we have established, that the ultimate tensile force depends on the thickness of glue. Moreover a simple, rapid method was worked out by the measurement of the evaporation rate of the solvent for the qualification of the industrial adhesives.

STORING STABILITY OF SWEET HUNGARIAN PAPRIKA (*CAPSICUM ANNUUM* L.) IN REFRIGERATED AND CONTROLLED ATMOSPHERE

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Authors give account of their several years' storing experiments. Raw material was sweet, white flesh (bell group) Hungarian paprika. Cultivars were Fehérözön Synthetic and Fehérözön Super. Freshly picked paprika can be stored in normal atmosphere for 15-18 days (at temperature 6-7 °C and relative humidity 85-90%). Whereas the storing period can be extended up to 44-46 days in CA. Storing conditions are: 8-9 °C air temperature, 88-93% relative humidity, 0-1% carbon dioxide + 4-5% oxygen + 94-96% nitrogen. Marketable quantity is high. Damage in outer and inner quality of paprika is restricted. Though change in vitamin C content and consistence is significant.

EFFECT OF STORAGE ON RESIDUE-LEVEL OF ZINEB AND MANCOZEB IN SOME PRODUCTS OF APPLE AND TOMATO

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A method using gas liquid chromatography with electron capture detector was used to follow the effect of storage on pesticide-residues in tomato and apple products.

Treatments with Mancozeb and Zineb were carried out according to the recommendation of Hungarian Ministry of Agriculture. All products were divided into two equal parts, one of them was washed and processed to various products

directly after treatment, and the other part was processed after 15 days (safety interval).

Products were stored for three months in sealed clean bottles at room temperature to elucidate the effect of storage on residue levels of Zineb and Mancozeb.

Although the residue content before storage was lower than the acceptable maximum concentration, the three month storage of the preserved apple and tomato products reduced the Mancozeb and Zineb content to the mere traces of their established maximum residue level (MRL).

PHYSIOLOGICAL CHANGES OF NEW HUNGARIAN APPLE CULTIVARS DURING STORAGE

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We have chosen some parameters that are important from the point of view of physiological changes during apple storage. The way to find the basis of softening is to determine the changes of the pectic substances, i.e. changes in total amount, fraction rate, chemical composition, molecular weight. The structural changes may be observed in the middle lamella and primary cell wall during storage. There are significant differences in enzyme – especially PPO – activity. The Idared, Redspur, Jonagold, Gloster, Golden delicious were the most important investigated varieties. It was also found that the permeabilities of cell membrane differ during the storage.

The physiological changes are caused partly by the distribution changes of electrolytes, mainly of the calcium. This longitudinal and other distribution changes are compared with different varieties related to harvesting dates and storage periods, as well as the calcium-chloride treatments and storage conditions.

EXPERIMENTAL PRODUCTION OF INULIN AND ITS HYDROLISATES

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Under the Hungarian soil-, climatic, and economic conditions Jerusalem artichoke (*Helianthus tuberosus* L.) is the most applicable agricultural product for both fructose and inulin production.

We had laboratory experiments to choose the most economic varieties for processing on the bases of chemical components and their changing during the storage. The 6 varieties investigated had the following properties: soluble solid content 20-24 Bx, pH value 6.1-6.5, acid level 0.1-0.3%, ash 1.0-1.24%, total sugar content 12.5-15.5 g per 100 g, reducing sugar content 0.17-0.45 g per 100 g, inulin content 10-16 g (75-85% fructose).

A product line was developed for Jerusalem artichoke to process natural syrups with high fructose content, pure fructose, dried products rich in inulin, pure inulin concentrate, nature and sweet pulps.

SWEETENERS IN FRUIT JUICES RICH IN FIBRE

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In the past years the increasing application of sweeteners was pressed by several factors:

- the demand on low energy foods increased
- the so-called ills of civilization (i.e. diabetes mellitus) do not allow carbohydrate consumption for steadily increasing part of humans.

The consumption of soft drinks, juices etc. increased in the last decades because of tourism, working place catering, etc.

Newer and newer compounds as sugar substitutes have been introduced.

Products with sweetening agents are now available on the food market, however the fibre enriched juices are still missing.

Taking the social composition into consideration the fibre enriched fruit juices are qualified for supplying the nutritional demand of aged people of increasing number.

Authors prepared fibre enriched apple and apricose drinks in various composition using beside traditional saccharose Nutrasweet or Sunett sweeteners, independently or in appropriate combination.

Storage experiments were also carried out at ambient temperature and in refrigerated storage room investigating the changes in sensory and chemical properties.

The results of experiments were evaluated by mathematical-statistical methods.

EXPERIMENTS TO INCREASE THE DIGESTIBILITY OF KERATINE CONTAINING WASTE MATERIAL

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Authors showed results of their new method which is convenient to increase the digestibility of waste material containing keratin (feather, bristles).

The applied method is nonpolluting so in this way there are not as dangerous polluting by-products as in the previously declared acid and basic hydrolysis.

Hydrolysis executed alkalescent agent with urea in autoclave resulted in digestibility as high as 80% but it meant less decomposition.

The amino-acid content, the ratio of the free amino-acids, degree of the racemization which refers to decomposition of the biologically important L-modifications were specified in the hydrolysed material.

Applicability is verified in pilot-scale test.

BIOENGINEERING PROBLEMS IN THE FERMENTATION AND DOWN STREAM PROCESS OF THE INDUSTRIAL ENZYMES

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The elaboration of the technology for enzyme production requires a complex research programme which includes the strain improvement, the optimization and the scale up of the fermentation, the concentration and the recovery of the product, purification of the enzyme according to demands.

The problems of the biotechnological research are introduced by various topics:

- the strain breeding of *Bacillus licheniformis* producing alpha-amylase and *Aspergillus niger* producing glucoamylase
- the mathematical design of experiments and the scale up for the glucoamylase fermentation
- the down stream processes for both the production liquid glucoamylase enzyme and the highly purified glucose oxidase preparations.

Beside the technological solutions the economic points were also taken into consideration.

PRODUCTION OF BIOACTIVE COMPOUND WITH CULTIVATION SHIITAKE *LENTINUS EDODES*

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Medical effects of the Shiitake mushroom have been studied by many researchers, and remarkable effects to remove serum cholesterol and antiviral or antitumor activities are certified.

At the beginning of our experiments we wanted to produce Shiitake in submerged cultures. In order to enhance the biomass the three main components – glucose, yeast extract, $(NH_4)_2SO_4$ – were added in various concentrations, and after the preliminary measurements a "Total Factor Plan" was made.

On the other hand we wanted to make certain the occurrence of erytadenine (its medical effect is remarkable) in mycelia. *Lentinus edodes* strains Le–4 and Le–3343 were received from Czechoslovakia and Germany.

More than one gram dried Shiitake mycelia was obtained from 100 cm^3 medium consisting of 3% glucose, 0.2% $(NH_4)_2SO_4$ and 0.8% yeast extract in case of strain Le–4, while strain Le–3343 produced 0.2–0.4 g dried mycelia under similar conditions. To obtain fungal mycelia a BIOSTAT system (B. Braun) has been used.

Lentysine was isolated from *Lentinus edodes* with column chromatography [Amberlite IR–120 (H^+) and Amberlite IRA–400 (OH^-)], for the identification and measuring the GC–MS and LC–MS technics were applied.

KARYOTYPING OF YEASTS BY PULSED FIELD GEL ELECTROPHORESIS

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Pulsed Field Gel Electrophoresis (PFGE) developed by Cantor and Schwartz (1984) has given the possibility for the determination of karyotypes of yeast and fungal strains. One of the recently developed version of this method: RFE (Rotating Field Electrophoresis) was used in our laboratory for the determination of chromosome patterns and CLPs (Chromosome Length Polymorphism) of yeasts.

Comparison of karyograms of different strains belonging to *S. cerevisiae* according to the currently accepted classification of yeasts by Kreger-van Rij (1984) was carried out. Considering the number and size of chromosomes the order of CLP increase compared with *S. cerevisiae* was as follows: *S. pastorianus, S. bayanus* and *S. paradoxus*.

Electrophoretic karyotyping of somatic hybrids proved to be an efficient method for the selection of improved yeast strains when brewer's yeast strains with glucoamylase activity were constructed and selected. The isolated primary hybrid clones were further screened according to their karyograms, and clones which proved to be the most similar in their chromosome patterns to the parental brewer's yeast strain were selected.

AMPLIFYING OF DEX GENE BY PCR

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The DEX or STA genes are responsible for production of glucoamylase enzymes. These genes can be found in some yeast strains called *Saccharomyces diastaticus*. A gene bank containing the 2.6-4.4 kb long fragments has been created in *E. coli* by means of shuttle plasmid vector. The recombinant plasmids have been transferred into laboratory yeast strain. The transformants have been screened for degrading starch but the enzyme activity has not been detected. In our opinion the cloned fragments might not have had the promoter sequence. The whole DEX2 gene which is 2753 bp long have been tried to amplify by PCR. For this reason two primers have been designed called P1 and P2 flanking on the two 5' ends of desired gene. An approximately 0.7 kb long product has been detected although 2.7 kb long was expected. It probably happened because P2 primer had homology sequence 0.7 kb far from one of the 5' end. The specificity of PCR reaction has been tried to enhance by glycerol and two-temperature PCR, respectively but without any success. Since there is a unique restriction site of EcoRI inside of the gene two further primers called P3 and P4 have been designed on two sides of the above-mentioned restriction site. The gene has amplified in two pieces. Because these two fragments have an about 200 bp long overlapping sequence the whole gene has been constructed by recombinant PCR.

PRODUCTION OF YEAST ENRICHED WITH TITANIUM

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Under given conditions yeasts are able to accumulate large amounts of microelements in their cells and incorporate them into organic compounds. In our investigation titanium has been introduced *Saccharomyces cerevisiae* cells. The nutrient media has been supplemented with titanium-ascorbate solution: at 20-50 μg cm^{-3} Ti-concentration the titanium uptake resulted in 1500-6000 μg Ti per g dried

yeast. The addition of Ti-ascorbate is more suitable in the exponential phase of yeast growth: in this case titanium is bound to the cell components instead of adsorption on the surface.

PRODUCTION OF Zr CONTAINING YEAST

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Accumulation of Zr by *Saccharomyces cerevisiae* and *Candida utilis* species were examined. The Zr was added to the media by several ways in form of different compounds. The Zr concentration of the samples was measured with spectrophotometry, the other elements were detected with ICP.

Saccharomyces cerevisiae proved to be superior in accumulating Zr in the cell, the highest concentration were reached by a complex made of $ZrOCl_2$ and ascorbic acid.

THE ROLE OF MICROELEMENTS IN BEER FERMENTATION

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In the beer fermentation some microelements play positive, others play negative role. In the Hungarian beers shortage of zinc was detected using inductively coupled plasma emission technique. The brewer's yeast consumed almost the whole amount of inorganic zinc at the first day of fermentation. For the separation of inorganic zinc and that bound in complex an ion-exchange technique was worked out. The method was controlled with consecutive dilution and standard addition technique. Using the method, in some cases during beer fermentation shortage of inorganic zinc was determined.

PHYSIOLOGICAL INVESTIGATION OF LACTIC ACID BACTERIA IN A CHEMOSTAT SYSTEM

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Lactose and citrate utilization of *Lactococcus lactis* subsp. *lactis* var. *diacetylactis* was investigated in a continuous chemostat system ($D = 0.2\ h^{-1}$, pH = 5.8, T = 30 °C), using different limiting carbon sources.

In the first period of fermentation (12.5 mmol lactose + 10 mmol citrate) the metabolism of microbe was homofermentative in 65% percent. Significant amounts of formic acid, acetic acid and ethanol were formed next to the lactic acid. Changing the carbon source to 12.5 mmol lactose resulted in homofermentative process in 95%. In the presence of 25 mmol glucose as carbon source the lactic acid bacterium strain showed heterofermentative features. In the last part of fermentation the conditions were the same as in the beginning (12.5 mmol lactose and 10 mmol citrate) and that caused a change in the end products. These changes might occur due to the changes in the plasmid profile of the microorganisme.

The presence of LAC and CIT plasmids were investigated on indicator agar. The results demonstrated that the lactose metabolizing ability of the microorganism is an unstable property, while the fermentation in the absence of citrate did not cause the loss of citrate utilizing ability, that is the loss of CIT plasmid.

The results indicate that the application of this organism can cause a selection of undesirable population, to avoid this, a careful establishment of the optimum condition is required.

NEW METHODS FOR HEAT TREATMENT CALCULATIONS IN CYLINDRICAL CANS

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Proposals are presented for the improvement of an existing program: "Heat Treatment Inside a Finite Cylinder". Formulae were created to include the heat capacity of the container when calculating the temperature field. Activation energy (E_a) and reference rate constant (k_r) were also introduced into the calculation system, which handles empirical changes of n-th order. Dependence or independence of rate-constant (k) and time constant (D) versus initial value of a quality attribute were also considered.

New equations have been developed for the calculation of the equivalent heat treatment time (F). These are based on interpolation between a number of fixed temperatures, if rate-constant or time-constants are known from experiments at these temperatures.

EXAMINATION OF THE USEFULNESS OF EMPIRICAL KINETIC EQUATIONS FOR DESCRIBING THE FORMATION OF 5-HMF IN GRAPEFRUIT JUICE

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Formation of 5-hydroxy-methyl-furfural in grapefruit juice was investigated at four temperatures and five heat-treatment times. Measured data were evaluated by relatively new methods: search for an adequate transformation by minimizing the ratio of variances around regression lines and within groups; applying the method of mean square of successive differences, Bartlett's test for homogeneity, unweighted and weighted regression analysis with different conditions for the initial transformed concentration. Reaction order, according to the empirical kinetics of formation was $n = 0.31$. Rate constants at four temperatures and one common initial transformed concentration were also determined. Special analysis based on the least squares method provided activation energy and reference rate constant. Original concentrations varied according to power functions of slightly autocatalytic nature.

DEVELOPMENT AND TECHNOLOGICAL APPLICATION OF A SOFTWARE SYSTEM FOR HEAT TREATMENT

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A unique, communicative software system has been discussed, summarizing the numerous different softwares developed to solve various tasks related to heat processing. The programs written formerly in Commodore and IBM BASIC as well as in Turbo Pascal computer languages, were improved to Lotus 1-2-3 R3.1 MACRO language. The new system gives more emphasis on the aspects of practical use, as well.

A part of the system is the program which generates different technological alternatives on heat processing equipments. This part is completed with the most up-to-date divided hydrostatic sterilizer types. Beside the former 6 Hunister types the so-called "large can" Hunister and the "Novoster" are included. The principles of rapid generation of technological alternatives for tunnel pasteurizers and retorts are also given.

Another part of the system is the program for evaluation of the heat penetration data measured and collected by fix or mobile type of heat penetrometers. Data presentations are in table forms and in graphs. The program for the simulation of the effects of different heat processing technologies on the microbiological and quality status of heat-treated food is now getting to be fitted into the package.

A very important goal is to prepare a uniform data bank usable in the practice for the technological engineer until the national network joining the computer systems of the individual companies would be built, and to elaborate a comprehensive program system for heat treatment of foods.

HEAT CONDUCTION MEASUREMENT OF FOOD INDUSTRIAL PRODUCTS

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The applicability of two measuring devices for thermal conductivity (the Guarded Hot Plate, GHP, and the Heat Wave methods) was investigated. The instruments were tested and some practical proposals for the measurement with the GHP instrument were given. Although the equipment worked on different measuring principles acceptable results were obtained for milk protein concentrate powders, using corrections for etalon and temperature. The results well agreed with the thermal conductivity data for similar milk powders.

ERROR ANALYSIS OF HEAT CONDUCTIVITY COEFFICIENT

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The author investigated the applicability of heat penetration curves deriving from the heat treatment of foods in rectangular cans for the determination of the thermal diffusivity. As much as 15% error can be expected in the final result with the Olson-Jackson equation with the implication of the influence of the Biot number. This error can be reduced with careful insertion of thermocouple to 10% which is already a good accuracy in engineering calculation.

SOME APPLICATIONS OF HEAT FLUX MEASUREMENTS IN FOOD PROCESSING

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In this paper a heat flux sensor and its calibration are described. Some possibilities of the application of heat flux sensors in food processing are mentioned. Heat flux measurements help to find the warming places in food stuffs storaged in bulk. Heat losses through walls of chambers with controlled climate, of refrigerating houses, buildings, pipes may be measured. During heat processing the integral of the heat flux gives the total heat absorbed by a canned food, from that the internal temperature can be calculated, if the thermal conductivity, the thermal diffusivity and the specific heat of food are known. Surface thermal resistance can be determined with measuring the heat flux and temperature difference too.

COSTHERM, A SOFTWARE FOR THE PREDICTION OF THERMOPHYSICAL PROPERTIES OF FOODS

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Proper experimental determination of the thermophysical properties of foodstuffs is quite difficult. It is also of limited use since the properties of the same food vary with factors such as water content and temperature. This has prompted the development of mathematical expressions to predict the properties of a food based on its composition and the properties of its constituents.

In this paper a simplified model developed in the COST – 90 Working Group by Chris Miles and Charles Veerkamp and a software COSTHERM written by Hans Pol and co-workers is introduced.

The model estimates the food properties by using the composition data (fractional composition of water, proteins, carbohydrates, fats and minerals in the food), the density and the initial freezing point of the product as the input. The model also uses the thermodynamics of diluent solutions to predict the ice and water fraction in a frozen food.

The outputs of the model are the thermal conductivity, the thermal diffusivity, the specific heat, the enthalpy and the density of the food on the desired temperatures.

TEXTURE MEASUREMENT OF CUCUMBERS FOR PROCESSING

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The knowledge of physical properties of vegetable is necessary to develop advanced harvesting systems and processing methods assuring a high product quality. This knowledge concerning different varieties of vegetables has also to be regarded for their suitability in existing harvesting and processing techniques. Especially in producing pickled cucumbers the utilization among existing varieties is very different. Therefore there is an increasing interest of the processing industry and breeders for exact test methods, referring to quality of harvested cucumbers, related to the pickled cucumbers. Among several quality characteristics the texture of the cucumbers, defined as the quasi modulus of elasticity, is one of the most important properties.

VERIFICATION OF A FINITE DIFFERENCE MODEL FOR TRANSIENT HEAT CONDUCTION IN FINITE CYLINDER BY ANALYTICAL METHOD

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H – 1118 Budapest, Ménesi út 44. Hungary

An assessment of the accuracy of a finite difference model for predicting temperature profiles during thermal processes in cylindrical containers was made using an analytical method. The analytical method was applied by solving ten roots for each Biot number and thus the first ten members of the infinite series were used to get proper accuracy. Calculations were made for one-dimensional shapes as the infinite slab and the infinite cylinder by both analytical and numerical methods. The

solutions were compared to verify if the used space intervals were sufficiently small to give accurate prediction.

It was stated that 15 nodal points in both the radial and axial directions were sufficient to keep the average of deviations under 0.1% and the maximum deviation under 0.5% of the initial temperature difference between cylinder and its surrounding medium.

PROGRAMMABLE LOGIC CONTROL FOR PROCESS CONTROLLING

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The development of the programmable logic control systems for technological and manufacturing processes are described in the paper. The benefits of the PLC systems are as follows:

- easy modification of the programme
- user friendly operation of the system
- data storing and data transfer capability
- visualization of the process on computer display.

The mentioned benefits are demonstrated for a CIP cleaning process by the means of operator console lights and display visualization.

A CALCULATION FORMULA AND THE MEASURING SYSTEM OF THE DEFORMATION IN TIN CANS DURING HEAT PROCESSING

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The pressure change in the sterilization equipment has an effect on the deformation of tin cans during heat treatment.

On the other hand the inside pressure also changes due to the temperature change which again cause deformation. It is important to known the total extent of

these effects, because it can be used to calculate the critical pressure differences during heat treatment to avoid irreversible deformations.

It is possible in two ways:

- by measuring the deformation with a special instrument
- by setting up a calculation formula with the knowledge of different parameters of the tin can (thickness of plate, etc.).

Both the collection of measured data and the setting up of the calculation formula were performed by the project.

STORING AND HANDLING PHYSICAL DATA OF FOOD INDUSTRIAL MATERIALS ON PC

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Probably it has happened to you that the number and the structure of your databases, the claims to your DB manager program changed. If you have too much money and time it does not matter, you only have to have it rewritten or modified; wait and pay for your program.

Else by the DataBase Manager program you can solve almost any database handling task that does not require relational database.

- You can create any type of generally widespread DBASE standard DB and modify the structure of database already done and filled.
- The environment required:
  format of display (list, record forms), order of records filter conditions, parameters of copying commands (search, assignment, sum, print, etc.), format of printing, etc.
  can be edited and modified by the aid of HELP that is available on every state of the program.
- And after that, the functions can be arranged through menues even by an unexperienced user.

The main aim of the programmer was to fuse the advantage of a

- database handler language (DBASE, CLIPPER, FOX...) flexible yet demanding familiarity with programming, and a
- program, user-friend yet developed for only
  a fixed given task with the least compromise.

Advantages: Almost full flexibility
Comfortable database handling even in NetWork.

COMPUTING PROBLEMS AT MEASURING PROGRAMS OF PENETROMETERS

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Our penetrometer is called Fructometer. This instrument has been connected to a Commodore 128 personal computer since 1988. The analog to digital converter and a measuring interface was developed at our department. There are two sorts of task which have to be solved by the program.

- Doing the predestined analyses on the data.
- Eliminating the irregularities automatically or by the intervention of the operator.

The most important problems or irregularities which we must respect are described in the followings.

The sample has not reached the measuring tool. The analog to digital converter has not completed the conversion. The need to detect that the measuring is finished. To eliminate the data which measured repeatedly. To delete the wrong data. To eliminate the data received when the instrument was stopped too late. To eliminate the force values which are greater than the rupture limit (e.g. when the measuring tool reached the core). To compute the Young's module precisely when too large speed was set by the operator. To handle the errors of the disk drive. To correct the enlarging factor at graphic displaying according to the actually measured values. To accommodate to the altering values (e.g. when skin and flesh strength measuring is altered).

AUTOCAD IN THE EDUCATION OF FOOD ENGINEERS

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Nowadays technical people, engineers meet nearly day by day the computers or any kind of application of them, and it is necessary to know how to use these equipments.

Institutes of higher educations have to ensure that graduated people possess the knowledge of computer.

The Food Industry College in Szeged started to teach the CAD program two years ago.

The subject covers two semesters, two hours practice a week. The students having attained the bases of computers get two independent tasks.

One of them is a so-called "modelling" when a student constructs a workshop drawing of a simple part by AutoCAD.

The other subject is more complex, which requires deeper knowledge of the program. They have to construct a screw-type jack from different parts. Of course the drawings should fulfil the role of mechanical drawing. Our experience shows that we successfully establish into our education a new subject which increases the level of education of industry people.

SECTION OF FOOD TECHNOLOGY – BIOTECHNOLOGY

CROP YIELD OF AUTUMN WHEAT VARIETIES TREATED WITH FERTILIZERS, THEIR GLUTEN TESTS AND PROTEIN CONTENT EVALUATION

L. TANÁCS, Á. KŐVÁRI, K. KOVÁCS, L. CSÉCSI, I. BEREK and L. GERŐ

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H–6724 Szeged, Mars tér 7. Hungary

Gluten tests, the SDS-value and the raw protein content unambiguously show that following oilflax increased rations of N-fertilizer lead to an increase in the SDS-value of gluten and protein content, while this effect is depreciated by P and K. In our opinion P and K may have hampering effect on the take-up of N. Considering the yield although, the joint use of P and K resulted in a significant growth of the crop.

Amino-acid examinations have shown that increasing the level of N at a constant level of P and K results in an increase in amino-acid content, while increasing the level of P and K at a constant level of N results in a decrease in the amino-acid content.

Summarizing our test results – and taking into account ecologic, economic aspects and aspects of nature conservation as well – it can be recommended that wheat varieties should be examined in this way. It is specific, how different wheat varieties respond to fertilizers. For this reason it is necessary to investigate the reaction to chemical fertilizers of any new wheat variety individually, and to find the optimal dosage of fertilizers.

THE EFFECT OF SOME INGREDIENTS AND ADDITIVES ON THE RHEOLOGICAL PROPERTIES OF DOUGH AND ON THE QUALITY OF BREAD

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The effect of different ingredients and additives – such as whole wheat meal, ewe-cheese cream, whole meal of four cereals, oil seeds – on the rheological characteristics of dough and on the quality of baking tests was studied. On the basis of the results of valorigraph and spread tests and baking experiments it was stated that most of these additives has an unfavourable effect on the structure of gluten, on the volume and porosity of bread and on the rheological properties of the bread crumb. The "non wheat dough components", for example oil seeds, dilute the gluten and interrupt the structure of dough. But by means of the above rheological methods optimum concentration ranges or combinations of these ingredients and dough improvers can be found which, in addition to higher biological value, result in baked products with acceptable dough structure and good baking quality.

POSSIBILITIES FOR THE PRODUCTION OF CARBOHYDRATE-BASE MACARONI DOUGH

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[b] Cereal Research Institute, H-6726 Szeged, Alsókikötősor 9. Hungary

Maize flour-base macaroni dough was produced by the authors. The cooking features were determined, sensory evaluation was carried out to test the dough quality. It was concluded that the sensory and cooking qualities can be improved by applying the emulsifiers. The degree of quality improvement depended on the type of the applied emulsifier.

A STUDY OF THE EFFECT OF EMULSORS WITH SDS-PAGE METHOD

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Macaroni dough model-systems were produced with emulsors from aestivum flours by the authors. The cooking quality and the sensory features of the different kinds of dough were found to be optimal at 0.65% emulsor concentration.

The changing of the dough structure was followed by solvent fractioning. The distributions of the molecular weight in the fractions produced (salt-, carbamide-, SDS- as well as SDS and β-mercapto-ethanol-soluble) were examined with SDS-PAGE method. The evaluation of the different kind of gel was carried out by a Video Densitometer. The molecular weight distribution of the fractions was changed by the emulsors and cooking.

CLASSIFICATION OF GROUND PAPRIKA BY COLOUR-MEASUREMENT

M. FEKETE-HALÁSZ, E. HUNN-HATVANI and P. RACS-ZÁHONYI

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CIELab colour coordinates L*, a*, b* were determined in 60 different samples of paprika, by Labscan spectrocolorimeter. Samples were divided into six lots according to their pigment contents and each paprika lots were separated to five different lots by sieving. It was established that the a* colour coordinate increased with increasing pigment content and there was no change in the b* coordinate.

The b* coordinate changed significantly by particle size but there was no significant change in the a* coordinate. The L* coordinate changed by both pigment content and particle size. A classification method was suggested by the colour coordinates.

AN UP-TO-DATE EVALUATION OF REGULATIONS OF MINISTRY OF AGRICULTURE IN THE YEARS 1936–38 ON THE PRODUCTION AND QUALIFICATION OF RED-PAPRIKA POWDER

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College of Food Industry, University of Horticulture and Food Industry,
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In Hungary the production and trade of red-paprika powder have been regulated by the state since 1917. It is reasonable to assume that 40–50% of the red-paprika powder of the world market is produced in Hungary, and this powder has special taste and flavour characters that make it different from any other similar product.

The production and trade of red-paprika powder under free market conditions were regulated by the MA (Ministry of Agriculture) decrees of 1936–38 (121300/1936, 27000/1936, 140100/1937, etc.). In the past 40 years ground red-paprika can be produced by only two state companies and the quality control was ruled by an MA decree 10/1988 MÉM (Ministry of Agriculture and Food Industry) and by the Hungarian Standard MSZ 11851.

In these days anyone can produce ground red-paprika, but the control of quality does not completely serve the interest of consumers.

The author suggests to renew the regulation of state in the quality control and to apply the adequate sections of 27000/1936 (16–18 paragraphs) and 121300/1936, respectively.

The application of certification cards and closing seal would serve the benefit of producer and consumer as well.

Protection of the "Hungaricum" character and quality of red-paprika powder is of special importance for Hungary.

PRODUCTION OF STARTER CULTURES OF EDIBLE MUSHROOMS IN FERMENTOR

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College of Food Industry, University of Horticulture and Food Industry,
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Using modified Van der Walt culture medium the optimal parameters for the production of *Pleurotus ostreatus* and *Lentinus edodes* starter cultures were characterised.

The time of cultivation necessary for the appearance of fruit bodies could be decreased by half. Moreover sterile method for the large scale mushrooms production was developed.

OBJECTIVE METHOD FOR MEASURING CHEESE RIPENING DEGREE IN DIFFERENT CHEESES

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The suitability of penetrometric, Shilovich, formol and dialysis methods for measuring cheese ripening degree was investigated.

Samples of Pannonia and Trappist cheese were examined as a function of ripening time. Ripe samples were also examined.

The three chemical methods provided suitable informations on the evaluation of cheese ripening. The penetrometric method was not suitable for it.

The relationship between dialysis ripening index values and Shilovich and formol ripening indices was linear and highly significant. The values obtained by chemical methods also positively correlated with the age of cheese. The data of ripe cheese were evaluated by variance analysis.

The use of the three simple and rapid chemical methods together with the usual organoleptic tests is recommended for the routine determination of proteolysis, i.e. for the objective qualification of cheese ripening.

INTENSIFICATION OF FOOD- AND BIOTECHNOLOGICAL OPERATIONS BY MICROWAVE ENERGY

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Industrial microwave equipments have gradually been used in the countries with highly developed industry during the last few decades. Great electric density can be produced by microwave energy, and sometimes the operation time connected with heat and substance transmittance can be reduced by one order of value. Tolerant operation of low temperature can be so insured, making the quality of final production much better. The characteristic feature of microwave is that it ensures the homogeneous operation in the whole volume of substance owing to its very short length of wave, the large penetrating depth and the selective absorbtion.

Microwave technics can be used with success in several bio- and food-technological operations. In the paper its wide scale adaptation with some descriptive examples is presented.

EXAMINATION OF ULTRAFILTRATION

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Experiments carried out by the authors showed that UF can be substituted successfully for conventional clarification without the use of chemical separation aids. Work undertaken on the chemical analyses of treated wines and mathematical analyses of the process UF made it possible to evaluate the treatment, the fouling constant etc.

Crossflow filter using membrane in spiral-wound form 100 kDa has been tested for this duty. Significant correlation was found between the flux vs time and flux vs volume either in power of exponential equation model.

Acta Alimentaria, Vol. 22 (3), pp. 265–267 (1993)

BOOK REVIEWS

Fruit storage

P. SASS

Mezőgazda Kiadó, Budapest, 1993. 364 pages

Content of the book gives new and many-sided knowledges concerning the theory and practice of fruit-storage. Inspite of dealing mainly with apples and pears, good information can be found about stone-fruits (cherry, sour-cherry, apricot, peach, nectarin, plum), berry-fruits (strawberry, black- and redcurrants, raspberry, bramble, gooseberry) and table-grapes.

The author describes in detail the physiological background of the above mentioned fruits (fruit development, quality factors, ripening and determining ripeness), the environmental factors and growing conditions (meteorology, variety effects, soil, fertilization, irrigation, yield-regulation, plant-protection, harvesting date, transportation).

This is followed by the description of storage methods and techniques (simple storage, refrigerated storage, different controlled atmosphere storages, i.e. the most up-to date ones as LO, ULO storage, hypobaric storage etc. and transportation in CA).

The storage conditions (temperature, relative humidity, composition of air, chemical treatment, length of storage period) are dealt with in details.

Special attention is paid to the most important storage losses and diseases; correlations among fruit quality, meteorological factors and storage losses are discussed. The engineering knowledge (architecture, machinery, equipments) can be found only in such a proportion that is necessary for storage technologist.

Finally a short summary of storage technology is given including the most characteristic parameters, as well as the history of storage and its world-wide situation. The text is illustrated with 200 tables and figures. The author refers to 350 internationally acknowledged experts, strengthening his own research work.

The book could be used either as an academic text-book, or as a practical guide. Some parts may seem to be too technical, but the average readers may skip these, without misunderstanding the major conclusions. Managers of medium-sized or even small orchards could use it with profit. The book can be recommended especially for high-school and university students, practitioners in the storage industry and fruit production as well as for academic and applied researchers.

P. TOMCSÁNYI

Akadémiai Kiadó, Budapest

Rapid methods for analysis of food and food raw material

W. BALTES (Ed)

Technomic Publishing Co. Inc., Basel, 1990. 392 pages

This edition is the English translation of B. Behrs Verlag GmbH Company's book, Schnellmethoden zur Beurteilung von Lebensmitteln (1987).

This publication discussed the possibilities, the limits and importance of rapid methods for food analysis. Rapid methods for food analysis are required not only for industrial process control but also for obtaining a fast overall view of the state of a food.

This book is a comprehensive collection of different methods for food analysis. Twenty-two leading German and 1 Australian food science experts report on the applicability of rapid methods in food industry. The book includes detailed discussion of the methods – such as electroanalytical measurements, Atomic Absorption Spectrometry (AAS), Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES), Thin-Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), Headspace Gaschromatography (HSGC), Near-Infra-Red Spectroscopy (NIRS), low resolution Nuclear Magnetic Resonance Spectroscopy (NMR), enzymatic rapid methods, immunochemical methods, isotachophoresis. Besides these chemical methods physical methods for rheology, consistency and particle size measurements, microbiological methods as well as sensory analysis of foods are illustrated, too. The book deals with the rapid sample preparation techniques for instrumental analysis and presents some equipment for rapid methods in food quality control.

These method-oriented representations are explained using some important examples.

Each chapter contains a selection of references covering the subjects.

This book is a valuable and useful source with information for all those dealing with the quality control and safety assurance in the field of food production and of official food control.

M. VÁRADI

Automation in the food industry

C. A. MOORE

Blackie, Glasgow and London, 1991. 212 pages

This book is a guide to automation for industry, especially for food processing. It consists of 10 chapters. Chapter 1 examines food processing as an industry, and the role of automation within this. Stages of manufacturing system are discussed from raw material handling to final product. Chapter 2 discusses the "total system" suggestion. The adoption of total systems makes the job easier, achieving greater consistency in performance and with the ability to change more readily in the future. In Chapter 3 integrated factory system is described. This concept is strictly related to the computer integrated

manufacturing philosophy. Chapter 4 gives specification for success, explaining the control system concepts as well as hardware and software requirements.

The various types of computers available are examined in Chapter 5. Chapter 6 illustrates the operator functions. Chapter 7 deals with important terms, such as system accuracy and reliability. Chapter 8 reviews how food processes can be automated in practice. The final two chapters identify what could become an advanced technique in creating friendly, controllable process plants within the food industry.

The chapters have been written by engineers, computer experts, control and system experts, who have many years' experience in the field of food processing automation.

A glossary of definitions is included at the end of the book.

The book is highly recommended for process engineers, electrical engineers and food technologists working in the food industry. It provides a valuable source of references to those who are interested in purchasing new equipment or designing automated systems.

M. VÁRADI

Continued from back cover

Continued from back cover

Continued from back cover

MAGYAR
TUDOMÁNYOS AKADÉMIA
KÖNYVTÁRA

RECENTLY ACCEPTED PAPERS

Storage of canned shelled Brazil nuts (*Bertholletia excelsa*): effects on the quality
RIBEIRO, M. A. A., REGITANO-D'ARCE, M. A. B., LIMA, U. A. & NOGUEIRA, M. C. S.
Catalytic deamidation of canola proteins
IGOR, S. D., DIOSADY, L. L. & RUBIN, L. J.
Free radicals and scavengers in plant and animal foods
DWORSCHÁK, E.
Selenium content of some higher fungi
VETTER, J.

NOTICE TO CONTRIBUTORS

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

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

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

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

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

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

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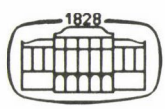

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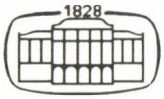

AKADÉMIAI KIADÓ
BUDAPEST

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FLUORINE REMOVAL DURING PRODUCTION OF KRILL PASTE AND KRILL PROTEIN CONCENTRATES

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Fluorine removal during production of krill paste (KP) and krill protein concentrates (KPCs) was analyzed. KP and KPCs with significantly low fluorine levels were obtained when treated by traditional methods, to which either an organic acid extraction was added at pH 4.5 followed by water washings, or simple water washings were applied. Nearly 99% of the fluorine was removed. The residual fluorine contents – 5–21 $\mu g\ g^{-1}$ (dry-matter) – observed in both KP and KPCs are acceptable for human consumption. Simultaneous reduction of cadmium (78%) was also observed during the fluorine removal. Questions related to the effect of treatments on the finished products are also considered.

Keywords: fluorine removal, krill, krill paste, krill protein concentrate

Antarctic krill – *Euphausia superba*, Dana – has been proposed as an alternative source of animal protein. This crustacean's high fluorine content, however, brings up doubt as to its suitability as a large-scale source of human food (SOEVIK & BRAEKKAN, 1979).

Selectively, most of the fluorine (over 99% of the total) is concentrated in the exoskeleton. Depending on storage conditions and processing, however, krill products can be contaminated by fluorine through fluorine migration into the soft tissue and by very small fragments of exoskeleton (ADELUNG et al., 1987; CHRISTIANS et al., 1981; CHRISTIANS & LEINEMANN, 1980 and 1983).

Technically, fluorine contamination can be reduced by adequate exoskeleton removal immediately after the krill is caught (BUDZINSKI et al., 1985; CHRISTANS et al., 1981, 1982; KARL, 1986; KARL et al., 1986). However, there are situations where exoskeleton removal is not entirely possible, since, at present, the operation is not very easy. On the other hand, an alternate technological possibility, following the mechanical removal of the exoskeleton, is the chemical treatment with citric acid to remove additional fluorine from krill products (CHRISTIANS & LEINEMANN, 1980; MANTHEY & SCHREIBER, 1983; KARL, 1986).

This paper describes the possibility of reducing the fluorine content to levels acceptable for human consumption, by modifying the proposed methods of obtention

during production of krill paste and krill protein concentrates. Effects of treatments on the finished products are also analyzed.

1. Material and methods

1.1. Samples

Antarctic krill – *Euphausia superba*, Dana – was caught in March 1987 by the Research Vessel "Prof. W. Besnard" of the Institute of Oceanography (University of São Paulo) during the Brazilian Antarctic Expedition. Immediately after the catch, the krill samples were packed in polypropylene bags distributed in layers of 2–3 cm of thickness with weight of 400–500 g each and were frozen to −25 °C. The samples were stored from 3 to 12 months at −25 °C until analyzed.

1.2. Fluorine removal during the production of krill paste

To remove fluorine during the production of krill paste the traditional method (BUDZINSKI et al., 1985) was modified by adding organic acid extraction followed by three consecutive water washings. The entire frozen (−25 °C) sample was crushed in a meat grinder equipped with a plate with holes of 13 mm in diameter and was manually pressed against a perforated (hole diameter: 1 mm) stainless steel surface.

After the exoskeleton was eliminated, the resulting juice was heated at 98 °C for 20 min in a water-bath, cooled to room temperature (22–25 °C), and centrifuged at 4920 G for 10 min to separate the liquid formed. Water and acid compounds (3 parts) – citric, tartaric and malic acids or lemon juice (*Citrus aurantiifolia*) – were added to the isolated material (1 part) until pH 4.5 was reached. The mixture was then stirred for 30 min with a magnetic stirrer and centrifuged at 4920 G for 10 min. Three parts of water were then added to the product (1 part) extracted with acid solution and was homogenized for 2 min with a magnetic stirrer and centrifuged at 4920 G for 10 min. This water washing operation was repeated two more times.

1.3. Fluorine removal during the production of krill protein concentrates (KPCs)

Many processes have been suggested for producing fish protein concentrates, including methods using alkaline and acid treatments (FINCH, 1970). To remove fluorine during the production of two types of KPC, alkaline and acid treatments, followed by three consecutive water washings, were used.

Alkaline process: the entire frozen sample (1 part) was crushed in a meat grinder equipped with a plate with holes of 4 mm in diameter. Five parts of water were then added, homogenized and filtered through a metallic sieve (hole diameter:

1 mm), thus eliminating the exoskeleton. To the resulting filtered suspension 2 mol l^{-1} NaOH was added until pH 11 was reached, then stirred with a magnetic stirrer for 45 min at room temperature (22–25 °C) and filtered under vacuum through a four-ply gauze in a Buchner funnel. Two mol l^{-1} HCl was added to the filtrate until pH 4.5 was reached, then centrifuged at 4920 G for 10 min. Water (3 parts) was added to the precipitate (1 part) thus obtained, then homogenized for 2 min by magnetic stirring and centrifuged (4920 G for 10 min) three times. The isolated protein was successively defatted first, with ethanol (1:3), then with ethanol–hexane (1:1) and, finally, with ethanol, for 60 min each, with magnetic stirrer, at 22–25 °C. The solvent phase was separated by centrifugation (4920 G for 5 min). The KPC obtained was exposed to room temperature (22–25 °C for 3 h), dried in an air circulating oven at 40 °C for 1 h, powdered in a mortar and sieved (0.297 mm^2).

Acid process: the alkaline process was modified by (a) replacing the alkaline treatment at pH 11 by acid treatment at pH 2 using 2 mol l^{-1} HCl; (b) eliminating the gauze filtration; and (c) protein precipitation with 2 mol l^{-1} NaOH at pH 4.5.

1.4. pH correction and estimation of thawing drip

The krill paste subjected to fluorine removal had its pH value corrected to neutrality by adding only Na-bicarbonate, and also by addition of food additives – Na-pyrosphosphate (0.5%), Na-tripolyphosphate (0.5%) or Na-chloride (3%) – followed by an addition of Na-bicarbonate (REHBEIN, 1980).

For the estimation of thawing drip (%, w/w), the krill paste was weighed (10 g) in a polypropylene tube (25 × 100 mm) and frozen at –25 °C for 15 h. After thawing at room temperature (22–25 °C) for 4 h, the exuded liquid was decanted and the sample weighed. The difference in weight was determined and taken as drip loss (REHBEIN, 1980).

1.5. Analytical procedures

Fluorine was potentiometrically quantified (expressed as μg per g) by using an Orion selective electrode (94–09) and a Procyon SA 720 ion analyzer (DOLAN et al., 1978). Moisture, ash and cadmium, respectively, were estimated by: oven drying at 105 °C for 15 h; incineration in a muffle furnace at 550 °C; and polarography (HORWITZ, 1980). Protein was determined by micro-Kjeldahl method (HORWITZ, 1980), using $CuSO_4 \times 5H_2O$ (70 mg) to replace the HgO. After the ash was dissolved in 1 mol l^{-1} HCl and pH adjusted to 5.0–5.5 with 12.5% (v/v) NH_4OH, the calcium was quantified by the chloranilate method (FERRO & HAM, 1957). Lipids were measured by gravimetry, after extraction with chloroform–methanol–water

(1:1:0.9) (KONING et al., 1985). Chitin was evaluated by gravimetry, after extraction of lipids with chloroform–methanol (1:1), alkaline deproteinization and acid demineralization (REHBEIN, 1980).

2. Results and discussion

2.1. Fluorine removal during production of krill paste

The krill paste is traditionally obtained by pressing the protein juice out of the krill, thermally coagulating the juice and separating the coagulated protein (BUDZINSKI et al., 1985). Like all krill products, the paste is also subject to excessive fluorine contamination, especially if the exoskeleton has not been adequately removed. The high and varied levels observed for untreated krill paste – 559.42 ± 5.51 – 1695 ± 0.72 (dry matter) due to contamination by exoskeleton were therefore not entirely retained by the pressing process employed. This conclusion is confirmed by the presence of chitin detected in this paste.

Considering the high fluorine levels detected – 559 to 1696 $\mu g\ g^{-1}$ (dry matter) – associated with the high bioavailability of the krill fluorine observed in chicks (SOEVIK & BRAEKKAN, 1979), rats (SIEBERT et al., 1982; ALVARENGA & TENUTA-FILHO, 1993) and humans (TRAUTNER & SIEBERT, 1983 and 1986), the untreated krill paste (control) cannot be used as food due to its toxicological unsuitability.

Through extraction with acid solution at pH 4.5, followed by three additional successive water washings (added to the traditional method of obtaining krill paste), nearly 99% of the fluorine was removed (Table 1). At the same time, no significant loss of protein (< 120 mg per 100 g) protein was observed by solubilization.

The residual fluorine contents – 1.22 to 5.75 $\mu g\ g^{-1}$ (wet matter) or 5.53 to 20.86 $\mu g\ g^{-1}$ (dry matter; Table 1) – observed in the extracted and washed krill paste are low, and suitable for human consumption. The maximal levels for safe and adequate dietary intakes of fluorine are estimated to be 2.5 and 4.0 mg per day for younger age groups and adults, respectively, while daily fluorine intakes higher than 6.0 mg (by children whose teeths were developing) and 0.10 mg kg^{-1} body weight (equivalent to 7.0 mg for adult of 70 kg) were associated with the occurrence of dental fluorosis (NATIONAL ACADEMY OF SCIENCES, 1989; WARDLAW & INSEL, 1990; OPHAUG, 1990). According to Table 1, to reach the fluorine level intake of 6.0 mg per day an unlikely consumption (higher than 1000 g per day) of krill paste would be necessary.

Table 1

Fluorine removal during the production of krill paste[a]

Treatments[b]	Fluorine	
	Residual[c] ($\mu g\ g^{-1}$)	Reduction (%)
Citric acid	5.74 – 15.78 ±0.70 ±0.27	98.76 ± 0.79
Tartaric acid	7.33 – 19.31 ±0.38 ±0.02	98.61 ± 0.71
Malic acid	5.53 – 20.86 ±0.23 ±0.02	98.76 ± 0.61
Lemon juice	7.89 – 13.91 ±0.14 ±0.48	98.84 ± 0.81

a: Arithmetic means and ± standard deviations of five samples analyzed in triplicates
b: Krill paste (1 part) extractions with acid solutions (3 parts), at pH 4.5 for 30 min and successive water washings (3 times)
c: Dry matter

In general, fluorine content in food varies greatly, and is higher in marine foods and tea. On the other hand, residual fluorine levels – 1.22 – 5.75 $\mu g\ g^{-1}$ in wet matter or 5.53 – 20.86 $\mu g\ g^{-1}$ in dry matter – observed for the krill paste under study (Table 1) are significantly low when compared to those shown by certain foods such as fish, deboned meat and even other krill products mentioned in the literature. Levels from 2.70 to 10.8 $\mu g\ g^{-1}$ (wet matter) were reported for cod, haddock, herring and dogfish fillets (KE et al., 1970), cod with skin, and salmon, pilchard and canned herring (WALTERS et al., 1983). For manually and mechanically deboned beef, pork and poultry, products subjected to fluorine contamination by small bone particles during processing, levels between 0.70 and 34.4 $\mu g\ g^{-1}$ (wet matter) (WALTERS et al., 1983; DOLAN et al., 1978) were reported. For krill meat obtained by the roller-peeling method, 15 $\mu g\ g^{-1}$ (wet matter) was reported (BUDZINSKI et al., 1985); for minced, raw krill meat obtained by separation from the residual exoskeleton by a decanting centrifuge process, levels of 106 $\mu g\ g^{-1}$ (dry matter) (CHRISTIANS et al., 1982) and 121 $\mu g\ g^{-1}$ (dry matter) (KARL et al., 1986) were found, while for minced krill meat thermally coagulated and treated with citric acid at pH 6 and 5, rates were 100 $\mu g\ g^{-1}$ (dry matter) and 73 $\mu g\ g^{-1}$ (dry matter), respectively (KARL, 1986).

Table 2

Effects of the fluorine removal during the production of krill paste on the minerals[a]

Treatements	Fluorine		Ash		Ca		Cd	
	Residual[c] ($\mu g\ g^{-1}$)	Reduction (%)	Residual[c] (g per 100 g)	Reduction (%)	Residual[c] (mg per 100 g)	Reduction (%)	Residual[a] ($\mu g\ g^{-1}$)	Reduction (%)
Citric acid extraction[b]	243.73 ±116.53	71.14 ±1.07	5.38 ±2.35	58.39 ±2.25	- -	- -	- -	- -
Water washings[b]								
-1st	55.78 ±16.58	92.94 ±1.59	3.00 ±2.13	78.69 ±5.41	- -	- -	- -	- -
-2nd	17.87 ±2.24	97.51 ±1.14	2.25 ±2.28	85.47 ±8.89	- -	- -	- -	- -
-3rd	11.02 ±1.93	98.41 ±0.85	2.23 ±2.17	85.50 ±8.50	23.82 ±19.95	98.77 ±0.72	0.04 ±0.01	77.62 ±4.37

a: Arithmetic means and ± standard deviations of five samples analyzed in triplicates
b: See Table 1 (footnote)
c: Dry matter
d: Wet matter

More detailed fluorine removal carried out by using citric acid showed that the fluorine is extracted by acid while the water washings apparently serve to remove it in soluble form (Table 2). As expected, it could be observed that the mineral fraction was greatly affected, but this nutritional change is relatively unimportant. Fluorine, ash and calcium were reduced by 98%, 86% and 99%, respectively, and a very high correlation coefficient ($r = 0.997$) was observed between fluorine and ash.

KARL and co-workers (1986) proposed cadmium removal together with fluorine removal during krill processing because of the high cadmium content in different organs of the krills' cephalothorax. With the process described in this paper for fluorine removal it was also possible to remove 78% of the cadmium from krill paste (Table 2). The cadmium detected in untreated (control) and treated krill paste – 0.17 ± 0.03 $\mu g\ g^{-1}$ and 0.04 ± 0.01 $\mu g\ g^{-1}$ (Table 2), in wet matter, respectively, is very low if compared to the Brazilian legal limit ($1.00\ \mu g\ g^{-1}$) for cadmium content in foods (BRASIL, 1965), and to krill paste ($1.15\ \mu g\ g^{-1}$) reported by LEE and co-workers (1985). In the literature, cadmium levels of $0.00016\ \mu g\ g^{-1}$ and $0.00049\ \mu g\ g^{-1}$ (wet matter) were reported for krill meat blanched in fresh water and salt water, respectively (BYKOWSKI et al., 1980), 0.220 (0.170–0.275) $\mu g\ g^{-1}$ (wet matter) for boiled krill (without shells), 0.048 (0.036–0.066) $\mu g\ g^{-1}$ (wet matter) for krill muscle meat, 0.500 (0.470–0.570) $\mu g\ g^{-1}$ (wet matter) for boiled and spray dried krill (without shells) (STOEPPLER & BRANDT, 1979). For whole krill, 0.170 (0.070–0.360) $\mu g\ g^{-1}$ (wet matter) (STOEPPLER & BRANDT, 1979) and $1.14\ \mu g\ g^{-1}$ (wet matter) (LEE et al., 1985) were cited, and for whole krill meal, 1.200 (1.080–1.600) $\mu g\ g^{-1}$ (dry matter) (STOEPPLER & BRANDT, 1979).

After fluorine removal, krill paste acidity must be conveniently adjusted to reduce the sour taste and to avoid drip thawing. About 20% of exuded water – corresponding to 28% of the moisture, is lost after thawing if pH is not corrected (Table 3).

The effects of treatments on the sensorial quality of krill paste are not available in this paper. Apparently flavor was one of the most affected sensory quality attributes and, for this reason, research on flavor correction is in progress.

The average chemical composition of the krill paste is as follows: moisture 76.77 ± 0.72; ash 0.49 ± 0.06; protein 10.31 ± 0.05; lipid 10.95 ± 0.38; chitin 0.74 ± 0.08 (g per 100 g); and fluorine 2.86 ± 0.24 $\mu g\ g^{-1}$ (wet matter) or 11.02 ± 1.93 $\mu g\ g^{-1}$ (dry matter). Compared to traditional krill paste (LAGUNOV et al., 1974), that is studied here is similar for moisture (70–78 g per 100 g) and lipid (3–10 g per 100 g) but lower in ash (1–3 g per 100 g) and protein (13–20 g per 100 g) levels.

Table 3

Effect of the pH correction on the krill paste thawing drip[a]

Krill paste	pH	Thawing drip (%)
Control[b]	7.0 – 7.4	0.00
	7.2 – 7.5	0.00
Treated[c]	4.4 – 4.6	20.38 ± 2.65
	4.5 – 4.9	21.02 ± 1.61
Treated[c] and corrected for pH[d]	6.2 – 6.5	0.00
	6.3 – 6.7	0.00
Treated[c] additives added[e] and corrected for pH[d]	7.1 – 7.5	0.00
	6.8 – 7.4	0.00

a: Arithmetic means and ± standard deviations of five samples analyzed in triplicates
b: Untreated
c: For fluorine removal with citric acid (Tables 1 and 2)
d: With $NaHCO_3$
e: $Na_4P_2O_7$ (0.5%), $Na_5P_3O_{10}$ (0.5%) or NaCl (3.0%), isolately added

2.2. Fluorine removal during the production of krill protein concentrates – KPCs

During the production of the two types of krill protein concentrates (KPCs) about 99% of the fluorine was removed in relation to whole krill (Table 4). The results suggest an effective acid treatment by fluorine extraction just as occurred with the krill paste (Tables 1 and 2). Furthermore, the fluorine may have also been extracted under the alkaline conditions employed (PARK et al., 1988).

To protect the proteins against chemical, principally alkaline, effects, the treatments were carried out at low temperature (22 – 25 °C) and for short periods (45 min). The alkaline treatment used was milder than others in which lysinoalanine – LAL – an unusual and toxic aminoacid – is formed. For fish protein concentrate, the maximum LAL formation reported was at pH above 13, 90 °C and over 2 h of treatment (FUJIMAKI et al., 1980). With fish muscle treated at pH 10, 90 °C, for 1 h, no LAL formation was observed; this formation occurred only at pH 12 or more (MILLER et al., 1983). Subjecting fat-free minced krill to alkaline digestion – 0.05% NaOH, at 37 °C, for 5 h, with pH varying from 11.5 to 8.0 – 0.5% LAL were formed in the functional KPC produced. This LAL level was considered relatively low

compared to others reported in the literature (OEHLENSCHLAGER & SCHREIBER, 1981).

Similar to that occurred with the krill paste analyzed above (Table 1), the KPCs residual fluorine contents – about 9 $\mu g\ g^{-1}$ (dry matter, Table 4) – are also low and acceptable for human consumption. As compared to the maximum fluorine level (100 $\mu g\ g^{-1}$) for fish protein concentrates permitted by the USA's FDA (FOOD AND DRUG ADMINISTRATION, 1967), the KPCs levels (Table 4) are about 10 times lower.

Table 4

Fluorine removal during the production of krill protein concentrates – KPCs[a]

Treatments/ products	Fluorine			
	KPC1 (alkaline process)		KPC2 (acid process)	
	Residual[b, c] ($\mu g\ g^{-1}$)	Reduction (%)	Residual[b, c] ($\mu g\ g^{-1}$)	Reduction (%)
Protein isolating[d]	82.55 ±32.63	90.05 ±1.29	155.02 ±5.35	85.55 ±0.11
Water washings[e]				
– 1st	26.82 ±3.71	97.24 ±0.38	28.35 ±0.49	97.36 ±0.16
– 3rd	8.83 ±1.07	99.10 ±0.10	22.09 ±0.18	97.94 ±0.10
KPCs[f]	9.15 ±0.18	99.18 ±0.14	9.11 ±1.17	99.13 ±0.11

a: Arithmetic means and ± standard deviations of five samples analyzed in triplicates
b: Related to the whole krill
c: Dry matter
d: Exoskeleton removal, adjusting at pH 11 (KPC1) or at pH 2 (KPC2), filtration (KPC1) or not (KPC2), and protein precipitation at pH 4.5
e: Protein isolated (1 part): water (3 parts)
f: After defatting, drying and powdering

Compared to others, the KPCs studied in this paper advantageously showed the lowest levels of fluorine and ash (Table 5). KPC2 showed chitin contamination since no filtration was made after acid treatment.

Table 5

Chemical composition of krill protein concentrates, g per 100 g

	Krill protein concentrates – KPCs				
	KPC1[a]	KPC2[a]	SIDHU et al.[b] (1970)	KUWANO & MITAMURA (1977)	OEHLENSCHLAGER & SCHREIBER (1981)
Moisture	13.79 ±0.74	8.80 ±0.05	0.00	7.91	5.60
Ash	0.26 ±0.08	0.54 ±0.03	14.00	8.46	5.73
Protein	83.31 ±1.51	86.82 ±0.63	77.50	80.30	84.00
Lipids	0.35 ±0.02	0.15 ±0.01	0.30	0.24	0.10
Chitin	ND	1.43 ±0.02	5.66	–	–
Fluorine ($\mu g\ g^{-1}$)	8.28 ±0.23	8.21 ±1.09	12.00	150.00	250.00

a: Table 4, arithmetic means and ± standard deviations of two samples analyzed in triplicates
b: Dry matter
ND: Not detected

The fluorine content of 12 $\mu g\ g^{-1}$ (dry matter) reported by SIDHU and co-workers (1970) is apparently underestimated. It appears to be impossible for a KPC produced with whole krill to show such a low level. This result was reported in 1970, before SOEVIK and BRAEKKAN (1979) first reported the occurrence of high quantities of fluorine in krill.

The level of 250 $\mu g\ g^{-1}$ reported by OEHLENSCHLAGER and SCHREIBER (1981) is relatively high, although some conditions used are favorable to fluorine removal, such as exoskeleton elimination, washings with diluted HCl at pH 4.5, alkaline digestion with 0.05% NaOH, at 37 °C, for 5 h and dialysis against water.

As it was observed above in relation to krill paste, the KPCs' residual fluorine (Table 5) is very low when compared to that of fish (KE et al., 1970; WALTERS et al., 1983), deboned meats (WALTERS et al., 1983; DOLAN et al., 1978) and other krill food products (BUDZINSKI et al., 1985; CHRISTIANS et al., 1982; KARL et al., 1986; KARL, 1986).

3. Conclusions

By modifying the traditional production methods, it was possible to obtain krill paste and krill protein concentrates with fluorine levels sufficiently low to allow human consumption.

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STUDY OF THE CONDITIONS OF FERMENTATION FOR THE PRODUCTION OF GLUCOAMYLASE

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The filamentous fungus *Aspergillus niger* (*A. niger*) plays an important role in industrial microbiology. During the fermentation of *A. niger* a broad assortment of extra- and intercellular enzymes and other products is produced. It has numerous advantageous properties from economical and technological aspects. With gene techniques, systems were developed for Aspergillus fungi that utilize the glucoamylase gene sequency favourably.

Glucoamylase is one of the most important industrial enzymes, the investigation of the conditions of fermentation is actually a current task, industrially as well as at pilot plant level.

A synthetical culture medium of known composition has been elaborated enabling the study of the utilization of the culture medium and of the enzyme formation by physiological conditions close to industrial fermentation.

Keywords: *A. niger*, intercellulase enzymes, glucoamylase gene sequency, filamentous fungi

Aspergillus niger belonging to filamentous fungi, plays an important role in the industrial microbiology. It produces a wide assortment of extra- and intercellular enzymes and of other fermentation products. It has numerous advantageous characteristics considering economy and technology, as well. In the past 5–10 years, stock production with gene techniques has been started for filamentous fungi, too. Expression systems have been elaborated for other Aspergilli, with the use of the elements of the glucoamylase gene of *A. niger* (ESSER & MOHR, 1986; BODE, 1966).

The genetically modified organisms were first tested under laboratory conditions, which were greatly different from the conditions of industrial fermentation. The investigation of the conditions of glucoamylase fermentation is today highly actual. Glucoamylase is one of the most important industrial enzymes being produced in the greatest quantities. *Aspergillus niger* is suitable for the production of numerous other products, as well (DIETZ & CHURCHILL, 1985).

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Our trials aimed at the preparation of a synthetic culture medium of known composition, that enables the study of the utilization of the culture medium and the formation of the enzyme by the physiological conditions close to industrial fermentation.

1. Materials and methods

Table 1 presents some characteristic factors of glucoamylase (GA) under industrial and laboratory fermentation conditions. As it can be seen, there are significant differences in the processes of metabolism. For the reduction of these differences the following concept was elaborated.

Table 1

Some industrial and laboratory characteristics of glucoamylase fermentation

	Industrial size	Laboratory size
Carbon source	complex, corn-starch groats, hydrolysate	single-complement, commercial saccharose or glucose
Nitrogen source	corn steep liquor	peptons (complex) or other N-salts
Substrate quality	changing often	mostly known, precise composition
Substrate concentration (%)	10 – 15	2
Water activity	low value	high value
Attainable enzyme activity (U cm^{-3})	10 – 30	0.2 – 0.3
Separability of mycelium	for corn groats it is not separable	separable
Growth	filamentous	growth, often pellet-like
Medium utilization measurement	indirectly	directly

There are numerous osmotolerants among Aspergilli. This osmotolerance has been utilized by the industry, when high (10–15%) substrate concentration was used for fermentation (BARTOSZEWICZ, 1986).

Increasing the concentration of the ingredients of the minimal culture medium, ionic power and osmotic pressure are also increasing. Minimal culture medium is the most suitable to follow the processes. Non-predictable interactions are few, the special requirements of the auxotroph stock can be precisely measured. In the case of *A. niger*, no literature data were found about the use of a minimal culture medium of so high concentration, and about its influence on enzyme formation. For this reason, the following trials were started and carried out (Table 2).

Table 2

Test values of concentration of the components of culture media

Base culture medium (g per 1000 cm^3)		
$NaNO_3$ (NH_4Cl)	3.0 (1.9)	salts of Czapek medium
KH_2PO_4	1.0	
$MgSO_4 . 7H_2O$	0.5	
KCl	0.5	
$FeSO_4 . 7H_2OO$	0.028	
$CoSO_4 . 7H_2O$	0.022	micro-element supplementation
$MnCl_2 . 4H_2O$	0.013	
$ZnSO_4 . 7H_2O$	0.018	
Maltodextrin	150.0	instead of saccharose
$CaCO_3$ per bottle	0.5	
120 h, 28 °C, 300 r.p.m., 100 cm^3/500 cm^3 flask		

Variations

	Nitrogen source:			
Multiple by salt concentrations × 1, 3, 5, 10, 15, 20	NO_3^-		NH_4^+	
Salts of Czapek medium	yes	yes	yes	yes
Micro-element supplementation	no	yes	no	yes

In the culture media, a carbohydrate mixture of known composition, the maltodextrin was used instead of simple sugar as carbohydrate source. Besides, the salts of the Czapek type medium (the minimal culture medium used for Aspergilli) were applied with/without micro-element supplementation. The $NaNO_3$, namely the NH_4Cl salt of the minimal culture medium was used as nitrogen source.

After a shaken cultivation of 120 h, the glucoamylase activity, out of the accompanying enzymes the alpha-amylase activity, the final pH and the quantity of mycelium were determined from the fermentation liquid.

The glucoamylase (GA) activity was given in Miles units: 1 cm^3 fermentation liquid frees 1 g glucose during 1 h at pH 4.2, at 60 °C from a starch solution of 4%. The freed glucose has been determined enzymatically (GOD, POD, O-Dianizidin reagent), with the inhibition of other disturbing alpha-glucosidases (ANON, 1973) with TRIS-buffer (GOD – glucose oxidase, POD – peroxidase).

The determination of alpha-amylase (AA) has been carried out with Phadebas tablets. The tablet contains a water-insoluble starch colour complex. During enzymatical hydrolysis, water-soluble coloured fragments are generated. The values of absorbance measured by colorimetry (620 nm) are proportional to the alpha-amylase activity that is considered to be a unit, when it hydrolyses 1 μmol glucoside binding pro minute at 60 °C temperature, at pH 7.0 (CESKA et al., 1969).

2. Results

Our targets were directed at three points:

The effect of the increased salt concentration (with a high carbohydrate concentration) on the growth and the enzyme production, for the use of NO_3^- or NH_4^+ as nitrogen source.

Study of the length of time of a fermentation period at the use of different carbohydrate sources (glucose, maltodextrin, starch).

The proportion of glucoamylase isoenzymes formed, and comparison of a complex and two minimal culture media during one period.

2.1. Effect of salt concentration

The effect of the growing salt concentration with the use of NO_3 as nitrogen source is shown in Table 3. Percentage at the enzyme activities means that the gained results were compared always to the activity of a complex, corn-based filtrate obtained after a fermentation of 120 h, with a substrate concentration of 15%.

Table 3

Effect of increasing salt concentration in case of nitrate as nitrogen source

M[1]	GA (%)	AA (%)	pH	Mycelium mass (g)
1	3.3	0.6	2.64	1.63
3	14.3	0.3	2.77	2.10
5	32.4	0.2	3.30	3.07
10	60.3	39.8	5.62	2.77
15	78.1	38.6	5.70	2.40
20	86.9	45.1	6.43	2.10
1 *m*	25.8	1.1	3.85	2.31
3 *m*	47.4	37.7	4.36	3.05
5 *m*	76.0	35.5	5.95	3.42
10 *m*	94.0	46.2	5.62	3.37
15 *m*	86.8	45.1	5.29	2.99
20 *m*	47.2	86.5	5.25	2.84

M[1]: multiplier factor of salt concentration in the basic culture medium
m: micro-element supplementation
100% GA activity: 6 g l^{-1} h^{-1}
100% AA activity: 8 U l^{-1}

For ammonium as N-source, the results are summarized in Table 4.

It can be stated, that using the salt concentration of the commercial minimal medium, mycelium mass has been produced even a high carbohydrate concentration and the enzyme formation remained at a very low level. From the initial value of 6.2, the pH decreased rapidly, indicating an unbalanced culture medium. As a consequence of this, AA activity was low. A high salt concentration of 15–20-times more would be harmful on the micro-organism. At the use of nitrate as N-source the 10 *m*, and for ammonium as N-source and the 15 *m* were found to be the best culture medium (*m*: micro-element supplement, indicated in Table 2).

Table 4

Effect of increasing salt concentration in case of ammonium as nitrogen source

M[1]	GA (%)	AA (%)	pH	Mycelium mass (g)
1	17.6	8.6	2.41	1.73
3	36.9	2.8	2.41	2.80
5	35.0	2.2	2.53	3.08
10	42.1	0.4	2.67	3.44
15	61.7	1.0	3.29	3.10
20	69.5	3.2	3.22	3.80
1 *m*	17.3	8.9	2.40	1.73
3 *m*	50.5	5.1	2.96	3.24
5 *m*	57.3	2.0	3.23	3.57
10 *m*	74.3	8.9	3.75	3.42
15 *m*	97.7	64.1	4.13	3.65
20 *m*	76.6	19.6	3.96	3.83

M[1]: multiplier factor of the salt concentration of the basic culture medium
m: micro-element supplementation
100% GA activity: 6 g l^{-1} h^{-1}
100% AA activity: 8 U l^{-1}

2.2. Use of various carbohydrate sources

Based on the results, we chose the composition with 20-times salt concentration and supplemented with micro-elements. The mycelium mass, the activities of enzymes GA and AA were measured for one fermentation period. In this trial, the effect of the carbohydrate source was studied, so the maltodextrin was changed to glucose and to starch. The growth is shown in Fig. 1. On starch as carbohydrate source, the fungus could not grow under these conditions, on the glucose the amount of the produced mycelia was slightly smaller than on maltodextrin. The glucoamylase activities measured from the fermentation liquid parallelled to the amount of mycelia formed. The catabolite repression caused by the glucose can be unambiguously observed in Fig. 2. The combination of the NO_3^- nitrogen source with glucose or with maltodextrin, respectively, resulted in a higher GA activity (Fig. 2).

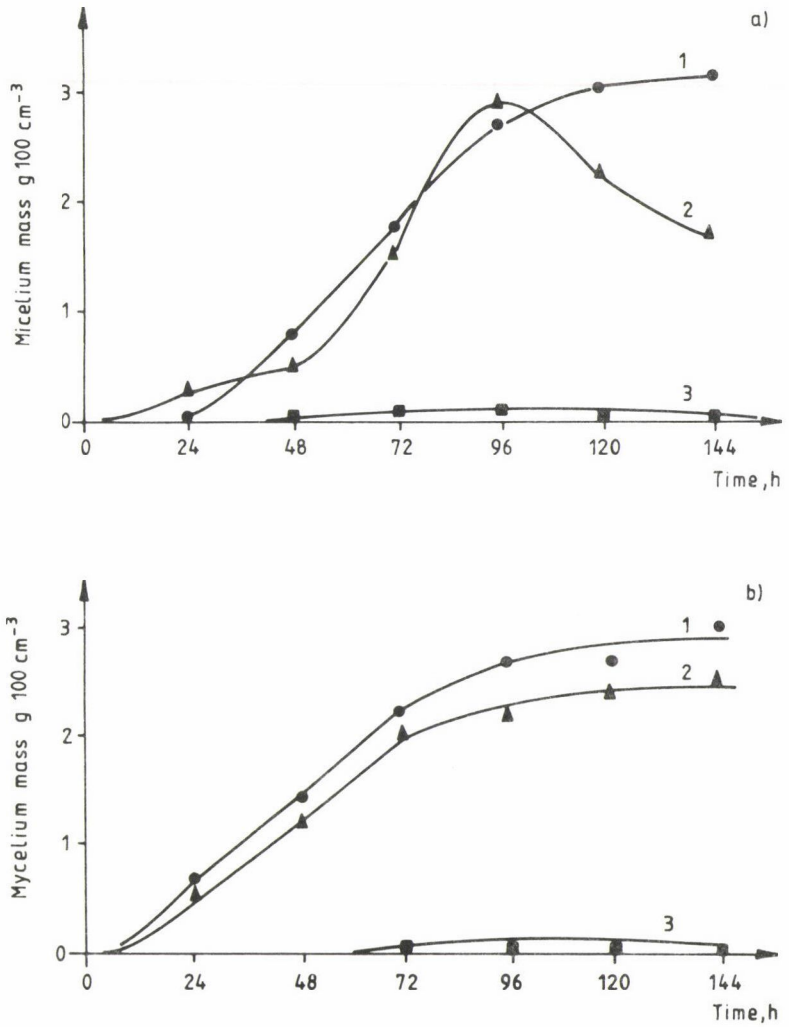

Fig. 1. Effect of various carbohydrate and nitrogen sources on the amount of mycelium. (a) NO_2^- nitrogen source; (b) NH_4^+ nitrogen source. Carbohydrate sources: 1: maltodextrin, 2: glucose, 3: starch

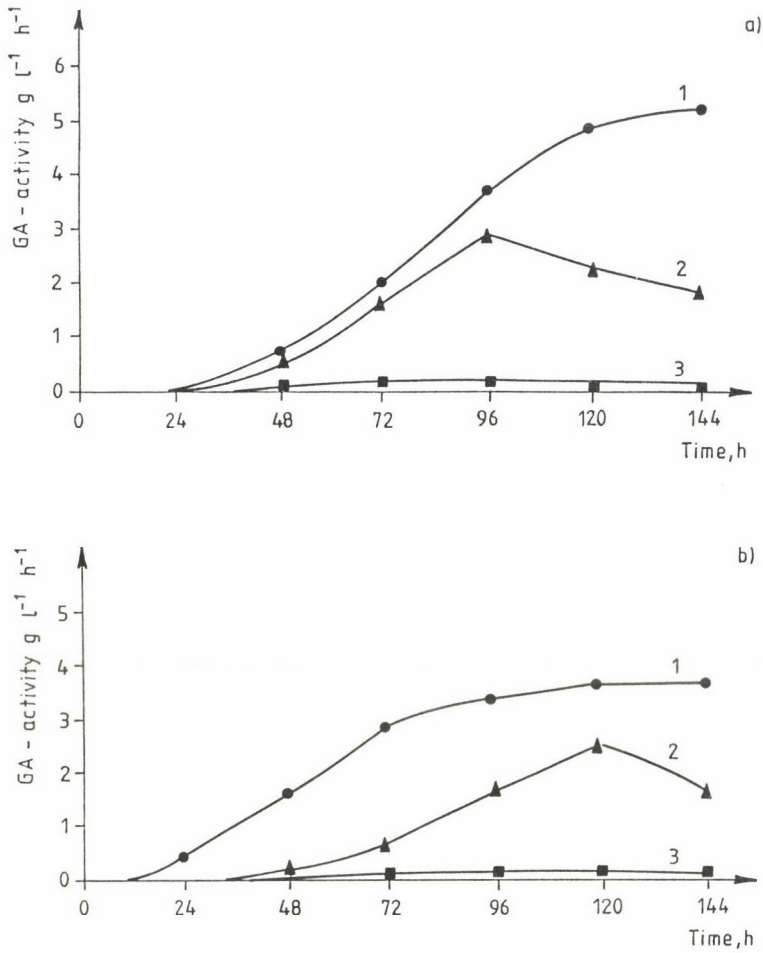

Fig. 2. Effect of various carbohydrate and nitrogen sources on the glucoamylase formation. a) NO_2^- nitrogen source; b) NH_4^+ nitrogen source. Carbohydrate sources: 1: maltodextrin, 2: glucose, 3: starch

The alpha-amylase activity values on Fig. 3 show, that in the contrary to GA, the AA was not repressed by the glucose, this is specially striking for NO_3^- as nitrogen source. At the effect of starch, the AA has been induced. This induction is of a higher degree in case of ammonium N-source than in case of nitrate N-source.

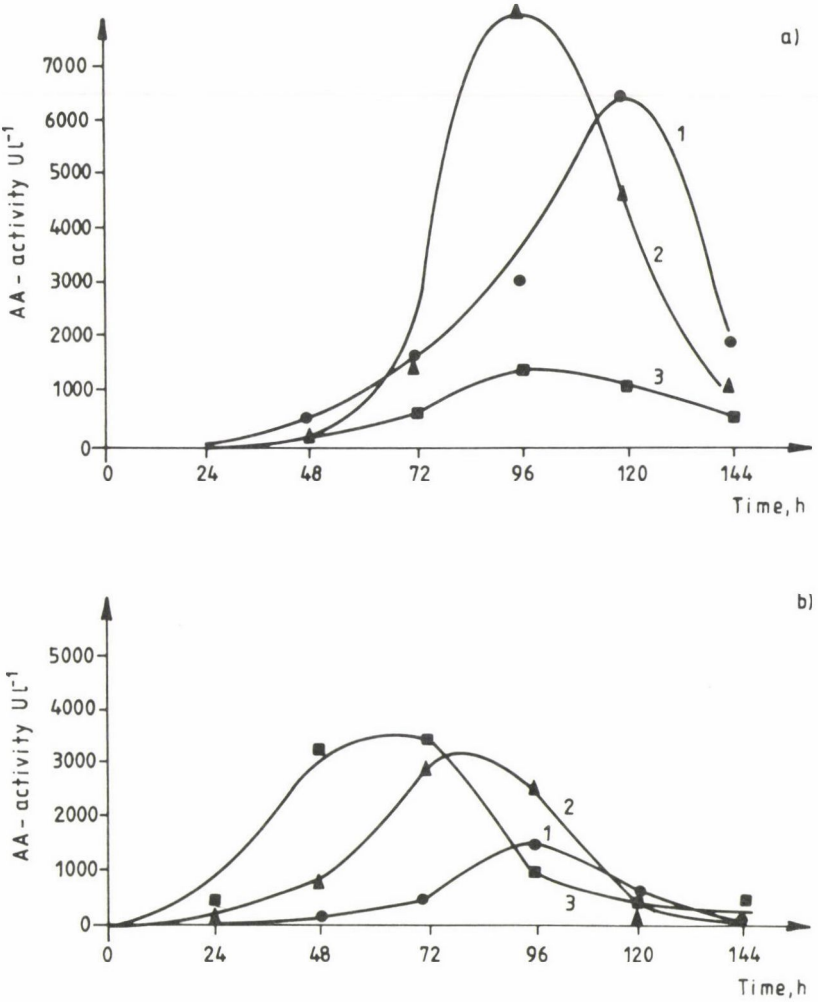

Fig. 3. Effect of various carbohydrate and nitrogen sources and the alpha-amylase formation. a) NO_3^- nitrogen source; b) NH_4^+ nitrogen source. Carbohydrate sources: 1: maltodextrin, 2: glucose, 3: starch

2.3. Investigation of the isoenzymes of glucoamylase

GA is secreted by *A. niger* in the form of two isoenzymes (GA I and GA II). The study of the effect on enzyme formation of the synthetic culture medium on corn-base is completed when the proportions of the two isoenzymes are followed during fermentation. The enzyme activities given in the Tables mean the total activity. The proportion of GA I and GA II can be determined after a separation

process only. The isoenzymes were thus separated with the help of polyacrylamid gel electroporesis (PAGE) method and the proportion was analysed by a BIOMED laser-densitormeter (B. Fullerton, California, USA).

Table 5

Glucoamylase activities measured on complex culture medium on corn base

Samples	Culture period (h)	GA activity ($g\ h^{-1}\ l^{-1}$)	GA I : GA II ratio	pH
1	48	2.61	88 : 12	4.48
2	72	4.04	90 : 10	4.30
3	96	5.25	91 : 9	4.24
4	120	6.62	92 : 8	4.12
5	144	8.06	100 : 0	4.05

Table 6

Glucoamylase activities measured on minimal culture medium with nitrate as nitrogen source

Samples	Culture period (h)	GA activity ($g\ h^{-1}\ l^{-1}$)	GA I : GA II ratio	pH
1	48	1.71	100 : 0	5.70
2	72	2.76	87 : 13	5.37
3	96	4.43	90 : 10	5.02
4	120	4.97	89 : 11	4.92
5	144	5.11	89 : 11	4.83

Table 7

Glucoamylase activities measured on minimal culture medium with ammonium as nitrogen source

Samples	Culture period (h)	GA activity ($g\ h^{-1}\ l^{-1}$)	GA I : GA II ratio	pH
1	48	1.62	72 : 28	4.48
2	72	2.88	82 : 18	4.12
3	96	3.31	100 : 0	3.88
4	120	3.64	100 : 0	3.55
5	144	3.64	100 : 0	4.29

It could be stated, that up to 120 h of cultivation, the proportion of GA I : GA II can be considered as similar, within the standard deviation, on minimal culture medium and on corn-based medium with nitrate N-source. On the medium containing ammonium as N-source, in a running of 96 h, the GA II disappeared (Tables 5–7).

3. Conclusions

The results of the trials were summarized in Table 8.

Table 8

Statements concluded on the basis of the studies concerning the industrial and laboratory characteristics of glucoamylase fermentation

	Industrial size	Minimal medium
Carbon source	complex, corn starch, hydrolysate	complex, water-soluble
Nitrogen source	corn steep liquor	nitrat, ammonium
Substrate quality	changes often	known
Substrate concentration (%)	10–15	15
Water activity	low value	low value
Attainable enzyme activity (U cm^{-3})	10–30	5–8
Separability of mycelium	non-separable at corn groats	separable
Growth	filamentous	filamentous
Measurement of medium	indirectly	directly

It can be stated, that the elaboration of the composition of a synthetic culture medium, where the glucoamylase formation of *A. niger* comparable to the results obtained on corn-based culture medium was successful. The GA activity could be increased from a value of 0.3 g cm^{-3} h^{-1} – 5 g cm^{-3} h^{-1}. This could be well used as a model for the optimization of industrial enzyme fermentation.

*

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STORAGE OF CANNED SHELLED BRAZIL NUTS (*BERTHOLLETIA EXCELSA*): EFFECTS ON THE QUALITY

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Shelled Brazil nuts were canned under three different atmospheric conditions and retail packed in polypropylene trays covered with PVC and stored for 12 months at normal ambient temperature. Monthly chemical analyses, comprising acid and peroxide values, iodine number and ultraviolet absorption measurements were performed on the nut oil. Brazil nuts stored in air presented increasing acid values up to 2 mg KOH/g and peroxide values that in the third month reached and kept values up to 19 meq O_2/1000 g till the end of the experiment. The presence of oxygen absorbers in the cans prevented oxidative deterioration, and kept the peroxide values lower than the nitrogen atmosphere (11 and 14 meq O_2/1000 g, respectively). Spectrophotometry at 232 and 270 nm ultraviolet range confirmed other analytical results. Considering the nut oil quality and its susceptibility to rancidity, storing shelled nuts in cans with oxygen absorber was the best way of preserving Brazil nuts. Nuts on the trays were considered inadequate for consumption after two months of storage.

Keywords: Brazil nuts, preservation, storage, canning, quality keeping, rancidity

Brazil nut trees (*Bertholletia excelsa*) are native plants of the Amazon region, located in the North of Brazil. Domestic consumption of the nuts is greatly reduced and most of the production is exported either shelled or in shell. Because of its richness in oil and protein, native people may live on nuts while in the forest collecting these fruits. Abroad it is known as a delicatessen. Nowadays Brazil nut trees are being planted in a rational way which in the near future will result in higher production. Due to the hot climate of this country and the nut's unique property prone to rancidity, Brazil nuts are offerred or found in the market only for Christmas seasons, and it is estimated that only 1% of the production is consumed in the country.

This work is part of the first author's Master's degree dissertation.

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As known, oxidative deterioration leads to rejection of the food. Previous work by JAN and co-workers (1988) verified that cans were the most efficient means for storing shelled whole nuts. Brazil nut oil presented oxidative stability coming somewhere between rubber seed oil and passion fruit seed oil, typical Amazonian plants. This low stability was associated with its high unsaturated fatty acid content (41.2% oleic and 36.1% linoleic), though iodine number was low (95.4) (ASSUNÇÃO et al., 1984). High unsaturated fatty acid levels and also the lack of the natural pelicule that separates and protects the kernel from the shell were also indicated to be responsible for the low stability of walnuts to storage (TAPPEL et al., 1957). Reduction of oxygen concentration inside the cans is a procedure that has already proved to be effective in prolonging shelf life of canned nuts (GUADAGNI et al., 1978; HOLADAY et al., 1979; KAYS & DULL, 1988). Use of open plastic trays for storing pecans, macadamias and almonds has guaranteed their quality for a maximum two-month storage (FOURIE & BASSON, 1989). An alternative way to reduce oxygen concentration inside food packages is the use of oxygen absorbers in the form of packets of powdered active iron oxide (LEGAN & VOYSEY, 1991; SMITH et al., 1986).

1. Materials and methods

Broken and shelled Brazil nuts, exportation type, were bought in 20 kg aluminum multifoliated bags under vacuum in the Amazon state, Brazil. 250 g of nuts were canned in 200 cm^3 cans FL 2.8/2.8, 73 cm in diameter and 94 cm in height, covered internally with epoxyphenolic enamel, produced by Rheem Empr. Ind. e Com. S/A, and were retail packed in $150 \times 280 \times 3$ mm white polypropylene trays, covered with a colorless polyvinylchloride (PVC) film for domestic use [16 μ thick, 320 g H_2O/m^2/day of vapor permeability (38 °C, 90% RH) and O_2 permeability between 4650 and 9300 cm^3 O_2/m^2 atmosphere]. The inner atmosphere of cans were as follows: atmospheric air, atmospheric air plus oxygen absorber ("Ageless" from Mitsubishi Gas Chem. Co.) and with nitrogen gas. These procedures resulted in 21% oxygen inside the first two treatments cans and 6% oxygen in the third at the beginning of the experiment, measured by gas chromatography (unpublished results).

The experiment lasted for twelve months. The cans and trays were stored in a room with natural ventilation and light, however no control could be exerted on these conditions. Therefore temperature and hygrometric degrees were registered three times a day and an average was obtained monthly. Minimum and maximum values of temperature were 17 °C and 31 °C and those of relative humidity were 65% and 78.5% within the twelve months. Every thirty days four cans were taken at random from each treatment and the nuts from each can were hydraulically pressed at ambient temperature for oil production and further chemical analyses. Moisture content of the nuts was determined monthly according to the analytical procedures of

the Instituto Adolfo Lutz (IAL, 1985). Acid (NGD C-10) and peroxide (NGD C-35) values, iodine number (NGD C-32) and spectrophotometric absorption (E 1% 1 cm) in the ultraviolet range (NGD C-40) were determined in triplicate according to the Italian Norme Grassi e Derivati (NGD) of the Stazione Sperimentale per le Industrie degli Oli e dei Grassi (1976). All the data were subjected to statistical analyses including the analysis of variance (ANOVA) with application of F test, and Tukey test on the chemical data.

2. Results and discussion

The data reveal that nuts stored in cans kept for much longer and with better quality than in trays, no matter which treatment they have been subjected to. This was obvious after two months of storage when nuts on the trays were oxidized. After four months fungal growth was observed on the surface of these nuts and they were not suitable for human consumption. Because of this, statistical analyses have been conducted on the data from the cans, only. Data presented in the form of graphs (Figs 1-3) had the purpose to facilitate visualization of nuts behavior. It should be understood that statistical analyses were also performed on these data and no differences at 1% significance level were detected. In Tables 1-3 the results obtained from the application of the Tukey test are presented, the comparisons among the mean values are considered only for the variables where the F test applied to the ANOVA was significant for each period of storage. The letters A, B and C were used to shorten the information about the results obtained from the comparison among the means, after the application of the Tukey test, being A > B > C, numerically. When results are followed by the same letter, they can be interpreted as means that do not differ statistically.

Figure 1 shows moisture behavior during storage of nuts either in cans or on trays. Initial moisture content was 2.8%. Average moisture content of the canned kernels after twelve months was 2.95%, consistent with a water activity of 0.54 according to BEUCHAT (1978). After six months of storage the highest moisture content in cans was 3.44% under nitrogen, while in the nuts from the trays it had reached 4.84%. YOKOYA and co-workers (1970) reported the equilibrium moisture in Brazil nuts kernels to be 4.3% at 70.4% relative humidity after two months. A visible white fungal growth was observed on kernel surface after six months at 80.5% RH (moisture content ca. 5.5%). According to BEUCHAT (1978), when Brazil nuts have 3.75% moisture content (average value in the first three months of storage on our trays), its water activity is 0.65 at 21 °C and the oil content is 68%. However, this was not equilibrium value for our nuts and so, water content slowly increased and fungi appeared. Agreeing with YOKOYA and co-workers (1970) its acid value also increased at a faster rate than for the canned nuts (Fig. 2) probably due to the high

moisture level. As expected, the nuts under atmospheric air showed the highest acid value of 2.13 mg KOH g^{-1} oil with an average 2.03. A similar behavior both for acid values and K270 may reflect the effect of free fatty acids on the oil specific extinction values at 270 nm, as pointed out by CATALANO and DE FELICE (1970). In fact, coefficients of correlation between acid value and K270 were over 0.9, except for data of the nuts kept under nitrogen. Peroxide value was a better indicator of quality evaluation (Table 1). Oils from nuts kept under atmospheric air showed higher peroxide values than those with nitrogen or oxygen absorber. None of them reached 20 meq peroxides per 1000 g oil which is considered as the maximum acceptable value in foods for consumption in Brazil (ABIA, 1991), although after seven months the nuts under atmospheric air showed 19.26. High peroxide values for nuts were also found in different kinds of packaging as reported by JAN and co-workers (1988), HOLADAY and co-workers (1979), KAYS and DULL (1988), and SATTAR and co-workers (1991).

Table 1

Peroxide values (meq O_2 per 1000 g) *of canned shelled Brazil nuts oil*

Months of storage	Can internal atmosphere			
	Air	Air+O_2 absorber	Nitrogen	F test
Initial		11.39		
01	14.04 A	9.22 B	13.11 A	13.95**
02	16.44 A	9.11 C	12.83 B	28.62**
03	18.48 A	9.06 B	11.80 B	49.49**
04	18.71 A	9.81 C	13.95 B	42.29**
05	18.69 A	8.59 B	11.17 B	58.62**
06	16.37 A	9.49 C	12.88 B	25.23**
07	19.26 A	9.48 C	12.55 B	53.23**
08	18.58 A	10.30 B	12.14 B	40.19**
09	17.54 A	8.98 C	12.49 B	39.48**
10	17.41 A	9.14 B	10.57 B	41.62**
11	18.48 A	10.43 B	11.37 B	39.96**
12	17.09 A	9.98 B	12.19 B	28.22**
Mean values	17.59 A	9.47 C	12.26 B	
Coefficient of variance		10.46%		

Mean values followed by distinct capital letters in the same row differ at 1% significance level according to the Tukey test

** significant at 1% significance level

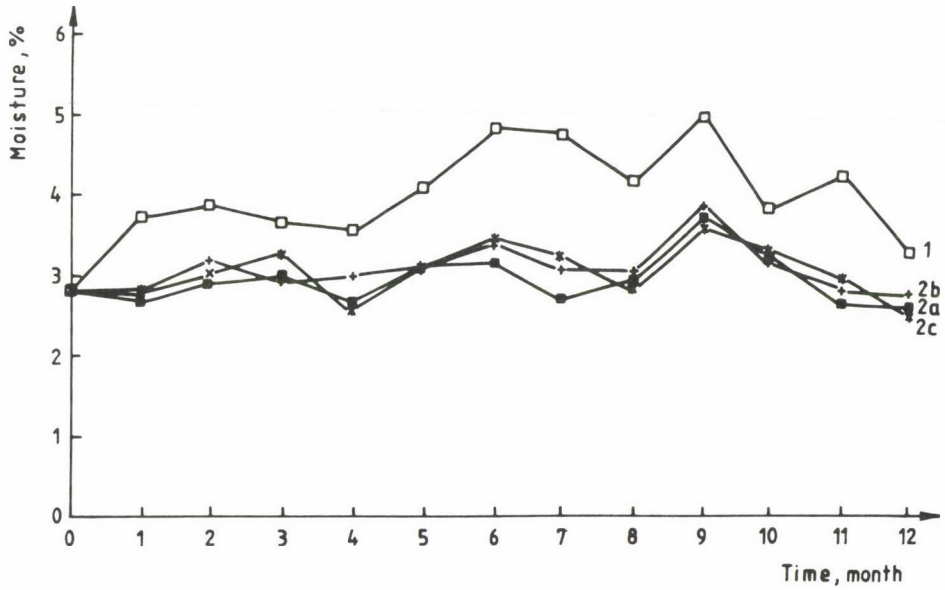

Fig. 1. Moisture content of shelled Brazil nuts. 1: Tray; (nuts in cans): 2a: air; 2b: air + O_2 absorber; 2c: nitrogen

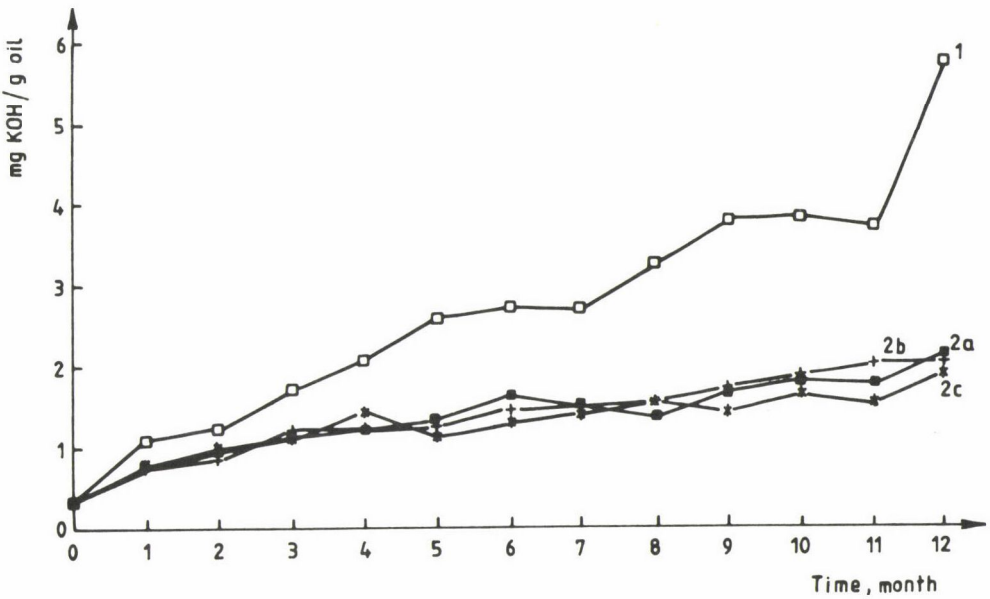

Fig. 2. Acid values of shelled Brazil nuts oil. 1: Tray; (nuts in cans): 2a: air; 2b: air + O_2 absorber; 2c: nitrogen

Alterations in iodine numbers (Fig. 3) were not large enough to reflect differences in oxidative changes of the oil as observed by FOURIE and BASSON (1989). Tables 2 and 3 show K232 and K270 values for oils from all the canned nuts: the antioxidative effect of the oxygen absorber, that was evident in Table 1, was demonstrated again. Peroxide values are related to K232 values. When peroxide is nil, no alterations are registered in UV absorption (or specific extinction at 232 nm), due to its catalytic action on the conjugation of the double links (GAMBA & MAZZINI, 1982). Tables 1 and 2 indicate a higher rate of breakage of initial peroxides and decrease in concentration of conjugated double bonds (K232) to generate smaller compounds (K270) than that formation of new peroxides in the oxygen absorber treatment. Figure 4 shows the data for the trays which were not submitted to statistical analyses and the constant formation of smaller and secondary compounds (K270) from hydroperoxides is evident and agrees with observations made by ROBERTSON and co-workers (1973). In general the oxygen absorber was an efficient way for keeping the quality of Brazil nuts.

Table 2

K232 values of canned shelled Brazil nuts oils

Months of storage	Can internal atmosphere			
	Air	Air + O_2 absorber	Nitrogen	F test
Initial		2.230		
01	2.156 B	2.232 B	2.614 A	9.26**
02	2.690 A	2.176 B	2.339 B	10.63**
03	1.930 A	1.803 A	1.819 A	0.71
04	2.741 A	2.143 B	2.438 AB	13.76**
05	1.927 B	2.038 AB	2.376 A	8.42**
06	2.694 A	2.304 B	2.601 AB	6.27**
07	1.755 A	1.560 A	1.602 A	1.63
08	2.760 A	2.041 B	2.357 B	19.93**
09	2.105 B	1.910 B	2.471 A	12.43**
10	2.750 A	2.122 B	2.356 B	15.43**
11	2.830 A	2.168 B	2.307 B	18.37**
12	2.590 A	2.087 B	2.363 AB	9.71**
Mean values	2.411 A	2.049 B	2.311 A	
Coefficient of variance		7.15%		

Mean values followed by distinct capital letters in the same row differ at 1% significance level according to the Tukey test

** significant at 1% significance level

MAGYAR
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KÖNYVTÁRA

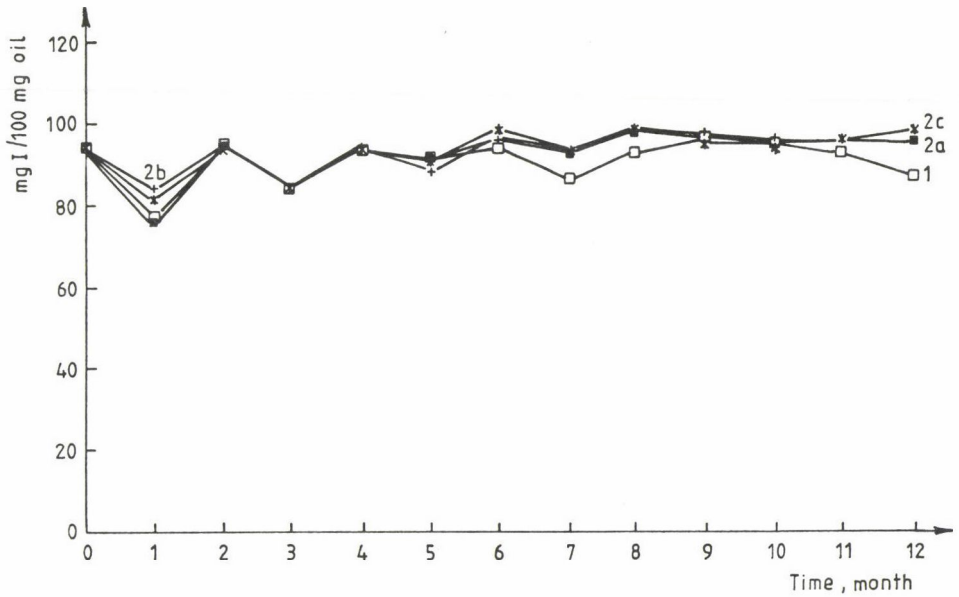

Fig. 3. Iodine values of shelled Brazil nuts oil. 1: Tray; (nuts in cans): 2a: air; 2b: air + O_2 absorber; 2c: nitrogen

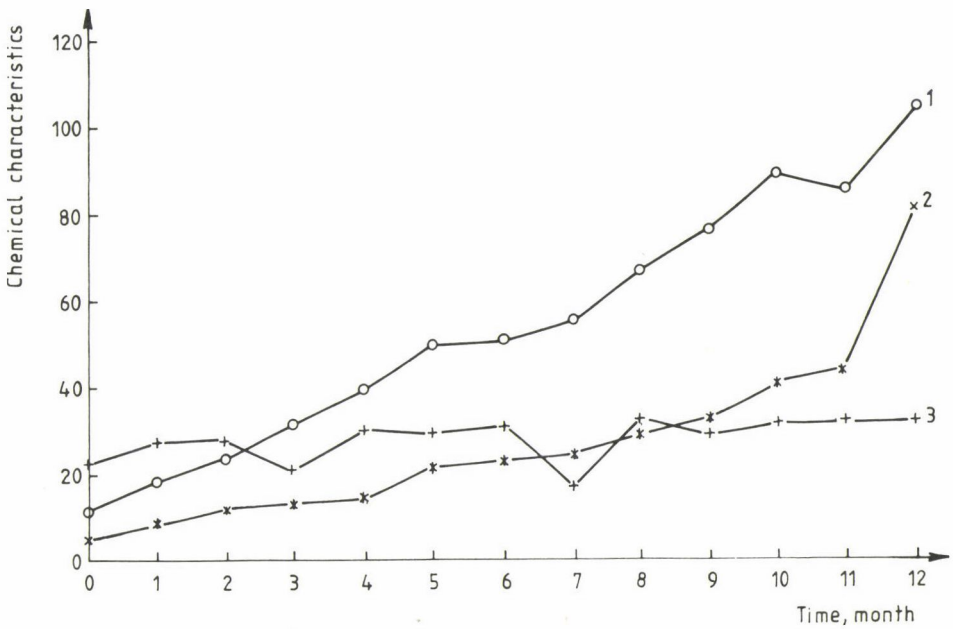

Fig. 4. Shelled Brazil nuts oil from the trays. 1: Peroxide value; 2: K232 × 10; 3: K270 × 100

Table 3

K270 values of canned shelled Brazil nuts oils

Months of storage	Can internal atmosphere			
	Air	Air+O_2 absorber	Nitrogen	F test
Initial		0.047		
01	0.070 A	0.060 A	0.072 A	1.40**
02	0.092 A	0.065 B	0.079 AB	6.74**
03	0.098 A	0.088 A	0.083 A	1.96*
04	0.098 A	0.077 AB	0.060 B	10.75**
05	0.112 A	0.085 B	0.081 B	9.77**
06	0.114 A	0.100 A	0.099 A	2.19**
07	0.129 A	0.098 B	0.101 B	9.87**
08	0.112 A	0.103 AB	0.086 B	6.24**
09	0.128 A	0.113 AB	0.098 B	7.73**
10	0.135 A	0.106 B	0.116 B	7.65**
11	0.144 A	0.123 AB	0.119 B	5.64**
12	0.123 A	0.108 A	0.102 A	4.12**
Mean values	0.113 A	0.094 B	0.092 B	
Coefficient of variance		12.29%		

Mean values followed by distinct capital letters in the same row differ at 1% significance level according to the Tukey test

* significant at 5% significance level

** significant at 1% significance level

*

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EFFECT OF SALT IN PICKLED OLIVES ON THE DIGESTIBILITY OF LIPID IN WISTAR RATS

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The effect of sodium chloride present in pickled olives was studied on the digestibility of dietary fat. Young Wistar rats fed on two different diets were used. One diet contained processed olives, and the other, the same type of processed olives with the salt removed. The results obtained indicate that the digestibility of total lipids is somewhat greater for the desalted olives, and that the digestibility of triglycerides and the oleic acid absorption are somewhat enhanced by the presence of salt. Also, the presence of salt diminished the absorption of the saturated fatty acids and increased that of polyunsaturated fatty acids.

Keywords: olives, sodium chloride, dietary fat, digestibility of lipids, triglycerides, absorption of fatty acids

The metabolizable energy (ME) of the diet is calculated from the energy conversion factors 4; 9; 4 cal g^{-1} referring to proteins, fats, and carbohydrates (FEDERAL REGISTER, 1993). Basically, the heat of combustion of the fat, protein and carbohydrate constituents of various food products is determined and corrected by a coefficient of availability, derived from digestibility experiments, to determine a specific energy value for each energy-containing nutrient (MILES et al., 1988).

The fats play an essential role in human nutrition, although an excessive consumption seriously risks health. Fats are absorbed in the small intestine; the most general concept is that they are broken down first in the duodenum and are then absorbed as small micelles of fatty acids. Following lipid digestion by enzymatic hydrolysis, the bile salts tend to form micelles. These act as transport medium, carrying the monoglycerides and free fatty acids – both are final products of lipid digestion – towards the epithelial cells. Difficulties in the prior hydrolysis phase logically cause deficiencies in fat absorption.

Dietary fat in cardiovascular disease is under study by numerous work groups (DYERBERG & BANG, 1979, 1980; RUIZ-GUTIERREZ & GARCIA, 1990; RUIZ-GUTIERREZ et al., 1990a, 1990b) as it can have important effects, for example, on the

composition of the cardiac lipids, fundamentally the phospholipids. It is known that the polyunsaturated fats rich in linoleic and linolenic essential fatty acids reduce the levels of cholesterol transported by high and low density lipoproteins (HDL, LDL). The monounsaturated fatty acids, such as those of olive oil, diminish the fraction of cholesterol transported by the LDL, but practically do not affect – and even increase – the fraction transported by the HDL (RUIZ-GUTIERREZ & GARCIA, 1990).

A reduction in total blood cholesterol is not necessarily beneficial, such as the reduction in the cholesterol associated with HDL (SHELDON, 1987). It has been demonstrated that a reduction in HDL cholesterol increases the risk of heart disease (MILLER et al., 1977). This paper studies the influence of sodium chloride on the digestibility of lipids from pickled green olives. Young Wistar rats were used as test models.

1. Materials and methods

1.1. Samples

Pickled green olives (Manzanilla var.) were used. After treatment with sodium hydroxide solution (2–5%), they were washed repeatedly with water and placed in brine (NaCl solution, 8%), where they underwent lactic fermentation (FERNÁNDEZ-DÍEZ, 1985). A portion of this sample (A) was washed repeatedly with water to eliminate the NaCl (B).

The olives were de-stoned and the flesh triturated until reaching a paste as homogeneous as possible. A sample of 5 g paste was weighed to 0.1 mg in a tared container, and put into the oven at 60–70 °C until constant weight, with a partial vacuum of about 25 mm.

1.2. Animals

Male Wistar rats (Iffa-Credo, Barcelona, Spain) weighing initially from 157 g to 159 g were kept in an air-conditioned room with a constant temperature (23–25 °C) and relative humidity (40–50%) and a 12 h light-dark cycle. They were housed in individual Letica metabolic cages which separated faeces from urine. After one week for adaptation, rats weighing around 165 g were divided randomly into two groups (eight animals in each group).

1.3. Diets

Two diets were used: dry olives with salt (A) and desalted (B), both supplemented with 10% skimmed milk (Moliko, Nestlé, Switzerland) and vitamin complex (Table 1). The diets were prepared on every third day to prevent deterioration. They were packaged in airtight flasks under N_2 atmosphere, and kept at 4 °C (7 days maximum).

Table 1

Composition of the experimental diet

Basal diet (g per 100 g)	Diet A	Diet B
Fat	59.40	57.29
Proteins	12.85	14.38
Sugars	5.84	5.61
Fibre	11.84	17.70
Vitamins[a]	3.00	3.00
Minerals[b]	2.00	2.00
NaCl	5.00	<0.01

[a] Vitamins (in 1 kg diet): retinyl acetate, 19800 IU; chocalciferol, 6000 IU; thiamine HCl, 20 mg; riboflavine, 15 mg; niacin, 70 mg; pirydoxine HCl, 10 mg; inositol, 150 mg; cyanocobalamin, 50 μg; ascorbic acid 170 mg; dl-α-tocopherol acetate, 40 mg; phylloquinone, 40 mg; Ca-panthotenate, 100 mg; choline-ClH, 1.36 g; folic acid, 5 mg; p-aminobenzoic acid, 50 mg; biotin, 0.3 mg.

[b] Minerals (in 1 kg diet): P, 7.75 g ; Ca, 10.0 g; K, 6.0 g; Mg, 1.0 g; Mn, 80 mg; Fe, 0.3 mg; Cu, 12.5 g; Zn, 45 mg; Co, 90 μg; I, 0.49 mg.

1.4. Faeces

The faeces obtained during the period of ingestion in the Letica metabolic cages were placed in flat-bottomed capsules and dried in a vacuum oven at 50 °C to prevent deterioration. All the animals, including the control group, were kept in metabolic cages for periods of five days, after two days of adaptation to this metabolic cages. A control group fed with a previously defatted diet was used to determine endogenous lipids.

The amounts ingested and excreted were strictly checked in the 5 days of the main period, when the animals ingested water and the corresponding diet "ad libitum". The faeces for each animal were collected and weighed daily, and kept in the freezer until the end of the experimental period. The fat was then extracted and preserved at −27 °C until analysis.

1.5. Analytical determinations

Gravimetric determinations were made of the fat in the diet ingested, total non-absorbed lipids, unsaponifiable fraction, free acidity, and fatty acid content.

1.5.1. Fat. The pickled samples, de-stoned and dried, were extracted in Soxhlet with hexane (b.p. 60–70 °C) for 5 h. The solvent was separated in a rotary evaporator at 40 °C and the oil dried in the oven at 100 °C.

1.5.2. Faeces lipids. The dry, pulverized faeces were extracted in Soxhlet with ethyl ether for 7 h. The solvent was eliminated in rotary evaporator (Butchi, mod. RE 111) under N_2 current until constant weight.

As part of the faeces lipids were possibly not extracted under these conditions, a second extraction was made after cold acid hydrolysis with HCl (TOULLEC et al., 1968; MÁRQUEZ et al., 1991). The two fractions were combined and representative aliquots were taken of the total non-absorbed lipids.

1.5.3. Unsaponifiable fraction. This was performed according to NORMA UNE (1973a). Separation by thin layer chromatography was carried out according to NORMA UNE (1973c). The unsaponifiable fraction of 10 g of fat, dissolved in isopropyl ether, was deposited in a band of thin layer, and developed with chloroform:methanol 70:30. The sterol bands were localized using a cholesterol standard (Rf = 0.51).

The components of the different fractions were analyzed using a Hewlett-Packard 5819 Series II gas chromatograph fitted with a flame ionisation detector, and HP-5 capillary column of 25.0 m. Nitrogen was used as carrier gas, the injector temperature was 280 °C, and the column isotherm temperature were maintained at 265 °C.

1.5.4. Fatty acids and methyl esters. These were obtained from the ethanol solutions of soaps and washing waters remaining after extraction of the unsaponifiable fraction, following the method of MÁRQUEZ et al. (1991).

1.5.5. Free acidity. This was determined according to NORMA UNE (1973b). The degree of acidity was expressed as percentage of oleic acid.

2. Results and discussion

The composition of the major fatty acids present in the pickled fruit was very similar in the two samples A and B (Table 2).

Table 2

Fatty acid composition in olive fruits lipids (%)[a]

Fatty acid	Olive with salt	Olive desalted
C12	0.24	0.32
C14	0.31	0.32
C16	12.45	11.89
C18:0	2.87	2.86
C18:1 (n−9)	71.81	72.89
C18:1 (n−7)	1.60	1.61
C18:2	6.96	6.92
C20:0	0.46	0.47
C24:0	0.31	0.28

[a] Duplicate values for methyl esters in standard mixtures by GLC analysis varied within 0.1%.

The characteristics of the non-absorbed lipids of both diets are shown in Table 3. Non-reabsorbed lipids and derivatives from the faeces of rats fed with the fat-free diet were determined. The non-absorbed lipids of the fractions from direct extraction and after acid hydrolysis, from the two diets representing extreme alteration conditions, were also determined. The total non-absorbed neutral lipids per 100 g of fat ingested were higher in faeces from B.

Table 3

General characteristic of the non-reabsorbed lipids (%)

Non-reabsorbed lipids	Total lipids	Unsaponified fraction (%)	Normal fatty acids	Acidity (oleic acid)
Fat-free diet	. –	26.2 ± 0.20	55.1 ± 0.01	. –
Diet A	17.2 ± 0.08	22.2 ± 0.15	47.8 ± 0.08	36.2 ± 0.01
Diet B	25.9 ± 0.65	25.4 ± 0.09	48.3 ± 0.05	37.8 ± 0.02

Diet A, with salt. Diet B, desalted.

The total non-absorbed lipids, and the corresponding values of apparent digestibility, are shown in Table 4. There was no significant difference in digestibility

between the diets. This is surprising as it might be expected that with an excessive fat ingestion, absorption would diminish because of saturation of the intestinal mucosa. Thus, the results may have been influenced by parameters or variables not controlled in the experiment, such as the impossibility of effective control of endogenous fat in obtaining net values of digestibility. Since the fat was corrected using a group of rats under identical experimental conditions, but with a fat-free diet, the correction depends on the time taken by the animals in consuming 100 g of fat (referred to the values of digestibility). That is, the lower the percentage of fat in the diet, the higher the content of endogenous fat in the non-absorbed lipids. Consequently the values of net digestibility should tend to equalise. Thus, the determination of endogenous fat is essential to establish the significance of the results.

Table 4

*Total lipids: Excreted (*g per 100 g *of diet ingested), and apparent digestibility*

Diet	Non-absorbed lipids	Digestibility
A	50.30 ± 2.32	59.60 ± 3.05
B	49.70 ± 1.13	61.75 ± 3.38

Diet A, with salt. Diet B, desalted.
Average values and ± s for eight animals in each group.

Table 5 shows the amounts of normal triglycerides ingested and excreted (per 100 g of fat), and the corresponding values of apparent digestibility. The determination was carried out by quantification with an internal standard. The digestibilities obtained were very much higher than those of the total lipids (Table 4), although the general conclusions are similar. Therefore, to arrive at definite conclusions, the same corrections suggested by later assays can be applied.

Table 5

*Normal triglycerides: ingested and excreted (*g per 100 g *fat ingested), and apparent digestibility*

Normal triglycerides	Diet A	Diet B
Ingested	94.7 ± 0.08	95.9 ± 0.17
Excreted	7.7 ± 1.09	9.5 ± 1.06
Digestibility	91.7 ± 1.39	89.2 ± 2.29

Diet A, with salt. Diet B, desalted.
The average values are the mean and ± s for eight animals in each group.

The mean amounts of unsaponifiable fraction and normal fatty acids ingested and excreted (per 100 g of fat) are given in Table 6. The amount of unsaponified fraction excreted was very much higher than that ingested. This demonstrates the importance of endogenous unsaponifiable matter. Also, it can be seen that in both diets, digestibility of fatty acids was very much higher than the values obtained for the total non-absorbed lipids (Table 4).

Table 6

Unsaponified fraction, and normal fatty acids: ingested and excreted (g per 100 g *fat ingested), and apparent digestibility*

Diet	Ingested	Excreted	Digestibility
		Unsaponified	
A	2.7±0.08	5.4±0.12	−37.4
B	2.9±0.04	7.3±0.09	−45.8
		Fatty Acids	
A	96.0±0.91	8.4±0.08	91.0
B	93.4±0.87	9.2±0.05	89.2

Diet A, with salt. Diet B, desalted.
The average values are the mean and ± s for eight animals in each group.

Although the research carried out up to now is sufficiently indicative of the relationship between endogenous lipids and composition of the diet, this study has gone deeper into the subject. Thus, two interesting compounds, cholesterol and choprostanol – the faecal neutral steroids of greatest physiological interest – have been quantified. Their absence in the diet guarantees that their later presence is related exclusively to the endogenous lipids. They were quantified from two samples of non-absorbed lipids in each group of animals fed with defatted, salted (A), and desalted (B) diets. The results obtained for those of endogenous origin and those present in the dietary fat (Table 6) show the composition of the fraction to be very simple. Separation and identification by LGC-MS demonstrated the major endogenous components to be cholesterol and choprostanol. In the non-absorbed lipids of the diets A and B, β-sitosterol also appeared. This is found in the sterols of olive oil in a higher than 90% proportion. Given the high percentage of endogenous sterols, the presence of the other dietary sterols was not noticeable. These compounds were identified from their chromatographic retention times and the similarity of their spectra with those of the respective standards.

Table 7

Quantitative determination of sterols (mg per 100 g *fat ingested)*

Sterols	Lipid free	Diet A	Diet B
Total	203.3 ± 1.4	435.5 ± 2.2	372.5 ± 1.8
Diet	-	53.7 ± 0.3	57.4 ± 0.6
Endogen	134.2 ± 0.8	183.2 ± 0.8	194.4 ± 1.0

Diet A, with salt. Diet B, desalted.
Average value and ± s for eight animals in each group.

2.1. Absorption of the fatty acids

The individual absorptions of the major fatty acids of the dietary fat in this study are shown in Fig. 1. The homogeneity of the results demonstrates that the presence of salt diminished the absorption of palmitic and stearic acids, and increased that of oleic and linoleic. That is, it aids absorption of the fatty acids in function of their unsaturation. The more unsaturated is the fatty acid (18:2 (n−6)), the greater is its absorption.

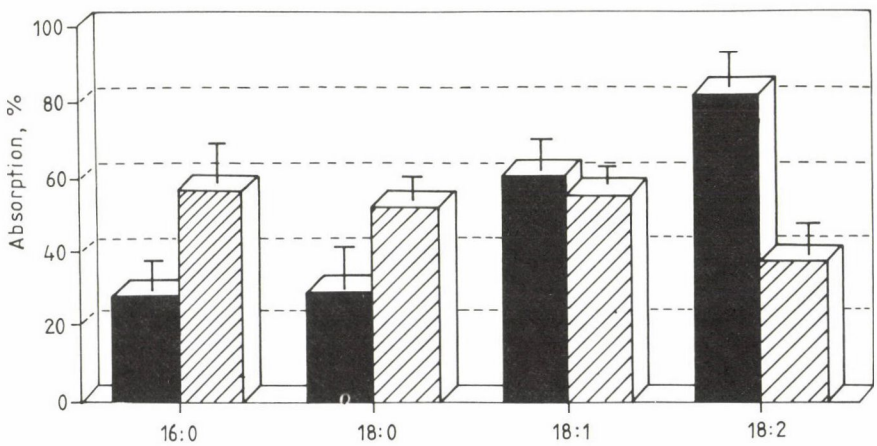

Fig. 1. Absorptions of the major fatty acids of the dietary fat, ■: with salt; ▨: desalted

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EFFECT OF HERBICIDES ON FLOUR QUALITY OF TWO WINTER WHEAT VARIETIES

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Two winter wheat varieties were treated with 11 different herbicides (Belgran, Aniten D, Banvell M, Lorgan, Assert, Puma, Illoxan, IP-Flo, Starane, Dikamin D and Dikotex 40) in field trials. The baking properties of their grain yields (wet and dry gluten content, gluten spreading, valorigraphic value and water absorption, falling number (FN), SDS sedimentation volume) have been determined.

Treatments with double doses of herbicides modified the dry gluten content, gluten spreading, water absorption, FN and the SDS sedimentation volume, respectively.

Considering the properties except for dry gluten, the interaction between varieties and herbicides seemed to be reliable, namely the quality of varieties was changed by the herbicide treatment. This variety specific effect of herbicides was different for every quality parameter.

Out of the 11 herbicides Logran and Assert were found to be the most favourable ones because the baking parameters have not been significantly damaged by them, moreover they were in some cases favourably influenced.

Keywords: flour quality, gluten, herbicide, winter wheat

Simultaneously with studies on the weed killer effect of different herbicides, experts often deal with their side-effects, first of all leaf burning, plant deformation and influence on yield of cultivated plants, too.

In spite of the fact that herbicide influence on the wheat quality has been known for a long time, this phenomenon has rarely been investigated.

Experiments of FAJERSSON (1958) in Sweden indicate that an overdose of 2,4-D reduces the yield of wheat by 8–20%, the test weight by 2–3.6 kg but the protein content is increased by 1.1–2.3%. In Hungary experiments carried out at the end of the 60's (MAJER-KISS, 1971) have shown that the protein content as well as the wet and dry gluten content increased in wheat grain yield when plants were treated with Dikonirt.

POLLHAMER (1973) investigated the effect of herbicides used at that time on the quality of Fertődi 293 and Bezosztaja 1 wheat varieties. These experiments

demonstrate that Dikonirt and Hedonal MCPP, depending on the year, increase the protein and gluten content but the gluten spreading, the Zeleny value farinographic value, water absorption, loaf volume and height-width ratio did not change significantly. Reduction in quality by Dikopur, Banvel D and Banvel M was observed, since the wet gluten content, the farinographic value number and the loaf volume decreased, while the gluten spreading, the ash content of flour and the height-width ratio of loaf increased.

According to ERDEI and SZÁNIEL (1975) the effects of several herbicides (Aretit, Pyramin, Diuron, etc.) on the quality are within the error limit of the experimental methods.

In another series of experiments POLLHAMER (1980) found that the wet gluten content in Mv-4 and Mv-5 varieties were decreased by Sys 67 and Tribunil Combi herbicides but an increase was observed when Arelon was used. In both wheat varieties the farinographic values was 100 when not treated with herbicides. This value was reduced by all herbicides tested and the largest damage was observed with HOE 23408, Sys 67, and Tribunil Combi herbicides. The same herbicides reduced the loaf volume and the flour water absorption but enhanced the gluten spreading. Treatment with Aretit, as well as Arelon influenced favourably the loaf volume.

Since 1977–79 PÉTER and co-workers (1985) investigated the effect of the double dose of Dikonirt, Dikotex 40, Gabonil and Aniten D on the quality change of GK Tiszatáj and GK Szeged winter wheat varieties. In both cases, all the four herbicides increased slightly the protein content, dry gluten content and the water absorption. The farinographic value and the loaf properties varied. Using double dose Aniten D, the farinograph value and the loaf height-width ratio increased significantly. Aniten D caused the largest loaf volume, too. Other herbicides exert no significant effect on these properties. The farinograph value and the loaf volume increased, the loaf height-width ratio decreased when GK Tiszatáj was treated with one of these herbicides.

Even from this short review of the literature it can be seen that the baking properties of wheat are influenced by the varieties, the active agents and dose of herbicides as well as by environmental factors.

In this paper we demonstrate the effect of the commonly used provocative dose of herbicides (as compared to the double amounts used in the practice) on the quality of two wheat varieties.

1. Materials and methods

Samples collected from provocative herbicide field experiments of GKI (Cereal Research Institute) were studied. Experiments were carried out on meadow chernozem soil with salinity in depth. Nitrogen supplying capacity of the soil was

good, availability of phosphorus and potassium was very good. NPK active ingredient was uniformly applied after bean forecrop in autumn at 210 kg ha^{-1} rate, in 1:1 ratio.

Herbicide sensitivity of KG Kata (early-ripening) and GK Csűrös (medium-ripening) wheat variaties were studied applying herbicides in provocative dose (double amounts of the suggested dose). Both wheat varieties are characterized with medium quality flour ($B_1 - B_2$) and high productivity.

Besides the untreated plants (control plot), the wheat plants were treated with 11 different herbicides (Table 1).

Table 1

Products, active agents and doses

Belgran	isoproturon + ioxinil + mekoprop	10.01 ha^{-1}
Aniten D	2,4 D + flurenol	6.01 ha^{-1}
Banvel M	MCPA + dicamba	7.01 ha^{-1}
Logran	triasulphuron	120.0 g ha^{-1}
Assert	imazametabenz-metil	1.21 ha^{-1}
Puma	fenoxa-prop-p-etil	2.81 ha^{-1}
Illoxan	diklofop-metil	6.01 ha^{-1}
IP-Flo	izoproturon	6.01 ha^{-1}
Starane	fluroxipir	1.61 ha^{-1}
Dikamin D	2,4 D	6.01 ha^{-1}
Dikotex 40	MCPA	8.01 ha^{-1}

All the 12 treatments had 4 replications in a random design. Reference (control) plots were mechanically weeded.

After harvest grain samples were taken from each of the 96 plots (2 varieties × × 12 treatments × 4 replications).

The wet and dry gluten content, gluten spreading, water absorption, valorigraphic value and the FN were determined according to the Hungarian standard (KARÁCSONY, 1970) and the modified method of MATUZ and co-workers (1986) was used for sedimentation test.

Data of the experiments were evaluated by means of variance analysis.

2. Results

The variance data show (Table 2) that the treatment factor (varieties + + herbicide) was significant for the quality properties. Significant difference could be found between the two wheat varieties ("A" factor) regarding the wet and dry gluten contents, water absorption, valorigraphic value, FN, but SDS values were identical.

Table 2

Analysis of variance of the wheat quality characteristics

Variance source	Degree of freedom	Wet gluten content MQ	Dry gluten content MQ	Gluten spreading MQ	Water absorption MQ	Baking quality Val. v. MQ	Falling number FN MQ	SDS sedimentation volume MQ
Replication	3							
Treatment	23	8.61*	1.39***	18.38***	7.45***	77.47*	3949.7***	0.61***
Variety (A)	1	125.08***	18.82***	348.08***	72.66***	1535.20***	49127.9***	0.12
Herbicide (B)	11	3.69	0.95**	3.15**	6.45***	9.79	2989.7***	0.58**
A × B	11	2.95	0.25	3.64**	2.52**	12.62	802.5*	0.69**
Error	69	4.25	0.35	1.18	0.87	11.01	409.3	0.18

*, **, ***: Significant at P = 5%, 1.0% or 0.1% probability levels

The herbicide treatments significantly influenced all quality parameters except for the wet gluten content and valorigraphic value. Although the variety × herbicide interaction (A × B) could not be proven in case of the wet and dry gluten content or the valorigraphic value but it was significant for the gluten spreading, water absorption, FN and the SDS value.

Table 3

Effects of herbicides on the quality of yield of wheat varieties

Treatment Herbicide	Variety	Wet gluten content (%)	Dry gluten content (%)	Water absorption (%)	Falling number FN (sec)	SDS sedimentation volume (cm^3)
Control	Kata	31.4	10.6	58.6	302	5.16
	Csűrös	29.7	10.0	60.8	371	5.03
Belgran	Kata	32.3	10.5	58.5	314	5.33
	Csűrös	29.4	9.8	61.6	345	5.03
Aniten D	Kata	30.9	10.3	59.0	315	4.98
	Csűrös	29.5	9.8	61.4	333$^-$	4.93
Banvel M	Kata	32.8	10.9	57.9	326	5.00
	Csűrös	30.0	9.9	60.9	378	4.80
Logran	Kata	33.0	10.6	58.7	327	5.08
	Csűrös	28.0	9.2	60.3	368	4.83
Assert	Kata	32.6	10.7	59.5	326	5.13
	Csűrös	28.9	9.4	61.7	381	5.30
Puma	Kata	31.4	10.6	60.4$^+$	304	4.83
	Csűrös	28.6	9.1$^-$	61.6	345	4.03$^-$
Illoxan	Kata	31.2	10.3	60.1$^+$	305	5.08
	Csűrös	29.2	9.2	61.3	392	4.30$^-$
IP-Flo	Kata	29.6	9.7$^-$	59.3	307	5.00
	Csűrös	28.6	9.2$^-$	61.6	361	4.23$^-$
Starane	Kata	30.4	9.6$^-$	62.7$^+$	288	5.03
	Csűrös	28.6	8.9$^-$	61.5	336$^-$	5.75$^+$
Dikamin D	Kata	30.2	10.2	61.1$^+$	285	4.75
	Csűrös	28.7	9.4	62.6$^+$	310$^-$	5.33
Dikotex	Kata	29.9	9.9	60.7$^+$	289	4.45$^-$
	Csűrös	29.2	9.3	62.2$^+$	311$^-$	5.40
LSD 5% for herbicide		3.0	0.9	1.3	30	0.61
Variety mean	Kata	31.3	10.3	59.7	307	4.98
	Csűrös	29.0	9.4	61.4	351	4.91
LSD 5% for variety		0.9	0.2	0.3	8	0.18

$^+$, $^-$ significantly higher or lower than the control (P = 5%)

Changes in quality properties of the two varieties and the effect of herbicide treatments are shown in Table 3 and Figs. 1–2.

Wet gluten content: Nonce of the herbicide affected it significantly. Generally, the gluten content was higher in the GK Kata than in the GK Csűrös.

Dry gluten content: Compared to the control all treatments caused a reduction in the dry gluten content but Banvel M and Assert herbicides slightly increased it in case of GK Kata. In both varieties the IP-Flo and the Starane treatments induced a significant reduction in dry gluten content. A variety specific decrease was found when GK Csűrös was treated with Puma herbicide.

The dry gluten content in GK Kata was higher than in GK Csűrös.

Gluten spreading (or flattening): This property differs significantly in the two varieties: gluten in the GK Kata is elastic but not viscous and has a low spreading value (4.2 mm); the Csűrös variety is characterized by more elastic gluten and by a larger (8.4 mm) spreading. In comparison to the control, a significant increase was caused in GK Kata by Assert, while in the GK Csűrös none of the herbicides caused reliable changes in gluten spreading (Fig. 1).

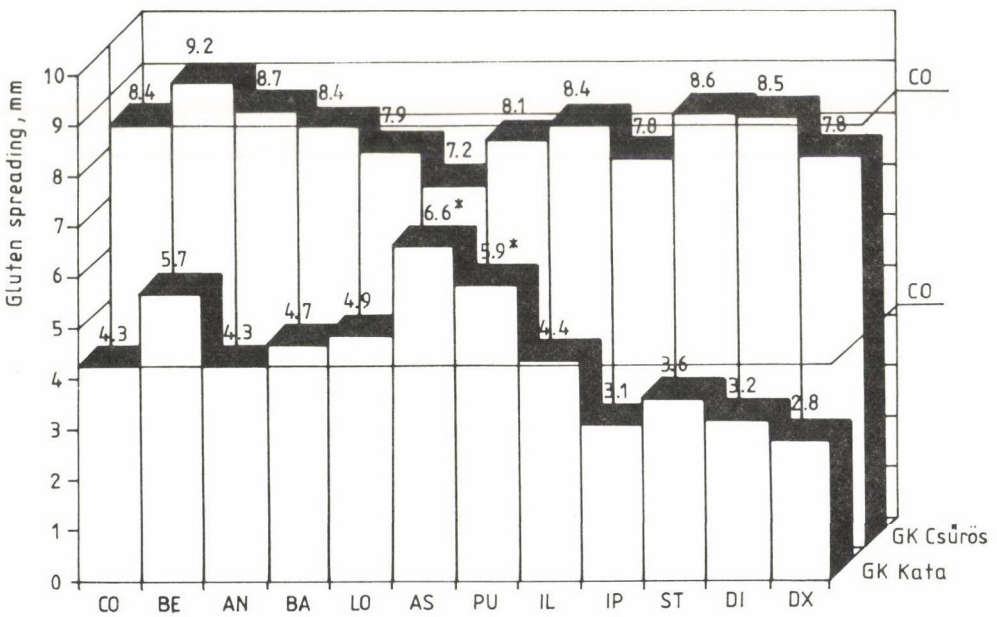

Fig. 1. Changes in gluten spreading of wheats affected by herbicides. Treatments: CO: control, BE: Belgran, AN: Aniten D, BA: Banvel M, LO: Logran, AS: Assert, PU: Puma, IL: Illoxan, IP: IP-Flo, ST: Starane, DI: Dikamin D, DX: Dikotex 40. *: Significantly differs from control at P = 5%

Water absorption measured with valorigraph: In comparison to the untreated control, Dikamin D and Dikotex 40 increased significantly the water absorption of

both varieties, but Puma, Illoxan and Starane herbicides increased it only in GK Kata. The water absorption of GK Csűrös was better than that of GK Kata.

Flour quality, measured with valorigraph: Compared to the mechanically weeded control wheat, every applied herbicide reduced the value number of GK Kata (B_2 quality 47.95). The reduction of quality caused by Belgran, Aniten D, Banvel M, Logran, Puma, Illoxan, IP-Flo, Starane was significant.

The quality of GK Csűrös was poor and this quality was not changed significantly by different herbicides (Fig. 2).

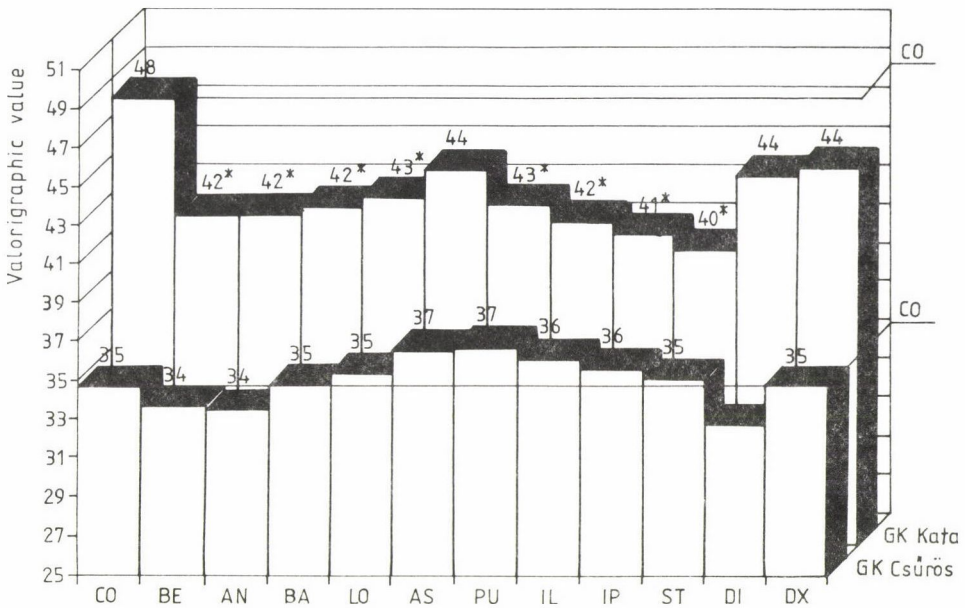

Fig. 2. Changes in four quality of wheat affected by herbicides. Treatments: CO: control, BE: Belgran, AN: Aniten, BA: Banvel M, LO: Logran, AS: Assert, PU: Puma, IL: Illoxan, IP: IP-Flo, ST: Starane, DI: Dikamin D, DX: Dikotex 40. *: Significantly differs from control at P = 5%

Falling number (FN): Considering this property the two varieties differ to a large extent: The FN in GK Csűrös was far higher (371) than in GK Kata (302). In GK Csűrös herbicides Starane, Dikamin D, Dikotex 40 and Aniten D decreased the FN significantly. The effect of other herbicides was different in both wheat varieties, but significant differences could not be observed.

SDS sedimentation volume: SDS value of the control treatment in both varieties was identical (5.16 and 5.03 respectively). Dikotex 40 in GK Kata, Puma, Illoxan and IP-Flo significantly reduced this value in GK Csűrös variety. SDS value of GK Csűrös increased reliably when Starane herbicide was applied. Neither of the varieties shows significant changes caused by other herbicides.

3. Discussion

Our results demonstrate that the different herbicides generate dissimilar changes in the quality of the wheat varieties, that is the effect of herbicides on wheat varieties and on the quality properties was specific.

The variety specific effect of herbicides on gluten spreading, baking quality and FN was very striking: e.g., the gluten spreading of GK Kata is enhanced but that of GK Csűrös is reduced by Banvel M, Assert, Puma and Illoxan. Gluten spreading of GK Kata is decreased, but that of GK Csűrös is slightly increased by Starane and Dikamin herbicides.

The "quality selective" character of herbicides manifested itself in the fact that the herbicides did not influence every property: e.g. Belgran, Banvel, Logran influenced only the baking value number by Starane effected on the dry gluten content, the water absorption, the valorigraphic value, the FN and SDS value as well. It is obvious that the best herbicides are those which have reliable weed killer effect and do not have any side-effect.

In this respect out of the 11 herbicides studied Logran and Assert proved to be the most favourable ones since they did not damage the baking parameters significantly even they sometimes exerted a favourable influence on them.

Out of the 7 quality properties examined:

none of the herbicides caused any significant changes in wet gluten content, and

in gluten spreading only 1 (Assert),
in dry gluten content 3 (Puma, IP-Flo, Starane),
in falling number 4 (Aniten D, Starane, Dikamin D, Dikotex),
in water absorption 5 (Puma, Illoxan, Starane, Dikamin D, Dikotex),
in SDS value 5 (Puma, Illoxan, IP-Flo, Starane, Dikotex),
and in valorigraphic value (in GK Kata only) 8 herbicides (Belgran, Aniten D, Banvel M, Logran, Puma, Illoxan, IP-Flo, Starane) caused significant changes.

It should be noted, that our observations on the interaction between herbicide and variety as well as between herbicide and quality have already been published previously (PÉTER et al., 1985). Variety specific effect of herbicides seems to be responsible for the fact that the (2,4-D, Aniten D and Dikotex 40) herbicides used in our experiments for a long time did not display that advantageous effects on the quality that MAJER-KISS (1971), POLLHAMER (1973) and PÉTER and co-workers (1985) found in other wheat varieties. Taking our results into consideration we find it worthwhile to continue our studies with a larger number of wheat genotypes.

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CATALYTIC DEAMIDATION OF CANOLA PROTEINS

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Methanol-ammonia/hexane defatted-canola meal has a protein extractibility of less than 60% at a pH of 12. In an effort to increase its extractibility a process for the partial deamidation of protein in canola meal was developed. Meal was slurried in aqueous HCl and treated with sodium dodecyl sulphate (SDS), at various levels of temperature, pH, and time. A two-level four-factor factorial experimental design was used to investigate initially the protein extractibility. The process thus developed increased the protein extractibility of the two-phase extracted meal from 58.0% to 96.3%.

An acceptable protein yield was obtained at a temperature of 90 °C, SDS concentration of 0.04 mol l^{-1} pH 2.5, and contact time of 30 min. Under these conditions 87.2% of the protein was dissolved. Coprecipitated SDS was removed with triethylamine acetate in acetone. The degree of deamidation was determined to be 38.6%, and some 7% of the protein was hydrolyzed to low molecular weight peptides.

The functional properties of the protein isolates produced at the optimum condition were similar to those of a commercial soy protein isolate.

Keywords: canola, protein isolates, deamidation, methanol/ammonia extraction, functional properties, SDS

Rapeseed contains toxic compounds such as glucosinolates as well as some antinutrients such as phytates, phenolic compounds and hulls. Although canola varieties first developed in Canada contain less than 30 $\mu M\ g^{-1}$ glucosinolates, the meal also contains high levels of fibre, phytate and polyphenols, making it unsuitable for human consumption. None of the many methods for the removal of glucosinolates developed over the past three decades is in commercial use due to protein losses, poor functional properties, or high processing costs.

A novel two-phase solvent extraction method developed in our laboratory uses methanol containing dissolved ammonia, and hexane to simultaneously extract the oil, and remove glucosinolates and some of the polyphenols. The oil produced is low in phosphorus. The resulting meal has a high protein content, it is free flowing, light in colour, but unfortunately it has a nitrogen solubility of only about 50% (RUBIN

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et al., 1986). The decrease in nitrogen solubility was attributed to protein denaturation or aggregation (TZENG et al., 1990). For effective protein isolate recovery, most of the nitrogen in the meal has to be brought into solution.

Chemical modifications of proteins by sulphonation, succinylation, phosphorylation and deamidation usually improve the solubility and functionality of oilseed proteins (SHIH and KALMÁR, 1987).

Deamidation is a particularly attractive chemical treatment method due to the fact that oilseed proteins contain a large number of amide groups. Canola protein contains about 30% glutaminyl and asparaginyl residues. The amide groups in the side chains of these amino acids are hydrophobic and thus inhibit the solubility of the protein. However, the amide groups are readily converted to their acid by relatively mild acid treatment.

The dependence of protein deamidation on acids and temperature has been well documented (MATSUDOMI et al., 1985). Deamidation also enhances other functional properties of oilseed proteins. MATSUDOMI and co-workers (1985) reported that 10% deamidation by treating soy protein isolate with 0.05 *N* HCl at 100 °C for 30 min resulted in significant improvements in water absorption, foaming stability, and emulsion capacity.

To be effective, deamidation has normally been conducted under rather severe conditions involving temperatures $\leq$ 100 °C and strong acids such as HCl at concentrations $\leq$ 0.5 *N*. Under these conditions excessive degradation of the protein often occurs, which could lead to bitter taste, off-flavours and loss of functional properties. To improve the deamidation process, two approaches have been suggested: enzymatic and catalytic deamidation.

KATO and co-workers (1987) used a protease at alkaline pH to deamidate food proteins, and reported considerable improvement in the functional properties.

Protein can be effectively deamidated in the presence of anions such as dodecyl sulphonate and sulphate in an acidic environment. The rate of hydrolysis depends on pH, temperature and the type of acid. The anions of dodecyl sulphate and dodecyl sulphonate, seem to catalyze the process (SHIH & KALMÁR, 1987).

The effectiveness of surfactants in protein hydrolysis depends on the hydrophilic-lipophilic balance (HLB) (NAWAR, 1985). Once a surfactant molecule is bonded to the protein, it may itself provide a further centre of attraction for other protein and/or surfactant molecules, by virtue of hydrophobe-hydrophobe interactions responsible for micelle formation. NAKAI and co-workers (1980) used surfactants such as SDS, sodium and potassium salts of myristic, oleic and linoleic acids for the solubilization of rapeseed protein isolates prepared from commercial meal, and the dispersability of the treated protein isolates were tremendously improved. In their study, they found that only the anionic surfactants were effective.

The objective of this research was to develop a mild deamidation process for increasing the solubility of methanol-ammonia/hexane extracted canola protein as the first step in producing a food-grade canola protein isolate.

1. Materials and methods

The starting meal was prepared by using the methanol-ammonia/hexane extraction process developed in our laboratory (RUBIN et al., 1986).

Fifty g meal was dispersed in 900 cm^3 solution of 0.02 or 0.08 mol l^{-1} SDS in a 1000 cm^3 beaker and was heated and continuously agitated using a Corning hot plate-stirrer. The pH was set to 1.50 or 3.00. The temperature was raised to the required value in 10–15 min and then it was kept constant for 30 or 60 min by the addition of 0.4 *N* HCl as required. At the end of the reaction time the slurry was cooled to room temperature.

The protein solubility of the slurry was determined by slowly raising the pH to 12.00 using 50% NaOH solution with constant stirring using a Corning hot plate-stirrer. The pH was kept constant at 12.00 for 30 min by adding 5% NaOH solution as required. After 30 min of extraction, the protein solution was separated by centrifugation at 5 000 r.p.m. for 15 min using an IEC B–22 centrifuge followed by vacuum filtration using a Whatman No. 41 filter paper. The meal was washed twice with 300 cm^3 distilled water at pH 12.00 each time and the solution recovered by centrifugation and vacuum filtration. The washing solution was added to the extraction solution. The protein content of the solution was determined by the Kjeldahl method (A.O.C.S., 1980).

The wet meal residue was washed with 500 cm^3 distilled water and dried in either a vacuum oven at 50 °C for 5 h or freeze dried, using a Labconco freeze dryer at −50 °C, and its protein content was determined.

The process of deamidation was optimized by carrying out a series of experiments at SDS concentrations between 0.03 and 0.06 mol l^{-1} 90 °C, and pH values between 2.00 and 3.00. The yield of protein in the alkaline extract, percentage deamidation, percentage peptide-bond hydrolysis, and cost of the process were considered in choosing the optimum conditions for producing canola protein isolates using the method of TZENG and co-workers (1990).

The residual sodium dodecyl sulphate present in the protein isolate in form of an SDS-protein complex was removed using the method of HENDERSON and co-workers (1979) with some modifications. Lyophilized protein was dispersed at an extractant-to-SDS ratio of 1 cm^3 10 mg in a solution of acetone containing triethylamine, acetic acid and water in ratio of 85:5:5:5 (v/v). The slurry was agitated for 30 min on a Bürell wrist action shaker, centrifuged at 2 000 r.p.m. using an IEC B–22 centrifuge for 20 min and vacuum filtered using a Buchner funnel with

Whatman #41 filter paper. The supernatant was discarded. The residue was re-extracted twice, and then washed three times with pure acetone to remove the extraction solution. The detailed flow diagram of the protein deamidation and protein-isolates production process is presented in Fig. 1.

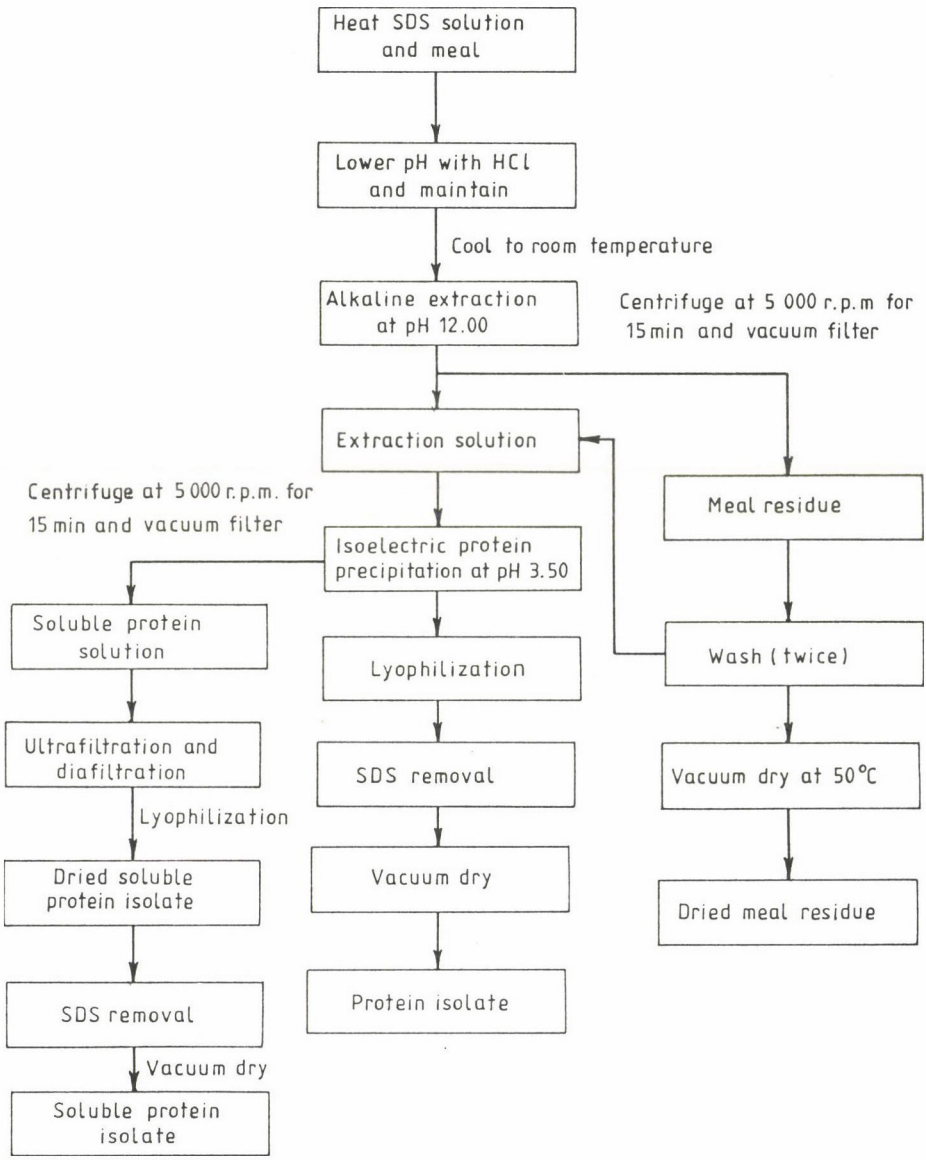

Fig. 1. Process flow diagram

The phytic acid content was determined by the ferric iron precipitation method used by NACZK and co-workers (1985).

The moisture content of the sample was determined by drying in a forced-air oven at 105 °C according to the A.A.C.C. Method 44–15 (A.A.C.C., 1976).

The non-protein nitrogen was determined by the TCA precipitation method of NACZK and co-workers (1986).

The degree of deamidation was determined using the method of JEFFERY and co-workers (1989) based on the use of Nessler reagent.

The residual sodium dodecyl sulphate was determined gravimetrically as sulphate after dissociation, by A.A.C.C. Method 40–66 (A.A.C.C., 1976).

The water absorption capacity and fat absorption capacity were determined as described by SOSULSKI and co-workers (1962). The whippability, foam capacity and foam stability, emulsion capacity and emulsion stability, nitrogen solubility index and protein dispersibility index were determined according to the method of NACZK and co-workers (1986).

Amino acid analysis was done on the deamidated canola protein and non-deamidated canola protein isolates by the Protein Analysis Services at the Medical Sciences Department, University of Toronto.

After hydrolyzing 7.66 mg of protein sample in 500 μl 5.7 *N* HCl at 107 °C for 22 h. The sample was dried and diluted to 100 μl with a buffer solution at pH 2.2. An aliquot of 0.5 μl hydrolysate was analyzed using Beckman Model 121M analyzer with single A/N/B.

2. Results and discussion

The amount of protein extracted at pH 12.00 was measured over a time period of 3 h. Since essentially all of the protein dissolution occurred before 30 min all protein extractibilities were determined by extraction for 30 min.

A two-level four-factor experimental design (Table 1) was used to assess the effect of deamidation on the solubility of canola protein. It was previously reported that the acid hydrolysis of asparagine to aspartic acid requires at least 30 min (MATSUDOMI et al., 1985). This is 100 times faster than the reaction of any other polar amino acid residue (HAN et al., 1983). Thus reaction times of 30 and 60 min were selected.

Table 1

A two-level four-factor experimental design

Factors	Temperature (°C)	SDS concentration	Time (min)	pH
Lower (−) level	60	0.02 mol l^{-1}	30	1.5
Upper (+) level	90	0.08 mol l^{-1}	60	3.0

Deamidation with sodium dodecyl sulphate (SDS) as a "catalyst" resulted in an increase in protein extractibility from 58% to 69.5% at the low level of addition and 96.5% at the high level (Fig. 2). These results show that with acid treatment in the presence of SDS, the solubility of canola protein in $CH_3OH-NH_3-H_2O$/hexane extracted meal could be increased substantially. The factors were analyzed in order to understand their contribution to the canola protein extractibility. Table 2 shows the result of the factorial analysis of the experimental factors. It is clear that temperature and SDS concentration have the largest effect. Time does not significantly influence solubilities, pH has a small but important effect, and there is a synergistic interaction between temperature and SDS concentration, pH and SDS concentration, and temperature, pH and SDS concentration. These interactive effects are overshadowed by the larger individual effects of these factors.

Table 2

Factorial analysis of the experimental conditions

Main effect of temperature:	87.88 − 76.10 = 11.78 ± 0.4
Main effect of pH:	79.28 − 84.67 = -5.39 ± 0.4
Main effect of SDS concentration:	88.18 − 75.81 = 12.37 ± 0.4
Main effect of time:	82.01 − 82.00 = 0.01 ± 0.04
Interaction of temperature and SDS concentration:	83.96 − 80.03 = 3.93 ± 0.04
Interaction of pH and SDS concentration:	83.16 − 80.82 = 2.34 ± 0.04
Interaction of temperature, pH and SDS concentration:	86.04 − 83.34 = 2.70 ± 0.04

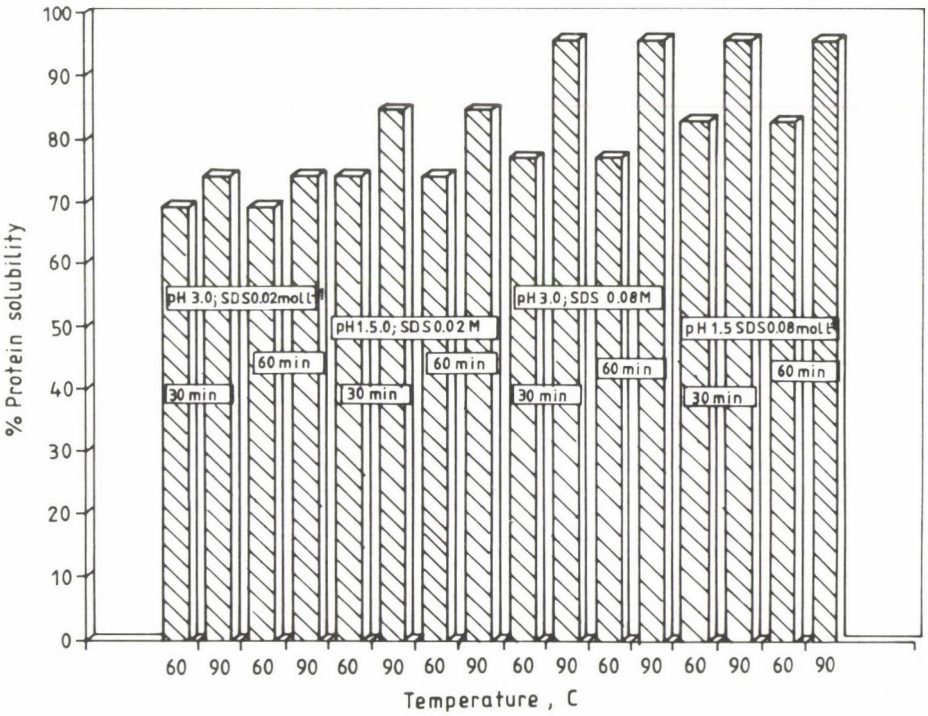

Fig. 2. Effect of temperature, pH, SDS and time on canola protein solubility

Based on these results we investigated a number of intermediate conditions to define processing values that would result in significant protein solubility increase with reduced SDS use. Based on the protein solubility, peptide loss, SDS usage and thermal exposure, we consider the best conditions to be pH 2.50, 0.04 mol l^{-1} SDS, 90 °C for 30 min. Under these conditions the protein extractibility was increased to 87.2%, with 38.6% deamidation and 7.1% peptide bond hydrolysis. These conditions were used to produce protein isolates used in the determination of functional properties.

The process developed by DIOSADY and co-workers (1989) yielded two products: an isoelectric and an acid-soluble protein isolate.

The isoelectric protein isolate was precipitated with 6 *N* HCl at pH 3.5. For the deamidated protein isolate, the protein precipitation started at ~ pH 11.5, immediately when acid was added, unlike the non-deamidated protein extract, which precipitated out in the pH range of 3.5 to 4.5. While the non-deamidated isolate contained ~ 98% (N × 6.25) protein, the deamidated protein isolate contained only 64.45% protein. The bulk of the deamidated isolate consisted of sodium dodecyl

sulphate (SDS) complexed with protein. This was not unexpected (MATSUDOMI et al., 1985 and SHIH & KALMÁR, 1987).

After treatment to remove the SDS the protein content of the isoelectric-precipitated protein rose to about 90% (N × 6.25) on a dry basis. The precipitated protein isolate alone accounted for 65.4% of the total protein present in the meal, or 81.6% of the extracted protein. The protein recovered as isoelectric and soluble protein isolates accounted for 80% of the protein originally present in the meal.

In addition to its high yield, the isoelectric protein isolate had a lower phytic acid content (0.26%) than the 1–1.5% previously reported by TZENG and co-workers (1990). The low phytic acid content is likely related to the negative charge of the SDS-protein complex, which could repel the negatively charged phytic acid ions preventing the formation of protein-phytic acid complexes.

The non-protein nitrogen concentration increased slightly due to peptide-bond hydrolysis during the deamidation process. As less than 8% of the protein in the canola meal was hydrolyzed this was not a major drawback of this approach, and it compared favourably with the results obtained by SHIH and KALMÁR (1987). Table 3 shows the composition of the various product streams after deamidation and treatment to remove SDS.

Table 3

The composition of the various product streams

Components	Meal	SPI	PPI	Meal residue
Weight (g)	50.00	9.35	20.52	21.55
Protein (%, w/w)	40.67 ± 0.04	31.57 ± 0.09	63.91 ± 0.11	14.45 ± 0.05
Phytate (%, w/w)	4.21 ± 0.01	5.23 ± 0.01	0.26 ± 0.01	4.44 ± 0.02
Non-nitrogen protein (%, w/w)	0.40 ± 0.01	0.78 ± 0.01	0.55 ± 0.01	0.66 ± 0.01
SDS (%, w/w)	0.00	16.49 ± 0.03	33.53 ± 0.02	4.93 ± 0.03

PPI = Precipitated protein isolate;
SPI = Soluble protein isolate;
SDS = Sodium dodecyl sulphate

The soluble protein isolate, consisting of the protein remaining in solution after the isoelectric precipitation, was recovered by concentrating the solution using ultrafiltration, purifying the concentrated solution using diafiltration and then lyophilization. The soluble protein isolate accounted for 14.5% of the protein in the meal. The protein content in the soluble protein isolate produced was only 31.8%. This is very low compared to the 98–104% protein reported by TZENG and co-workers (1990) for a similar but non-deamidated protein isolate.

Treatment with acetone containing triethylamine acetate increased its protein content only to 42%. The low level of protein in the soluble protein isolate and its low yield make it unsuitable food uses, and it was not investigated further.

The functional properties of the protein isolates produced were investigated to determine the effect of the deamidation. The deamidated isolates are lighter in colour than the untreated samples. The deamidated isolate was bland in taste, though the smell of ginger seems to persist, probably due to the acetic acid used in the removal of sodium dodecyl sulphate.

The water absorption capacity (WA), fat absorption capacity (FA), whippability (WHC), foam capacity (FC), foam stability (FS), emulsion capacity (EC), emulsion stability (ES), nitrogen solubility index (NSI), and protein dispersibility index (PDI) of the isolate were evaluated. The results are summarized in Table 4. The deamidated and non-deamidated canola protein isolates were compared with a soy protein isolate (Supro 500E), as control. The deamidated isoelectric protein isolate showed increased values in its functional properties, except for the nitrogen solubility index (NSI) and protein dispersibility (PDI). The NSI and PDI were somewhat increased, however, the increase was limited at pH 6.5 to 7.5, where the minimum nitrogen solubility occurred (Fig. 3). The water absorption capacity of the isoelectric protein isolate was the highest among the protein isolates tested. The water absorption capacity of the deamidated and non-deamidated soluble canola protein isolates was very low. The soluble canola protein isolate had the highest fat absorption capacity, but the deamidated soluble canola protein isolate also had a high fat absorption.

Table 4

Functional properties of the protein isolates

Products	Protein (%)	WA (%)	FA (%)	PDI (%)	EC (%)	ES (%)	WHC (%)
	AS-is						
Non-deamidated PPI	93.67	279	266	1.03	70	99	200
Non-deamidated SPI	93.66	51	567	12.5	64	99	175
Deamidated PPI	88.12	336	348	3.73	72	99	250
Deamidated SPI	42.43	48	426	13.12	50	96	180
Soy PPI (Supro 500E)	86.67	203	175	30.07	68	98	175
Tower meal (dry basis)	49.50	363	280	8.4	61	98	80

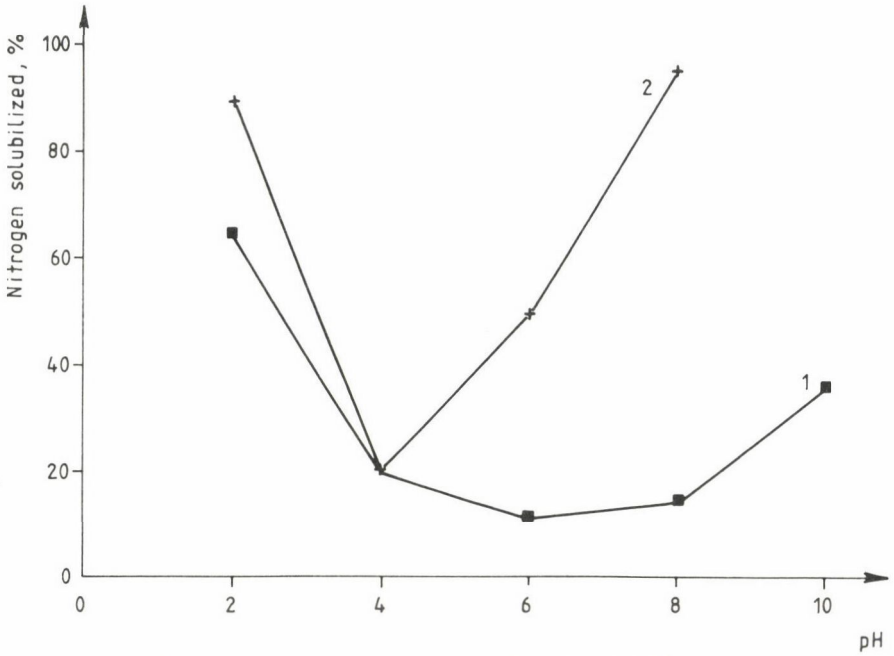

Fig. 3. The variation of nitrogen solubility index with pH
1: DPPI (deamidated precipitated protein isolate); 2: soy-isolate

The emulsion capacity and emulsion stability of the isoelectric protein were not affected and they remained higher than that of the soy protein isolate. The emulsion capacity of the deamidated soluble canola protein was reduced but its stability was not affected. The whippability of the deamidated isoelectric protein was better than those of both non-deamidated isolates and the soy protein isolate. The foam capacity of the deamidated canola protein isolate was also increased. The foam of the deamidated isoelectric protein had the highest stability among the protein isolates tested. The non-deamidated soluble protein isolate has higher stability than the deamidated soluble protein while the soy protein isolate possessed the least stability (Fig. 4).

The deamidated protein isolate possessed the highest foam stability and foam volume. Figure 4 compares the nitrogen solubility index of the deamidated canola protein isolate and soy protein isolate. The soy protein isolate has a higher nitrogen solubility than the deamidated canola protein isolate. The minimum nitrogen solubility of 10.92% was obtained around pH 6, rather than the minimum solubility point of pH of 3.5 observed with non-deamidated canola protein isolates.

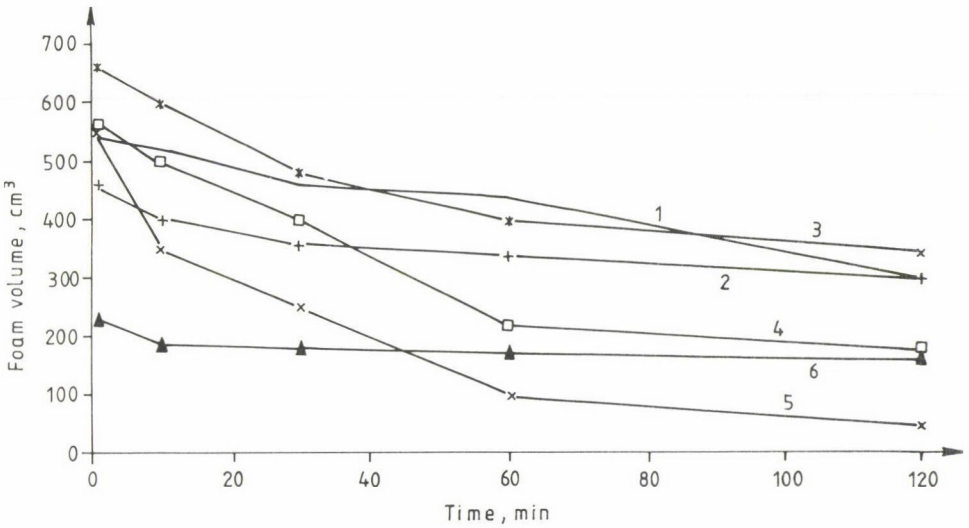

Fig. 4. Canola protein foam stability. 1: NPPI (non-deamidated precipitated protein isolate); 2: NSPI (non-deamidated soluble protein isolate); 3: DPPI (deamidated precipitated protein isolate); 4: DSPI (deamidated soluble protein isolate); 5: soy-isolate; 6: tower meal

3. Conclusions

The results indicate that the protein solubility of two-phase extracted canola meal could be substantially increased by treatment with sodium dodecyl sulphate in an acid medium. A maximum protein extractibility of 96% was obtained. The amount of protein recovered as protein isolates has been substantially increased to 80%. The isoelectric precipitated protein isolate accounts for 64.5% of the original protein in the meal which is higher than the amount reported previously. It contained only 0.26% phytic acid, about one-fifth of the value previously reported without deamidation.

The deamidation process enhanced most of the functional properties of the protein isolates. Unfortunately the process is impractical due to its complexity and very high SDS usage which must be removed by techniques that are incompatible with food uses. The improvement in yield and functional properties of the deamidated canola protein isolates warrant the investigation of deamidation with acid in the presence of other surfactants, or in the presence of appropriate ion exchange resins.

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INFLUENCE OF PHASE TRANSITION (FREEZING AND THAWING) ON THERMOPHYSICAL AND RHEOLOGICAL PROPERTIES OF APPLE PURÉE-LIKE PRODUCTS

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The effects of cyclic freezing and thawing on the rheological and thermophysical properties of some apple purée-like products were investigated. The results obtained showed that the phase transition temperatures of these systems depended not only on the mass fraction but also on the kind of solid matter, especially sugars, as well as on the phase of determination (before or after freezing).

It was observed that all the investigated products belonged to the group of thixotropic non-Newtonian systems, the consistency of which depended on their composition, i.e. on the substances added to them.

After freezing the changes of rheological properties were registered in all samples. Thus, the values of the consistency coefficients of apple purée decreased, while those of the products which had a more complex composition of solid matter, increased.

By cyclic freezing and thawing the viscosity and thixotropic character of the investigated products was changed. Consistency mainly became greater, while the thixotropic area changed depending on the kind of ingredients (sugar, starch, alginate) added.

Keywords: thermophysical properties, rheological properties, phase transition temperatures, freezing temperatures, thawing temperatures, apple purée, cyclic freezing and thawing

World production and consumption of frozen foods is constantly increasing primarily due to their high quality, which is mostly retained even during prolonged storage. However, by inadequate handling during either transport, storage or retail, partial or complete thawing may occur. In this case it often happens that the food is refrozen, with some changes in rheological as well as in thermophysical properties. Since those properties are closely connected to sensoric ones (e.g. the consistency), and consequently with the quality of the product, it is very important to know to which extent they change during the repeated freezing or recrystallisation.

Previous investigations showed that thermophysical properties, especially phase transition temperature, predominantly depend on the kind of the product (GUEGOV, 1980; CHANG & TAO, 1981; CHEN, 1986, 1987; CHEN & NAGY, 1987) as well as on

the nature and mass fraction of its dry matter (HEGEDUŠIĆ & LOVRIĆ, 1985; LOVRIĆ et al., 1986; HOO & MCLELLAN, 1987; SUCCAR & HAYAKAWA, 1990), while the rheological behaviour was additionally influenced by several others, like temperature (RAO et al., 1984), homogenization (COSTELL & DURAN, 1983; TANGLERTPAIBUL & RAO, 1987; CRANDALL et al., 1988; TRIFIRO et al., 1991), particle size, preservation (HEGEDUŠIĆ & LOVRIĆ, 1991; HEGEDUŠIĆ et al., 1991), storage conditions etc. (KIM et al., 1986).

The aim of this research was to investigate extensively the influence of cyclic freezing and thawing on the phase transition temperatures (freezing and thawing) as well as on the rheological properties of some apple purée-like products.

1. Materials and methods

Investigations were carried out with some purée-like products prepared from apple fruits of Ida Red variety. After peeling and dipping in 0.008% citric acid solution, apples were blanched in hot water and chopped in a blender. Sucrose, fructose, glucose, starch, Na-alginate and citric acid were added to the purée, in the proportions shown in Table 1.

The freezing and thawing temperatures of samples were determined by differential thermal analysis using the apparatus type MP ΔtPt-L by continuously scanning the temperature as well as the temperature difference between sample and reference material (quartz sand). As the results of the measurements, DTA cooling (freezing) as well as heating (thawing) curves (Figs. 1 and 2) were obtained. The beginning of DTA peaks was determined as the beginning of the phase transition (HEGEDUŠIĆ & LOVRIĆ, 1985).

Rheological properties were determined by using a rotational rheometer (Viscotester VT 24; Haake, Germany) with concentric cylinders fixtures type MV I, where the radius of the outer cylinder is 20.04 mm, and that of the inner cylinder is 21 mm. The height of the inner cylinder is 60 mm. Shear stress against the increasing shear rates from 1.66 to 53.00 1/s (rising measurement) was measured. At the highest shear rate, shear stress lasted two minutes. After that rotational rate successively decreased to the initial value (recurrent measurement). All measurements were done twice at 20 °C, and for further calculations arithmetic values of shear stresses were used.

Table 1

Ingredients used in the preparation of apple purée-like products

Sample	Ingredients	Mass fraction of ingredients (%)	Mass fraction of solid matter (%)
1	Apple purée	100.00	9.50
2	Apple purée	55.00	50.20
	sucrose	45.00	
3	Apple purée	55.00	50.20
	glucose	45.00	
4	Apple purée	55.00	50.20
	fructose	45.00	
	Apple purée	55.00	
5	sucrose	22.50	50.20
	glucose	22.50	
	Apple purée	54.00	
	sucrose	22.50	
6	glucose	11.25	51.10
	fructose	11.25	
	Na-alginate	0.55	
	citric acid	0.45	
	Apple purée	52.00	
	sucrose	22.50	
	glucose	11.25	
7	fructose	11.25	50.90
	Na-alginate	0.55	
	starch	2.10	
	citric acid	0.35	

Some of the results of such measurements are shown in Figs. 3 and 4. As they formed the thixotropic loop (that means that the viscosity changes with time and shear rate), the rheological properties of these products were calculated by power law equation (Ostwald – Reiner model):

$$\tau = k \gamma^n \quad (1)$$

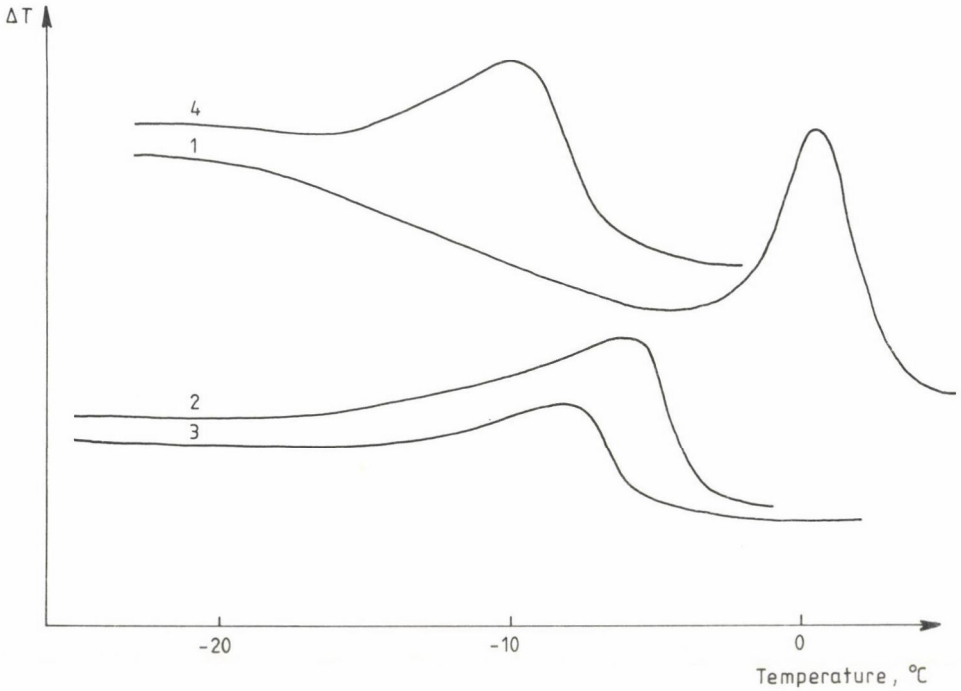

Fig. 1. DTA thawing curves of apple purée-like products. 1: apple purée, 2: apple purée + sucrose, 3: apple purée + glucose, 4: apple purée + sucrose + glucose + fructose + Na-alginate + citric acid

where

τ = shear stress (N/m^2)
γ = shear rate ($1/s$)
k = consistency coefficient (Ns^n/m^2)
n = flow behaviour index

For flow behaviour index determination (by calculating the logarithms of shear stresses and shear rates) only the values of the rising part of thixotropic loops were used.

With the aim of characterizing the thixotropity of the systems, the value of the thixotropic area was calculated using equation (2):

$$k_{tp} = \frac{1}{N} \sum_{i=1}^{i=N} \Delta \tau_l \qquad (2)$$

In order to investigate the influence of cyclic freezing and thawing on the rheological and thermophysical properties of such systems, the samples were frozen

in a freezer and held for 72 h at −20 °C. Then, they were left to thaw in the refrigerator (4 °C) for 14 h. The samples were then analysed and refrozen again in the same way. Each was therefore submitted to three successive cycles of freezing and thawing.

2. Results

The freezing and thawing temperatures were determined from corresponding DTA curves. Some of them are presented in Fig. 1. From the obtained results, it could be seen that the apple purée had the highest freezing temperature as well as the thawing temperature (−1 and −3.5 °C respectively), which could be expected, because of its small solid matter fraction (9.5%). The freezing temperatures of all other products (whose fraction of solid matter was cca 50%) were much lower and ranged from −17 to −23 °C depending on the sort of sugar added (Table 2).

Neither Na-alginate nor starch addition had significant influences on the phase transition temperatures mostly because of their relatively small fraction and high molecular weights.

The thawing temperatures were higher than the freezing temperatures which was already mentioned in some earlier investigations (HEGEDUŠIĆ & LOVRIĆ, 1985; LOVRIĆ et al., 1986; HEGEDUŠIĆ et al., 1991).

As already mentioned, all the products were submitted to cyclic freezing and thawing. After the first thawing, the freezing temperature of samples decreased by 1 to 4 degrees, while the initial thawing temperature remained the same or slightly increased. After the second and third thawing the freezing temperatures increased by several degrees, and at the end of the experiment, in most products, they were somewhat higher or equal to those before freezing. At the same time, the thawing temperatures remained mainly unchanged with reference to those after the first thawing.

Table 2

Freezing temperatures of investigated products

	Sample	T_f/°C	After first thawing T_f/°C	After second thawing T_f/°C	After third thawing T_f/°C
1	Apple purée	−1.0	−5.0	−2.0	−1.0
2	Apple purée + sucrose	−17.0	−18.0	−14.0	−11.0
3	Apple purée + glucose	−19.5	−21.0	−18.0	−19.0
4	Apple purée + fructose	−22.0	−24.0	−22.0	−18.0
5	Apple purée + sucrose + glucose	−19.0	−21.0	−20.0	−19.0
6	Apple purée + sucrose + glucose + fructose + Na-alginate + citric acid	−21.0	−24.0	−22.0	−19.0
7	Apple purée + sucrose + glucose + fructose + Na-alginate + starch + citric acid	−22.0	−25.0	−23.0	−22.0

The temperatures of DTA thawing peaks changed insignificantly during cyclic freezing and thawing. The greatest changes occurred in the apple purée products with sugar as well as with starch and alginate addition. From the results stated above it is evident, that the most significant changes in the phase transition temperatures occurred after the first freezing, while after the second and the third cycle of freezing and thawing these changes were minor. That could be explained by the phenomenon of rupturing of the product structure as well as by changes in the kind of binding of some components (starch, alginate, sugar) that were most intensive only during the first crystallization.

From the results concerning rheological properties it can be shown that the apple purée-like products belong to the group of thixotropic non-Newtonian systems (Figs. 3 and 4), that flow behaviour index values (n) range from 0.30 to 0.38, while the consistency coefficient values (k), regardless of almost the same fraction of solid matter, differ significantly (Table 4).

Table 3

Thawing temperatures of investigated products

Sample				After first thawing		After second thawing		After third thawing	
		T_{th} (°C)	T_p	T_{th} (°C)	T_p	T_{th} (°C)	T_p	T_{th} (°C)	T_p
1	Apple purée	3.5	0.5	−3.0	1.0	−3.0	1.0	−3.0	1.0
2	Apple purée + sucrose	−15.0	−6.0	−13.0	−6.0	−14.0	−6.0	−14.0	−7.0
3	Apple purée + glucose	−13.0	−8.0	−14.0	−9.5	−15.0	−9.0	−12.0	−9.5
4	Apple purée + fructose	−16.0	−9.0	−12.0	−9.5	−13.0	−10.0	−14.0	−10.0
5	Apple purée + sucrose + glucose	−14.0	−9.0	−14.0	−8.0	−14.0	−8.0	−15.0	−9.5
6	Apple purée + sucrose + glucose + fructose + Na-alginate + citric acid	−15.0	−10.0	−13.0	−9.5	−13.0	−8.5	−13.0	−7.5
7	Apple purée + sucrose + glucose + fructose + Na-alginate + starch + citric acid	−13.0	−9.0	−13.0	−8.0	−13.0	−8.0	−12.0	−7.5

T_{th}: thawing temperature (beginning of thawing)
T_p: temperature of the maximum of DTA thawing curve (end of thawing)

The highest consistency was registered for apple purée with starch and alginate addition (43.7 and 56.84 Ns^nm^{-2} respectively), whilst the lowest consistency was found in those into which only sugar was added. It is interesting to mention that the sugar addition, regardless of its kind (sucrose, glucose, fructose), effected the increase of consistency although the fraction of solid matter was ca 10 times greater.

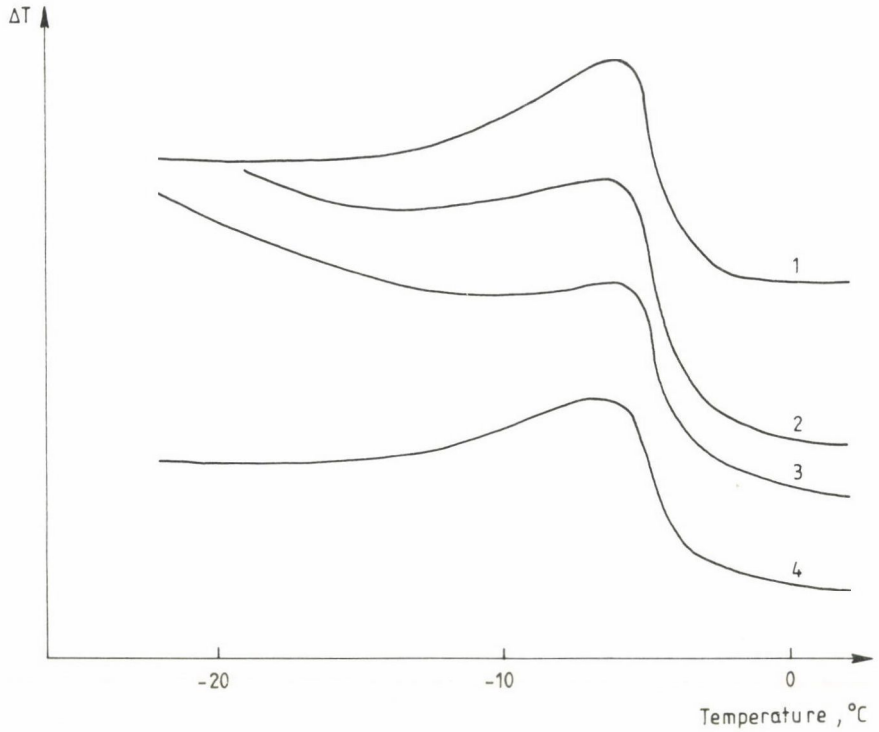

Fig. 2. DTA thawing curves of apple purée with sucrose addition determined before freezing (1) and after first (2), second (3) and third (4) thawing

This leads to the conclusion, that the consistency of apple purée-like products depends not only on the mass fraction but also on the kind of the solid matter that has been added (sugar, starch, alginate), which is in agreement with the results of the authors' earlier investigations. From the low coefficients of the thixotropic area it could be considered that apple purée has a transient behaviour between pseudoplastic and thixotropic systems. However, all other examined apple purée-like products exhibited a well-marked thixotropic character.

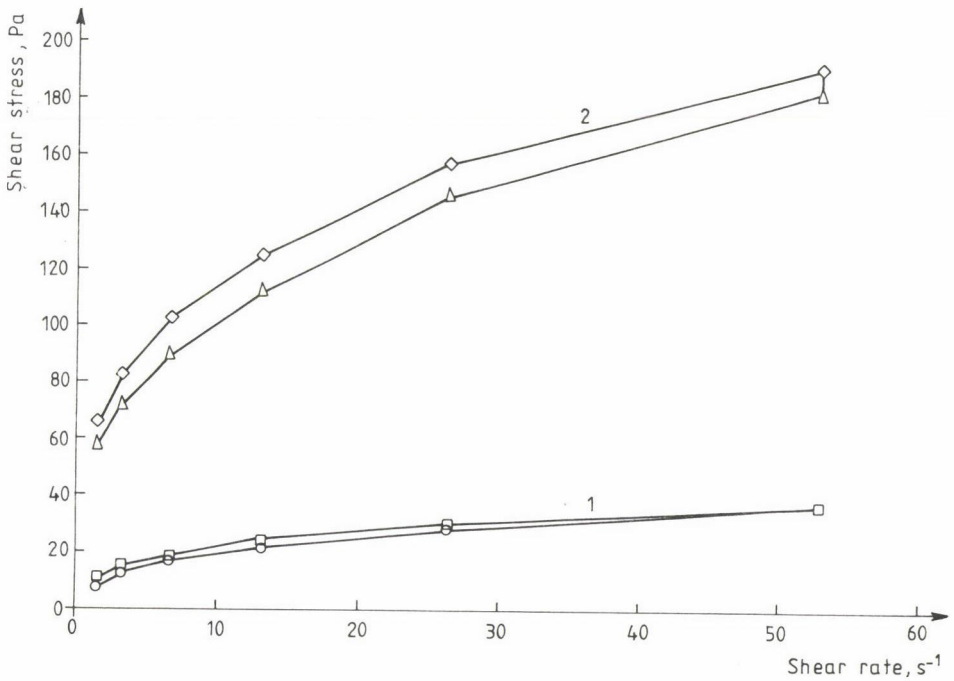

Fig. 3. Shear stress (τ) and shear rate (γ) relationship of some apple purée-like products. 1: apple purée, 2: apple purée + sucrose

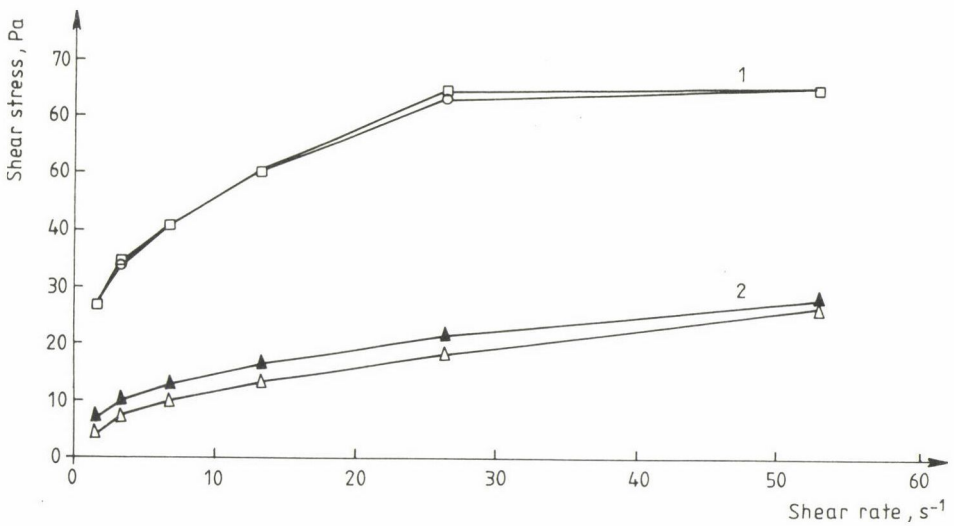

Fig. 4. Shear stress (τ) and shear rate (γ) relationship of some apple purée-like products. 1: apple purée + glucose, 2: apple purée + sucrose + glucose + fructose + Na-alginate + starch + citric acid

Table 4

Rheological parameters of apple purée-like products determined before and after freezing

	Sample	Phase of investigation	Coefficient of thixotropic area (k_{tp})	Flow behaviour index (n)	Consistency coefficient [k ($Ns^n m^{-2}$)]
1	Apple purée	before freezing	0.43	0.30	23.20
		after first thawing	0.43	0.30	16.60
		after second thawing	1.49	0.20	31.31
		after third thawing	0.61	0.25	30.12
2	Apple purée + sucrose	before freezing	2.43	0.37	6.23
		after first thawing	2.61	0.37	7.65
		after second thawing	3.03	0.35	16.14
		after third thawing	3.05	0.36	12.50
3	Apple purée + glucose	before freezing	1.90	0.34	9.85
		after first thawing	4.47	0.26	15.62
		after second thawing	3.40	0.32	15.92
		after third thawing	2.74	0.32	12.95
4	Apple purée + fructose	before freezing	2.47	0.30	7.45
		after first thawing	2.61	0.30	7.53
		after second thawing	3.76	0.30	10.75
		after third thawing	3.37	0.32	12.08
5	Apple purée + sucrose + glucose	before freezing	2.56	0.38	7.84
		after first thawing	4.20	0.35	16.52
		after second thawing	3.31	0.30	13.00
		after third thawing	4.62	0.30	13.42
6	Apple purée + sucrose + glucose + fructose + Na-alginate + citric acid	before freezing	9.59	0.31	43.70
		after first thawing	6.35	0.35	82.10
		after second thawing	7.07	0.30	62.53
		after third thawing	4.86	0.32	53.36
7	Apple purée + sucrose + glucose + fructose + Na-alginate + starch + citric acid	before freezing	11.40	0.31	56.84
		after first thawing	5.25	0.35	85.67
		after second thawing	6.27	0.25	84.67
		after third thawing	5.38	0.26	72.26

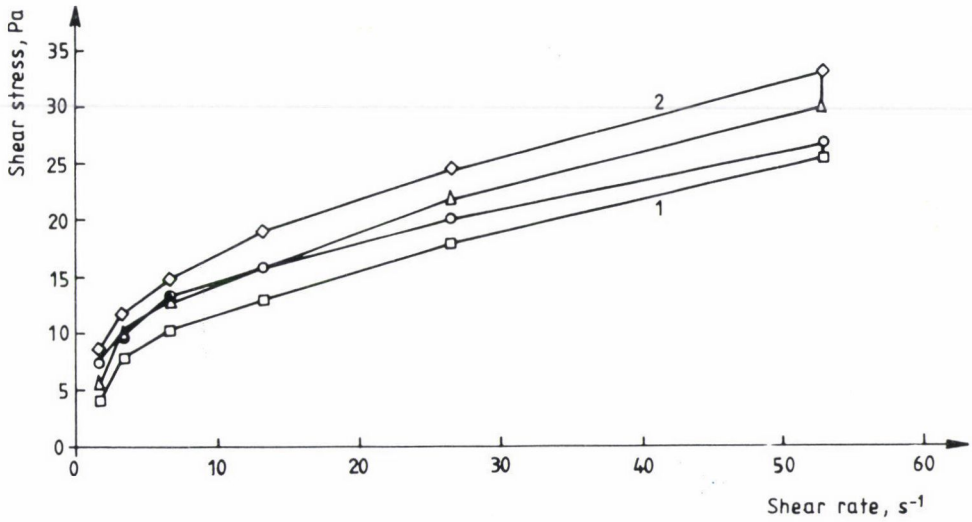

Fig. 5. Shear stress (τ) and shear rate (γ) relationship of apple purée with fructose addition determined before freezing (1) and after first thawing (2)

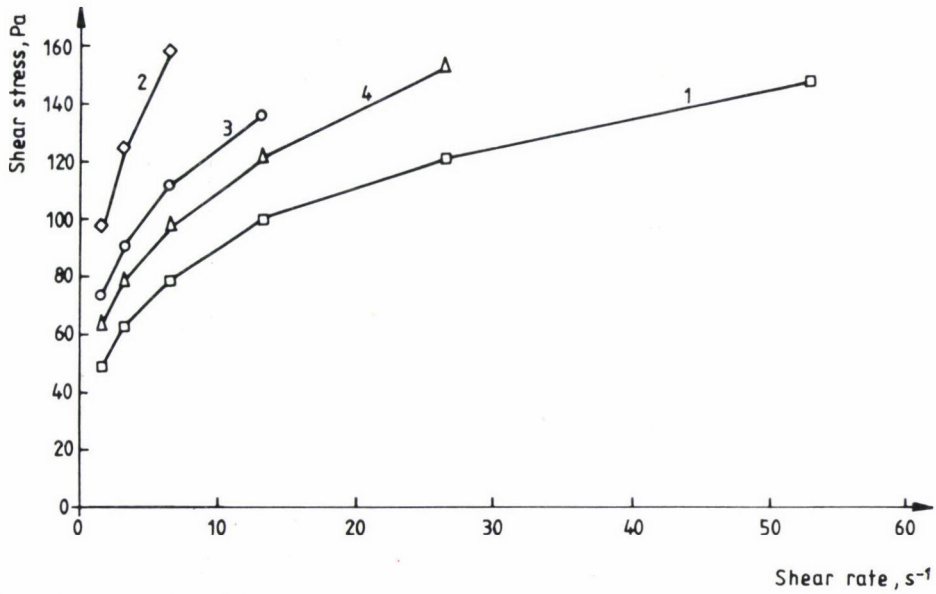

Fig. 6. Shear stress (τ) and shear rate (γ) relationship of apple purée with sucrose, glucose, fructose, Na-alginate and citric acid addition before freezing (1) and after first (2), second (3) and third (4) thawing (rising part of thixotropic loops)

Cyclic freezing and thawing caused also certain changes in the rheological properties of the products. All of them kept their thixotropic character (Figs. 5 and 6), but the thixotropic area values changed. These changes were lower as the composition of the products was simpler. Thus in the case of apple purée it could be neglected. The k_{tp} values of more complex products (samples 6 and 7) decreased significantly during freezing and thawing. It was also registered, that the thixotropic areas of the apple purées with sugar addition after freezing increased, and those with starch and alginate addition decreased, which could be connected with the nature of their water binding.

After freezing the consistency of all the products became quite different in relation to the consistency before freezing. In apple purée it decreased, but in all other samples, especially in those with alginate addition, increased.

Repeated freezing increased consistency in both apple purée and apple purée with sugar addition whilst when alginate was added to the same products, a decrease in consistency was observed.

After the third thawing, the consistency coefficient values of all samples were higher then before freezing.

3. Conclusions

The phase transition temperatures of the apple purée-like products investigated, depend both on the fraction and the kind of ingredients added, especially on the kind of sugar. After freezing they change, because of the rupturing of structure caused by the crystallization of water.

All of the investigated products belong to the group of thixotropic non-Newtonian systems whose consistency depends less on the mass fraction and more on the kind of the solid matter (sugars, starch, alginate). After freezing, the consistency of apple purée decreases. On the contrary it increases in the case of the products with more complex composition and with higher solid matter fraction.

Cyclic freezing and thawing affects the change of consistency of apple purée-like products as well as their thixotropic character. Whereas, the consistency mainly increases, the thixotropic area value changes, depending on the components (sugars, starch, alginate) which have been added.

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ENZYMATIC MODIFICATION OF BUFFALO MILK PROTEINS

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α-Chymotryptic hydrolysate of buffalo milk proteins was enriched in methionine by enzymatic peptide modification (EPM) to improve the biological value. Results revealed that part of the L-methionine ethyl ester was bound as methionine to the enzymatic hydrolysates of buffalo milk proteins. α-Chymotrypsin was the catalyst of the reaction. Methionine of the product (EPM4) was three times as high as that of the substrate protein. The covalent nature of the amino acid incorporation was supported by SDS polyacrylamide gel electrophoresis in the presence of urea. The isoelectric focusing zones of the products indicate that transpeptidation plays an essential role in the EPM reaction.

Results suggest that enzymatic peptide modification is a suitable method for increasing the nutritive value of buffalo milk proteins by the incorporation of a given quantity of the limiting essential amino acids.

Keywords: enzymatic peptide modification, EPM, SDS-PAGE, buffalo milk

The composition of milk has been a subject of interest to scientists for a long time. The composition of milk protein has generated great interest because of its nutritional and technological importance. Buffalo milk is popular in Egypt, as well as in other parts of the world, however, its proteins have not been fully investigated. HEWEDY and co-workers (1989) found that methionine content of buffalo casein micelles is 1.44%. EL-TOBGUI and ZAKI (1991) stated that buffalo caseins were somewhat more affected by coagulants than that of cow caseins. FUJIMAKI and co-workers (1977) recommended to the food industry to incorporate, into food proteins, the amino acids required by means of enzyme catalysis. Some results indicate that the transpeptidation has been considered as the mechanism of enzymatic peptide modification reaction (HOROWITZ & HAUROWITZ, 1959; YAMASHITA et al., 1970; HAJÓS et al., 1990). The enzymatic process is composed of aggregates held together by hydrophobic and ionic bounds (HOFSTEN & LALASIDIS, 1976; EDWARDS & SHIPE, 1978; SUKAN & ANDREWS, 1982). Transpeptidation in the course of enzymatic modification was also verified (HAJÓS & HALÁSZ, 1982; HAJÓS, 1986; HAJÓS et al., 1988b; HUSSEIN et al., 1991; HUSSEIN & HAJÓS, 1992). The enzymatic process allows enrichment of the proteolysis products whith essential amino acids. The amino acids

bound covalently to the C- and N-terminal end of the peptides (HAJÓS et al., 1988a; HAJÓS et al., 1989; LORENZEN & SCHLIMME, 1992).

The aim of this study was to improve the properties of the buffalo milk proteins by approaching a balanced essential amino acid composition.

1. Materials and methods

1.1. Materials

Raw bulk buffalo milk was obtained from the Faculty of Agriculture of Menofia University herd in Egypt. The milk was skimmed twice using a cream separator and freeze dried. α-Chymotrypsin, EC 3-4-21-Ir (Sigma) was used.

1.2. Preparation of protein hydrolysate

Buffalo milk protein was hydrolysed with α-chymotrypsin at pH 7.5, 37 °C for 2 h with stirring and after that freeze dried.

1.3. Enzymatic peptide modification (EPM)

The enzymatic peptide modification was carried out with α-chymotrypsin. The substrate concentration was 25% (w/v); the enzyme – substrate ratio was 1:100; pH 6.0; incubation temperature 37 °C; incubation time 16 h. L-methionine ethylester was added to the reaction mixture (Table 1). The EPM products were, after incubation, simultaneously dialyzed for 48 h through a cellophane membrane against distilled water. The nondialyzable fraction was freeze-dried.

1.4. Polyacrylamide gel electrophoresis (PAGE)

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS) was carried out with small modification of the method of WEBER and OSBORN (1975) using 10% polyacrylamide gels (bisacrylamide – acrylamide, 1:37) containing 0.1 mol sodium phoshate buffer (pH 7.2), 0.1% SDS and 6 mol urea. The electrophoresis buffer consisted of 0.1 mol sodium phosphate, 0.1% SDS, pH 7.2 and the sample buffer was 0.01 mol sodium phosphate, 1% SDS, 2% β-mercapto ethanol, pH 7.2. Samples were heated in sample buffer for 2 min at 100 °C, cooled and 6 mol urea was added. Electrophoresis conditions were 50 V for 1 h, followed by 100 V for about 4–5 h, until the dye front of added bromophenol blue had migrated about 9 cm. Protein staining was carried out with Coomassie Brilliant Blue R-250, involving the use of formaldehyde fixation to avoid losses of low molecular weight peptides. A

more sensitive staining was achieved by the silver staining procedure of MERRIL and co-workers (1982).

1.5. Densitometry

Densitometry of the gel slab was carried out by a Shimadzu corporation chart 200-91527.

1.6. Isoelectric focusing

Isoelectric focusing of EPM products and substrates was performed on glass slab 15 × 20 cm and 0.7 mm layer of 1.5 mol urea and 8.0% Sephadex containing 2% carrier ampholyte (Symolyte) pH 3.5 – 10.0. The prints obtained from the separations of Sephadex were stained for protein with Coomassie Brilliant Blue G 250.

1.7. Amino acid analysis

An aliquot of the samples was hydrolyzed with 6 mol HCl in a tube flushed with N_2 at 105 °C for 24 h and the amino acids were subjected to thin layer ion exchange chromatography. The amino acids were developed with an acetonic ninhydrin reagent by warming at 70 °C for 10 min.

In situ quantitative evaluation of the methionine was carried out by Biotech. Fischer densitometer (identified and compared to the control methionine).

2. Results and discussion

2.1. Electrophoretic patterns

The electrophoretic patterns and the densitograms of protein fractions of the buffalo milk protein, hydrolysate and EPM products separated by SDS-PAGE are shown in Figs. 1 and 2. These results illustrate that zones of small molecular mass α-chymotryptic hydrolysate of buffalo milk protein (in the range of 6000 – 20 000 Da) increased, whereas the yield in zones of high molecular mass decreased during the EPM reaction. The separation revealed little differences between EPM products (0.2 and 10), showed the greatest intensity on staining in the molecular range of about 5000 – 8000 Da

Minor zones were detected at positions corresponding to molecular weight about 14 000 and 22 000 Da. The results reveal also that the increase in the zones of high molecular mass and intensity became predominant above 20 000 Da of the EPM products (6, 8 and 10). These EPM products (6, 8, 10) showed definite zones ranged

from 6000 to 20 000 Da, these changes of the molecular mass and intensity are essentially influenced by the concentration of L-methionine ethyl ester in the reaction mixture.

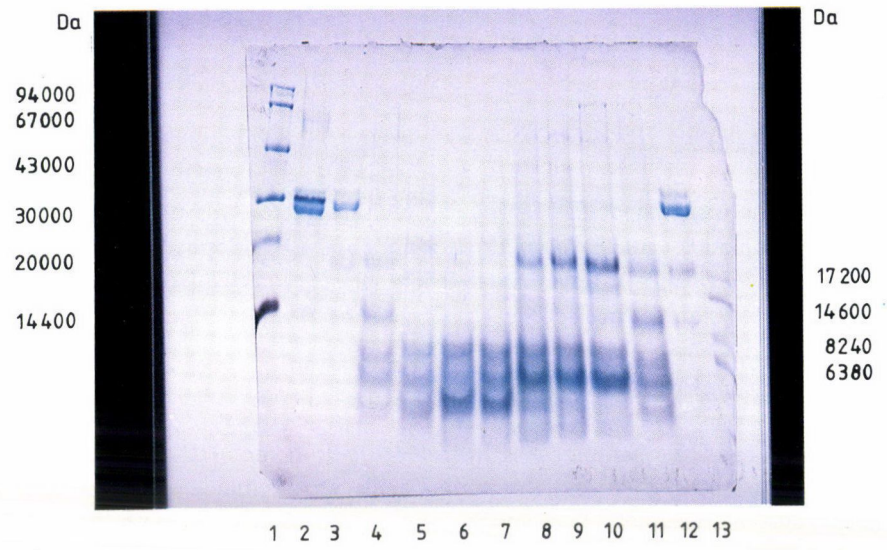

Fig. 1. SDS-PAGE patterns of the hydrolysate and modified buffalo milk proteins. 1: LMW, 2: Buffalo casein, 3, 12: Buffalo milk protein, 4, 11: Buffalo milk hydrolysate, 5: EPM 0; 6, 7, 8, 9, 10: EPM products with Met-enrichment (EPM 2, EPM 4, EPM 6, EPM 8 and EPM 10), 13: PMW

Figure 3 shows that considerable differences can be found between the peptides of the EPM products and those of the buffalo milk protein hydrolysate, and great differences between all EPM products. These differences may depend on the concentration of L-methionine ethyl ester in the reaction mixture. The changes in the zones indicate that transpeptidation is the major process in the enzymatic modification besides the amino acid enrichment.

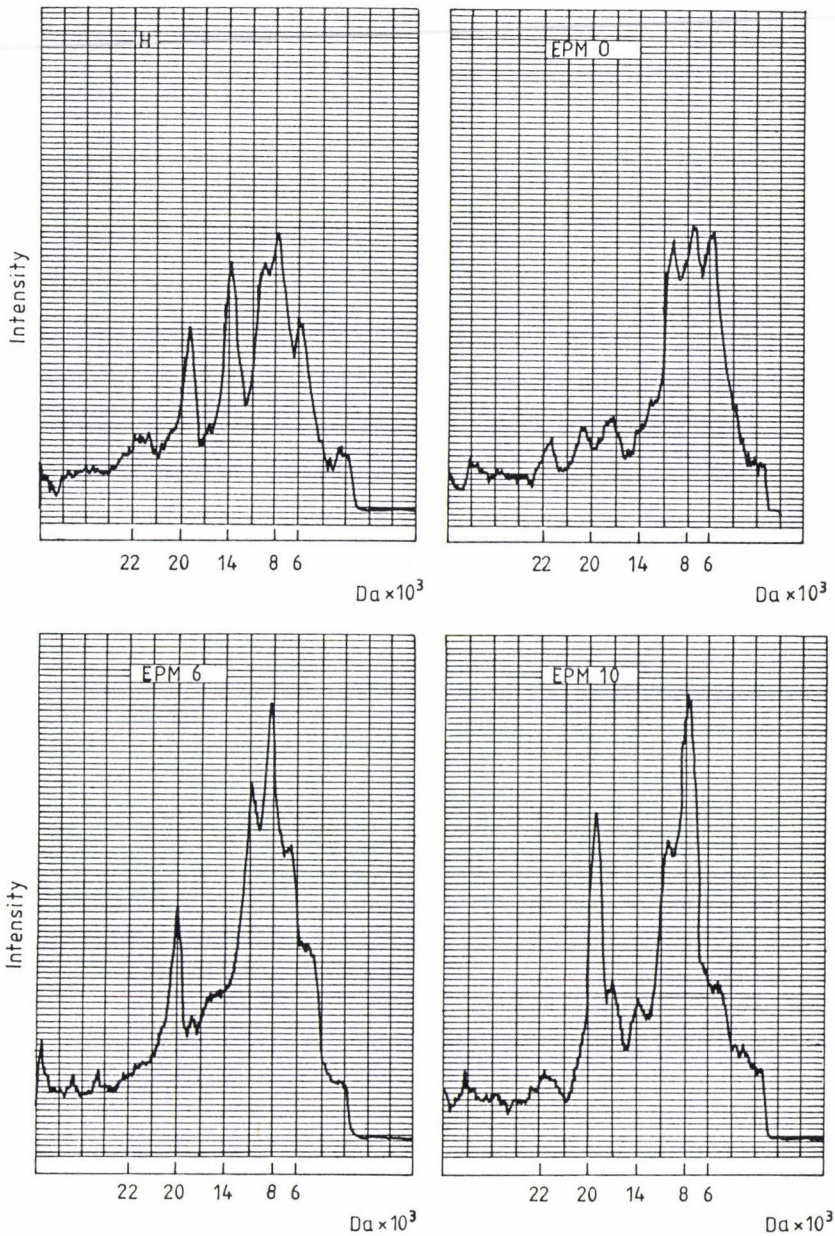

Fig. 2. Densitograms of protein fractions of the buffalo milk protein hydrolysate and EPM products separated by SDS-PAGE

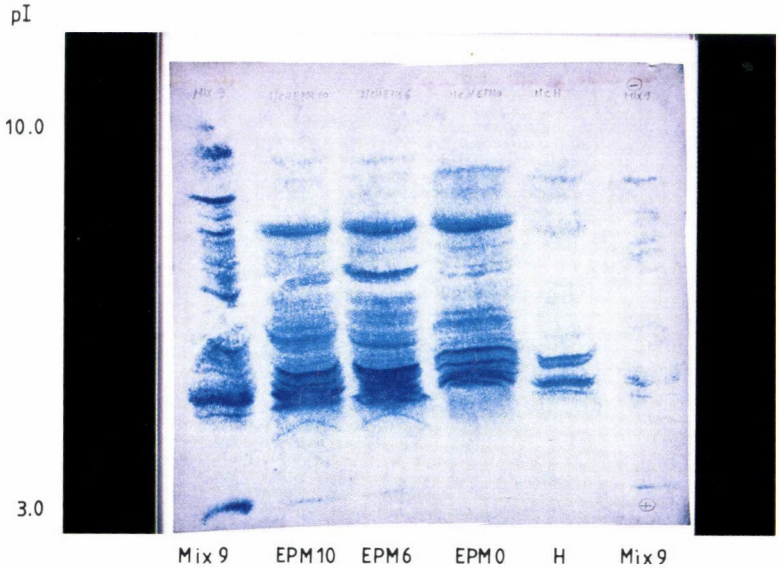

Fig. 3. Fractions obtained by isoelectric focusing on Sephadex of the EPM products and buffalo milk hydrolysates. Mix 9: control; EPM 10, EPM 6, EPM 0: enzymatically modificated proteins; H: hydrolysate of buffalo milk

2.2. Incorporation of L-methionine into buffalo milk proteins hydrolysate by enzymatic modification

Methionine content of buffalo milk protein, buffalo milk protein hydrolysate and EPM products are shown in Table 1.

This comparison shows methionine content in the EPM products with methionine incorporation higher than that of the substrate protein. Methionine content of EPM is greater by 3.8% than that of EPM 0. The optimum of L-methionine incorporation was found at a ratio of 34 g L-Met/100 g hydrolysate that is 48 g L-methionine ethyl ester/100 g buffalo milk protein hydrolysate.

Table 1

Methionine concentration in the reaction mixture and methionine content of the samples

EPM	Methionine added (g per 1 g hydrolysate)	Methionine covalently bound (g Met per 100 g protein)
0	0.00	1.58
2	0.14	3.21
4	0.34	5.44
6	0.48	3.77
8	0.63	6.61
10	0.91	5.65

Methionine content of buffalo milk proteins 1.52 g per 100 g protein

These results indicate that enzymatic peptide modification is a suitable method for increasing the biological value of buffalo milk protein by the incorporation of a given quality of the limiting essential amino acids according to the FAO/WHO recommendation (1973).

*

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COMPARATIVE STUDY OF SOME FERMENTATION PROPERTIES OF *STREPTOCOCCUS THERMOPHILUS* AND *LACTOBACILLUS ACIDOPHILUS* IN MILK AND MODIFIED MILK MEDIA

I. EFFECT OF THE NUTRIENT ADDITIVES AND FERMENTATION TEMPERATURE

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The growth and acid production of *Streptococcus thermophilus* and *Lactobacillus acidophilus* in single and mixed cultures were investigated. The bacteria were grown on sterilized cow's milk and milk media containing tomato juice and glucose at 40 and 45 °C, with inoculum of 3 and 5%. For the enumeration of the viable cell number, three nutrient media were compared. As a result of the statistical evaluation, the TGE medium (tryptone–glucose–yeast extract with 1% skim milk) proved to be the best in every case. Comparing the experiments, the mixed culture resulted in the highest acidity and cell count. Addition of 1% glucose to the milk had a slight effect comparing to the addition of 6% tomato juice. The addition of tomato juice to the milk media exerted a stimulatory effect on the growth of strains tested. Increasing starter percentage from 3 to 5% increased the acidity and the cell count. Comparing the effect of the fermentation temperature, the growth at 45 °C resulted in higher acidity and cell counts than the incubation at 40 °C.

Keywords: *Lactobacillus acidophilus, Streptococcus thermophilus*, mixed culture, nutrient medium

The therapeutic value of milk containing viable *Lactobacillus acidophilus* in controlling intestinal disorders has been known for a long time. Acidophilus milk or 'reform yoghurt' is the product obtained by fermenting milk with an authentic culture of *L. acidophilus*. However this microorganism grows slowly in milk. To overcome this problem some additives has been applied in the fermentation processes. Addition of 1% glucose to milk resulted in slight effect comparing to the β-galactosidase treatment (KHATTAB et al., 1986). Glucose and fructose have been reported to promote the growth of *Lactobacillus acidophilus* (MITAL et al., 1977;

SRINIVAS et al., 1990). Addition of tomato juice to skim milk has a similar effect and would be helpful in manufacturing acceptable acidophilus milk product. Tomato juice is a rich source of simple sugars, minerals and B-complex vitamins and its industrial application is suggested by many authors (DAVIS, 1970; LANG & LANG, 1975; MILLER & PUHAN, 1980).

STAMER and co-workers (1964) identified manganese as a stimulatory factor in tomato juice for promoting the growth of lactic acid bacteria. The tomato juice contained 6.1% total sugars, 9.0 mg magnesium per 100 g and 0.15 mg manganese per 100 g. NARKVIROY and RANGANNA (1976) found that the sugar content in tomato ranged from 2.44 to 4.70%. DAVIS and HOBSON GRAEME (1981) reported that tomato juice contained 11.0 mg magnesium per 100 g and 0.2 mg manganese per 100 g. The importance of the role played by starter cultures in successful production of fermented dairy products cannot be over-emphasized; they are the corner-stone upon which success or failure of the fermented dairy products processing depends. Years of research have been spent with studying their growth requirements, metabolism, preservation and selection as well as many other factors which influence their proper performance in fermented dairy products manufacture.

EL-SADEK and co-workers (1972) examined 50 random samples of Zabady from Cairo market for total microbial counts. The majority of the Streptococci isolates belonged to the *Streptococcus thermophilus* species. The majority of the Lactobacilli isolates belonged to the *Lactobacillus bulgaricus* species, followed by *Lactobacillus casei*, *Lactobacillus fermenti* and *Lactobacillus lactis*. Acidity is the main factor which controls the physical and chemical properties of fermented milks, also controls the extent of bacterial growth and the types of microorganisms growing (AMAL M. MOUSA et al., 1985). Comparative study on some chemical and bacteriological properties of yoghurt bacteria affected by the level of defatted soy flour in the milk media showed that the use of *Lactobacillus bulgaricus* for making fermented milk resulted in more acidity than *Streptococcus thermophilus*. RAO and GANDHI (1987) reported studies on microbial quality of acidophilus milk. Their results showed that the viable cell count of *Lactobacillus acidophilus* after 15-day storage at 5–8 °C in skim acidophilus milk was the highest (6.7×10^8 cm^{-3}), and had the greatest antimicrobial properties. Obviously acidophilus yoghurt can be made by adding concentrated culture of *Lactobacillus acidophilus* to normal yoghurt. BARIL and CARDWELL (1984) found that *Lactobacillus acidophilus* enhanced growth of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. HULL and co-workers (1984) reported the use of *Lactobacillus acidophilus* in manufacturing of yoghurt. CHOPRA and co-workers (1984) prepared a yoghurt-like product from soy milk alone, and after adding 10, 20 and 30% of skim milk supplemented with 1% sucrose. The starter cultures were single and mixed cultures of *Streptococcus thermophilus* and *Lactobacillus acidophilus*. Results showed that the addition of skim milk

enhanced the acid production and the mixed culture resulted in a better product than the single cultures.

GOH and co-workers (1990) studied the fermentation of ultrafiltration retentate by *Lactobacillus acidophilus* and *Streptococcus thermophilus*. The viable cells of lactic acid bacteria of the single culture of *Lactobacillus acidophilus* were similar in whole milk, UF retentate and evaporated milk, whereas the viable cells of the mixed culture of *Lactobacillus acidophilus* and *Streptococcus thermophilus* were higher in UF retentate. The mixed culture of *Lactobacillus acidophilus* and *Streptococcus thermophilus* produced more acid than the single culture of *Lactobacillus acidophilus*. The addition of minerals to whole milk, UF retentate and evaporated milk increased the acid production and the viable cell counts.

Present paper compares the growth and activities of the single and mixed cultures of *Lactobacillus acidophilus* and *Streptococcus thermophilus* in cow's milk, and studies the effect of addition of tomato juice and glucose.

1. Materials and methods

1.1. Cow's milk

It was obtained from the trade and sterilized before using. The composition of the milk: fat 2.8%, protein 3.4%, sugar 4.4%, ash 0.7%. Non-fat solid 8.5%, total solids 11.3%.

1.2. Cultures

Single strains of *Streptococcus thermophilus* and *Lactobacillus acidophilus* were obtained from the culture collection of the Department of Microbiology and Biotechnology of the University of Horticulture and Food Industry.

1.3. Preparation of tomato juice

Fresh, ripe and sound tomatoes were washed with water, sliced into small pieces and were heated at 90 °C for 5 min to facilitate pulping and inactivate pectin methyl esterase. After cooling the pulp to 40 °C and blending for about 30 sec, it was centrifuged at 1500 g for 5 min and sieved before sterilization. After a heat treatment of 121 °C, 15 min, a clear tomato juice was obtained.

1.4. Culture media for plate counting

The number of the viable cells was determined as colony forming units (cfu) in three culture media by plate counting. Total bacterial count was determined on Tryptone-Glucose-Yeast extract agar with addition of 1% skim milk (A.P.H.A., 1960). Lactic acid bacteria were determined on the Elliker (ELLIKER et al., 1956) and modified Elliker media.

1.4.1. Tryptone Glucose Yeast Extract (TGE) Agar

Tryptone	5	g
Yeast extract	2.5	g
Glucose	1	g
Agar	15	g
Distilled water	1000	cm^3

pH = 7.0

Before using 1% skim milk was added to the medium.

1.4.2. Elliker medium

Tryptone	20	g
Yeast extract	5	g
Gelatin	2.5	g
Glucose	5	g
Lactose	5	g
Sucrose	5	g
Sodium chloride	4	g
Agar	15	g
Distilled water	1000	cm^3

pH = 6.8

1.4.3. Modified Elliker medium. Elliker medium plus 8 g l^{-1} $CaCO_3$ and 2 cm^3 l^{-1} bromocresol green indicator (1.5% in 96% alcoholic solution).

1.5. Measuring of total acidity and pH

Total acidity was measured as lactic acid according to LING (1963). The pH values were measured with a RADELKISz OP-211/1 type Laboratory Digital pH Meter.

1.6. Experimental method

The fermentation experiments were run in Erlenmeyer flasks containing 200 cm^3 sterilized milk or modified milk media. The temperature was controlled by water bath with accuracy of ±0.1 °C. Samples were taken from the flasks and the total acidity, pH and cfu were determined in every other hour in case of single cultures, and in every hours in case of mixed cultures.

2. Results and discussion

2.1. Acid production and growth in milk

The experimental results of the fermentations are summarized in Tables 1–3. Mean values of log cfu were obtained by the calculation of the algebraical mean of three parallel log cfu results.

Table 1

Growth and acid production of Lactobacillus acidophilus *in milk media with 3% starter*

T (°C)	Time (h)	T.A. (%)	pH	Means of log cfu cm^{-3}		
				TGE	Elliker	M. Ell.
40	0	0.21	6.45	6.69	6.10	6.26
	2	0.25	6.38	7.16	6.75	6.58
	4	0.37	5.76	7.35	7.15	7.25
	6	0.66[a]	4.96	7.63	7.45	7.49
45	0	0.22	6.40	6.65	6.55	6.63
	2	0.27	5.99	7.27	7.01	7.05
	4	0.50[a]	5.39	7.55	7.35	7.38
	6	0.84[a]	4.56	7.95	7.58	7.63

T.A.: Total acidity in lactic acid (%)
[a]: Coagulated
95% confidence interval for log cfu means = ±0.12

Table 2

Growth and acid production of Streptococcus thermophilus *in milk media with 3% starter*

T (°C)	Time (h)	T.A. (%)	pH	Means of log cfu cm^{-3}		
				TGE	Elliker	M. Ell.
40	0	0.19	6.54	7.32	7.16	7.30
	2	0.29	6.05	7.78	7.52	7.58
	4	0.53[a]	5.35	7.89	7.73	7.74
	6	0.62[a]	5.20	8.05	7.99	8.01
45	0	0.21	6.45	7.15	6.98	7.07
	2	0.35	5.75	7.87	7.80	7.80
	4	0.56[a]	5.15	8.12	8.00	8.03
	6	0.77[a]	4.70	8.27	8.11	8.15

T.A.: Total acidity in lactic acid (%)
[a]: Coagulated
95% confidence interval for log cfu means = ±0.13

Table 3

Growth and acid production of Lactobacillus acidophilus *and* Streptococcus thermophilus *in milk media with 3% starter*

T (°C)	Time (h)	T.A. pH (%)	pH	Means of log cfu cm^{-3}		
				TGE	Elliker	M. Ell.
40	0	0.20	6.46	7.28	7.13	7.17
	1	0.23	6.41	7.49	7.32	7.37
	2	0.36	5.77	7.75	7.53	7.59
	3	0.54[a]	5.28	8.05	7.82	7.82
45	0	0.19	6.49	7.25	7.10	7.16
	1	0.29	5.96	7.71	7.57	7.60
	2	0.51[a]	5.36	8.10	7.87	7.81
	3	0.71[a]	4.83	8.23	7.99	8.03

T.A.: Total acidity in lactic acid (%)
[a]: Coagulated
95% confidence interval for log cfu means = ±0.10

As it is shown in Tables 1–3, the acid-production rate of the mixed culture was higher than that of the single cultures.

In case of the single cultures the time required to reach the coagulation of milk is 6 h at 40 °C and 4 h at 45 °C. The mixed culture reduces these times to 3 and 2 h. Acid production of the single and mixed cultures is shown in Figs. 1 and 2.

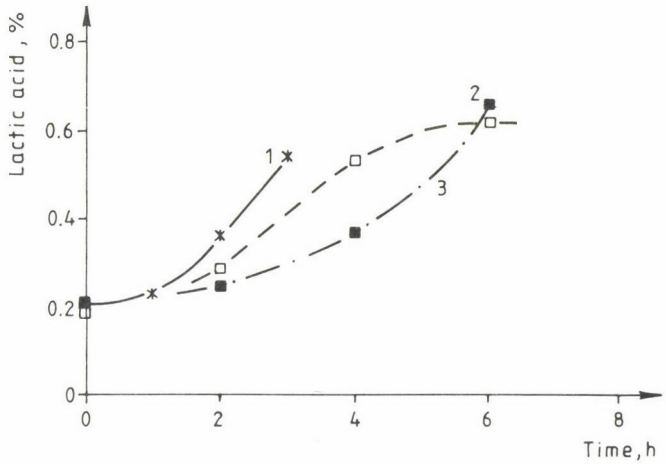

Fig. 1. Acid production in milk at 40 °C.
1: Lb. + St., 2: Lb., 3: St.; Lb.: *Lactobacillus acidophilus*, St.: *Streptococcus thermophilus*

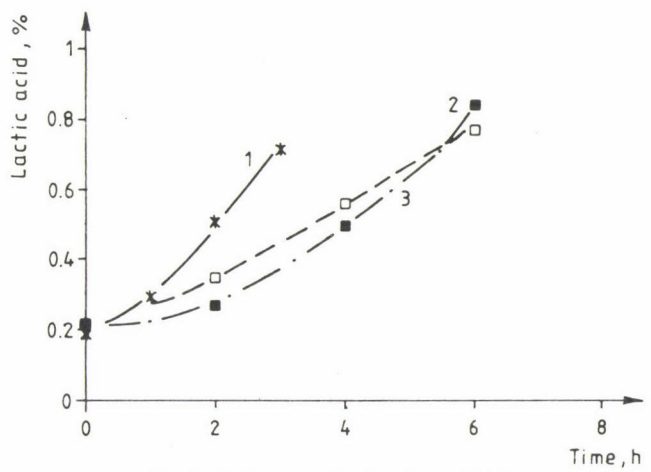

Fig. 2. Acid production in milk at 45 °C.
1: Lb. + St., 2: Lb., 3: St.; Lb.: *Lactobacillus acidophilus*, St.: *Streptococcus thermophilus*

The increased acid production of the mixed culture is based on the symbiotic growth of the two microorganisms.

Similarly to the acid production, significant difference exists between the growth of the single and mixed cultures of *Lactobacillus acidophilus* and *Streptococcus thermophilus*, as it is demonstrated by Figs. 3 and 4. The relative cell counts were calculated from the average of three parallel cfu values determined on the TGE agar.

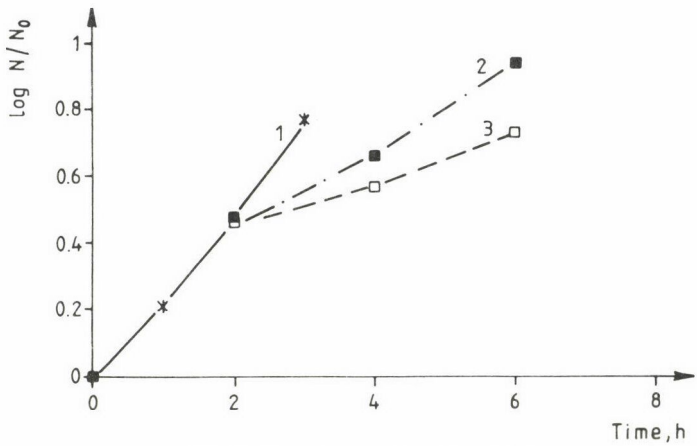

Fig. 3. Growth curves in milk at 40 °C.
1: Lb. + St., 2: Lb., 3: St.; Lb.: *Lactobacillus acidophilus*, St.: *Streptococcus thermophilus*

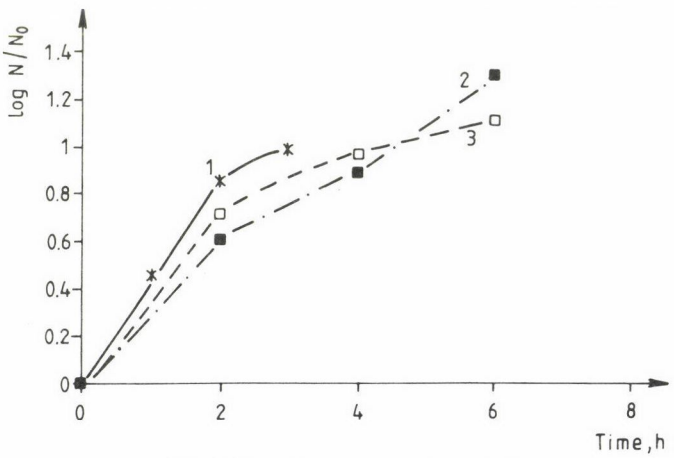

Fig. 4. Growth curves in milk at 45 °C.
1: Lb. + St., 2: Lb., 3: St.; Lb.: *Lactobacillus acidophilus*, St.: *Streptococcus thermophilus*

2.2. Effect of the recovery medium on the values of cfu

The effect of the recovery medium on the cell number determined by the several culture media was evaluated by analysis of variance, applying the STATGRAPHICS 4.0 program-package. The results are summarized in Tables 4–6.

Table 4

Analysis of variance for log cfu of Lactobacillus acidophilus

Source	Sum of squares	d.f.	Mean square	F
Main effects				
time	12.864017	3	4.288006	381.1
medium	1.044536	2	0.522268	46.4
temperature	0.728022	1	0.728022	64.7
Residual	0.731353	65	0.011252	
Total	15.367928	71		

Table 5

Analysis of variance for log cfu of Streptococcus thermophilus

Source	Sum of squares	d.f.	Mean square	F
Main effects				
time	9.171428	3	3.057143	233.1
medium	0.304753	2	0.152376	11.6
temperature	0.215606	1	0.215606	16.4
Residual	0.852408	65	0.013114	
Total	10.544195	71		

The residual values in the variance tables represent the variances of the three parallel plate counting results. As it is demonstrated by the significant F values, the log cfu is strongly affected by the recovery media. (The effect of the fermentation time and temperature was expected.)

Table 6

Analysis of variance for log cfu of Lactobacillus acidophilus *and* Streptococcus thermophilus

Source	Sum of squares	d.f.	Mean square	F
Main effects				
time	6.695304	3	2.231768	312.7
medium	0.483078	2	0.241539	33.8
temperature	0.610512	1	0.610512	85.5
Residual	0.463838	65	0.007136	
Total	8.252732	71		

The analysis of variance resulted in the same conclusion in the three fermentation, as it is demonstrated by Figs. 5–7.

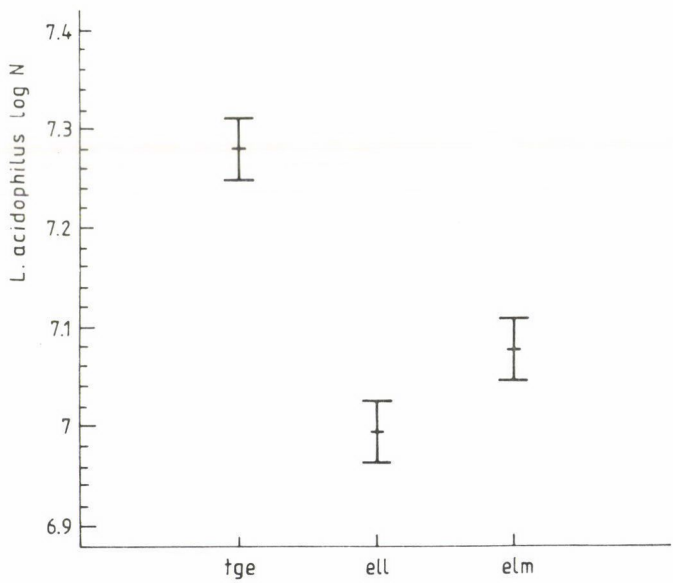

Fig. 5. Effect of the recovery media on the viable cell count of *Lactobacillus acidophilus*. Media: tge: TGE, ell: Elliker, elm: modified Elliker medium. 95% LSD intervals for means

The highest cell number can be obtained by using TGE medium. The cell numbers recovered on the modified Elliker medium were greater than those recovered on the Elliker medium, but the differences proved not to be significant in all cases.

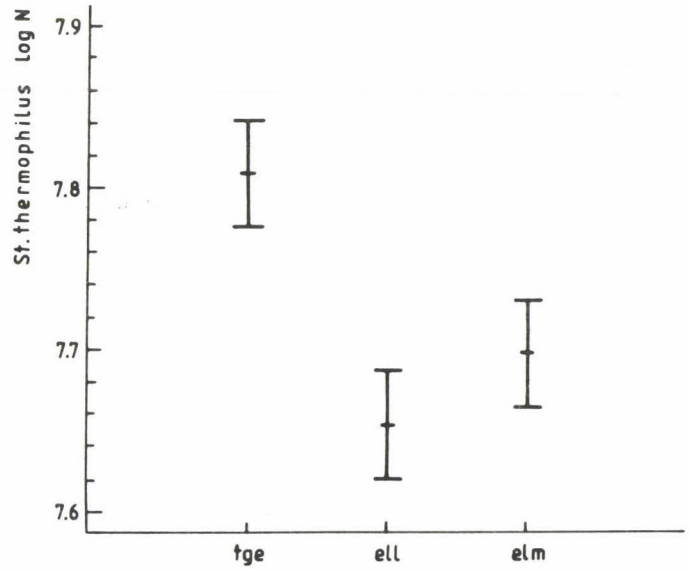

Fig. 6. Effect of the recovery media on the viable cell count of *Streptococcus thermophilus*. Media: tge: TGE, ell: Elliker, elm: modified Elliker medium. 95% LSD intervals for means

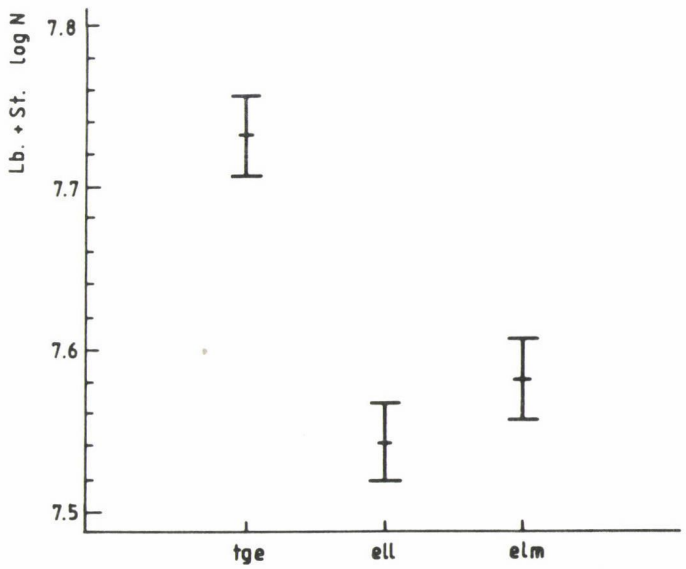

Fig. 7. Effect of the recovery media on the viable cell count of the mixed culture of *Lactobacillus acidophilus* and *Streptococcus thermophilus*. Media: tge: TGE, ell: Elliker, elm: modified Elliker medium. 95% LSD intervals for means

2.3. Acid production in modified milk media

The acid production of *Lactobacillus acidophilus* and *Streptococcus thermophilus* in single and mixed culture was studied in milk and modified milk media, applying different inoculum size and temperature.

The culture media were: cow's milk, cow's milk with 1% glucose and cow's milk with 6% tomato juice.

The measured values of the acidity and pH of the fermentations are summarized in Tables 7–9.

Table 7

Effect of inoculum concentration, nutrient additives and fermentation temperature on the acid production of Lactobacillus acidophilus

Time (h)	Cow's milk		Cow's milk + 1% glucose		Cow's milk + 6% tomato juice	
	T.A. (%)	pH	T.A. (%)	pH	T.A. (%)	pH
T = 40 °C	Inoculum = 3%					
0	0.20	6.43	0.20	6.41	0.23	6.36
2	0.25	6.16	0.25	6.15	0.27	6.10
4	0.34	5.77	0.35	5.75	0.41	5.56
6	0.64[a]	5.09	0.64[a]	5.08	0.71[a]	4.84
T = 40 °C	Inoculum = 5%					
0	0.22	6.40	0.22	6.38	0.24	6.34
2	0.29	6.13	0.29	6.12	0.31	6.05
4	0.41	5.55	0.42	5.53	0.50[a]	5.37
6	0.70[a]	4.69	0.71[a]	4.67	0.89[a]	4.39
T = 45 °C	Inoculum = 3%					
0	0.20	6.43	0.20	6.42	0.23	6.37
2	0.26	6.04	0.26	6.03	0.30	5.98
4	0.49[a]	5.38	0.50[a]	5.36	0.53[a]	5.32
6	0.79[a]	4.70	0.80[a]	4.68	0.85[a]	4.54
T = 45 °C	Inoculum = 5%					
0	0.22	6.40	0.22	6.38	0.24	6.34
2	0.30	6.00	0.30	5.98	0.34	5.93
4	0.56[a]	5.23	0.57[a]	5.20	0.62[a]	5.15
6	0.85[a]	4.58	0.87[a]	4.56	0.92[a]	4.47

[a]: Coagulated

T.A.: Total acidity in lactic acid

Table 8

Effect of inoculum concentration, nutrient additives and fermentation temperature on the acid production of Streptococcus thermophilus

Time (h)	Cow's milk		Cow's milk + 1% glucose		Cow's milk + 6% tomato juice	
	T.A. (%)	pH	T.A. (%)	pH	T.A. (%)	pH
T = 40 °C	Inoculum = 3%					
0	0.20	6.47	0.20	6.45	0.23	6.37
2	0.28	6.11	0.29	6.07	0.31	6.04
4	0.49[a]	5.37	0.50[a]	5.35	0.55[a]	5.22
6	0.59[a]	5.19	0.60[a]	5.16	0.64[a]	5.06
T = 40 °C	Inoculum = 5%					
0	0.22	6.42	0.22	6.40	0.24	6.34
2	0.32	6.00	0.33	5.96	0.37	5.90
4	0.54[a]	5.27	0.56[a]	5.21	0.58[a]	5.19
6	0.62[a]	5.14	0.63[a]	5.12	0.70[a]	4.85
T = 45 °C	Inoculum = 3%					
0	0.19	6.50	0.19	6.49	0.23	6.38
2	0.33	5.97	0.33	5.96	0.35	5.86
4	0.53[a]	5.31	0.54[a]	5.29	0.57[a]	5.24
6	0.74[a]	4.83	0.76[a]	4.80	0.79[a]	4.70
T = 45 °C	Inoculum = 5%					
0	0.22	6.42	0.22	6.40	0.24	6.36
2	0.34	5.85	0.35	5.86	0.40	5.76
4	0.57[a]	5.23	0.58[a]	5.22	0.63[a]	5.12
6	0.77[a]	4.73	0.78[a]	4.71	0.81[a]	4.69

[a]: Coagulated
T.A.: Total acidity in lactic acid

On the base of the experimental results the following conclusions can be drawn.

The difference between acid productions of single and mixed cultures was the same as in case of the fermentations in milk. The mixed cultures resulted in the highest acid production rate of all treatments.

Table 9

Effect of inoculum concentration, nutrient additives and fermentation temperature on the acid production of the mixed culture of Lactobacillus acidophilus *and* Streptococcus thermophilus

Time (h)	Cow's milk		Cow's milk + 1% glucose		Cow's milk + 6% tomato juice	
	T.A. (%)	pH	T.A. (%)	pH	T.A. (%)	pH
T = 40 °C	Inoculum = 3%					
0	0.19	6.47	0.19	6.47	0.23	6.38
1	0.23	6.38	0.24	6.23	0.26	6.10
2	0.33	5.96	0.35	5.72	0.38	5.68
3	0.50[a]	5.35	0.51[a]	5.31	0.57[a]	5.20
T = 40 °C	Inoculum = 5%					
0	0.22	6.44	0.22	6.41	0.24	6.35
1	0.25	6.17	0.26	6.10	0.27	6.08
2	0.36	5.70	0.37	5.69	0.42	5.59
3	0.57[a]	5.19	0.58[a]	5.18	0.62[a]	5.12
T = 45 °C	Inoculum = 3%					
0	0.19	6.55	0.19	6.53	0.23	6.40
1	0.26	6.10	0.27	6.06	0.29	6.02
2	0.48[a]	5.44	0.49[a]	5.39	0.50[a]	5.36
3	0.68[a]	4.88	0.70[a]	4.80	0.75[a]	4.72
T = 45 °C	Inoculum = 5%					
0	0.22	6.46	0.22	6.44	0.24	6.37
1	0.28	6.06	0.29	6.03	0.31	6.00
2	0.50[a]	5.37	0.52[a]	5.34	0.54[a]	5.31
3	0.70[a]	4.80	0.75[a]	4.74	0.78[a]	4.70

[a]: Coagulated
T.A.: Total acidity in lactic acid

Addition of 1% glucose to the milk resulted in slight effect comparing to the addition of 6% tomato juice in all treatments. The higher acidity of the fermentations performed with tomato juice caused partially by the acidity of this additive (0.02–0.04% lactic acid at the beginning). The difference in acidity is increasing during the fermentation and at the end of the measured period (6 h in single, and 3 h in mixed cultures) it reaches 0.07–0.08%. Taking the ending value of the acidity (0.7–0.9%) into account, the relative difference is about 10%.

The effect of temperature was as expected. The acid production rates at 45 °C were higher than those at 40 °C in every case.

Similarly to the effect of the increasing temperature, the increasing inoculum size resulted in higher acid production rate both in single and mixed cultures.

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FREE RADICALS AND SCAVENGERS IN PLANT AND ANIMAL FOODS

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Free radical reactions inducing lipid peroxidation were investigated in animal tissues and foods. Trace minerals may enhance free radical reactions in pigs as given in premix supplementation. The tissues of a new pig genotype kept in a natural lifestyle proved to be resistant against free radical formation. Characteristics of lipid peroxidation give a positive correlation with the fat content in the animal tissues. Chemiluminescence technique gives a rapid overall picture on the scavenger capacity and enzymic defense system in animal tissues and foods.

Keywords: lipid peroxidation, membrane damage, antioxidant scavenger, enzymic defence, chemiluminescence

Formation of free radicals in living organism is essential to maintain the normal physiological conditions. The functional balance is kept by defending mechanisms of chemical and enzyme origin. In unbalanced cases the excess of the reactive free radicals and their intermediates may damage the membrane structure. The changes in the permeability of tissue will cause degenerative processes and later various diseases in the living organism, and in a similar way some detrimental changes in the quality of foods.

The pathway of the mentioned process is given in Fig. 1. If the formation of free radicals is not controlled, it gives way to the degradative products of lipid peroxidation.

Our aim was to investigate the free radical formation in meat producing animals and also in various foods of animal and plant origin. We wanted to get more information on the exterior and interior effects influencing the free radical reactions and scavengers in food systems.

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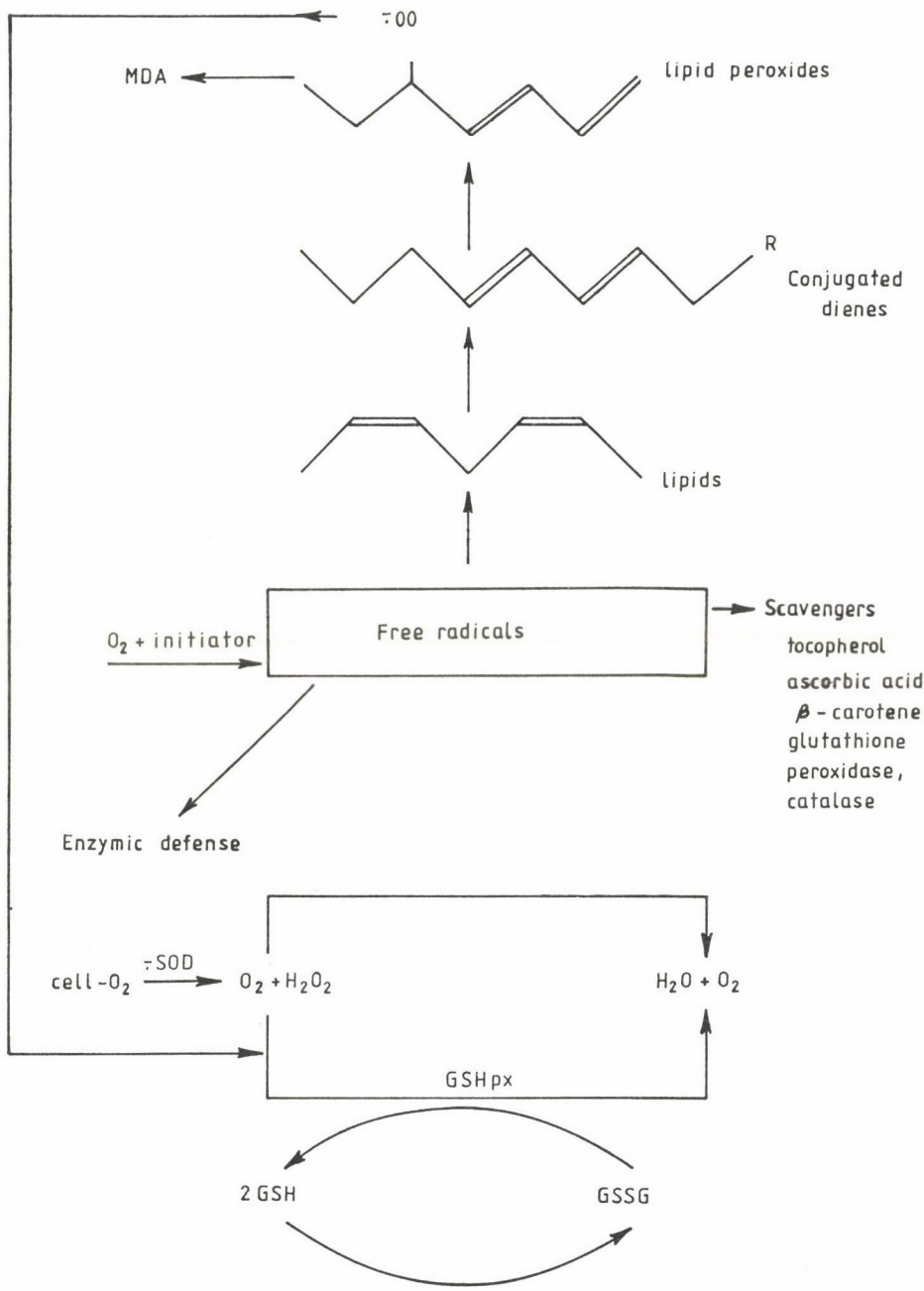

Fig. 1. Pathways of free radical reactions and the enzymic defense system

1. Materials and methods

Details of samples for analytical purpose are given in earlier publications (DWORSCHÁK et al., 1991; 1992).

The food samples were mixed with tenfold amounts of buffer solutions using a homogenizer (Thyristor, Janke and Kungel K. Gesellschaft) as described in methods. Homogenization took 5 minutes.

Two principles of the chemiluminescence technique were used: first the direct evaluation of free radicals; second: the defending enzyme (Superoxide dismutase = = SOD and Glutathione peroxidase = GSHpx) activities and the scavanger capacity of the meat samples (ZSINKA et al., 1988). The measurement was carried out with a CLD I Medicor-Medilab Luminometer (Hungary).

For the direct evaluation the luminol reagent solution consisted of 0.7 mmol l^{-1} luminol, 38 μmol l^{-1} hemin, 11.8 mmol l^{-1} Na_2CO_3 adjusted to pH 10 and deareated with N_2. Reagent of 200–4500 μl was added to the tissue homogenate (20 μl 1 mg cm^{-3} protein). The chemiluminescence intensity was expressed in mV, and it was recorded. Maximal intensity was 10 mV. The evaluation of a sample takes 60 s.

In the second case we measured the capacity of tissues to scavenge the superoxide radical evaluating the decrease of chemiluminescence intensity caused by the adrenaline-luminol reaction. The cuvette contained 20 μl tissue homogenate (1 mg cm^{-3} protein) 50 μl 10^{-3} mol l^{-1} adrenaline, 1000 μl luminol reagent and water up to the total volume of 1150 μl. Similarly the capacity of tissue to scavenge the peroxide radicals was determined by the reaction between luminol and hydrogen peroxide. The cuvette contained 20 μl tissue homogenate (1 mg cm^{-3} protein), 50 μl 8.8×10^{-5} mol l^{-1} H_2O_2, 1000 μl luminol reagent and water up to the total volume of 1150 μl.

Malondialdehyde (MDA) was determined photometrically with the thiobarbituric acid reagent (OHKAWA et al., 1979).

Conjugated dienes were assayed by the absorbance at 233 nm (A.O.A.C., 1984).

Among the enzymic defense, the activity of superoxide dismutase (SOD) was measured according to WINTERBOURN and co-workers (1975) on the basis of inhibition of adrenaline oxidation.

The activity of glutathione peroxidase (GHSpx) was evaluated by the photometric measurement of reduced glutathione after reacting with 5.5'-dithiobis (2-nitro-benzoic acid) (NOGUCHI et al., 1973).

Numerical data represent the average of four or five measurements. Standard deviations are also given in the text and in the figures, respectively.

For the statistical evaluation regression analysis was used where appropriate.

2. Results and discussion

2.1. Kahyb piglets reaching 30 kg body weight were divided into three groups. Control received 3% ISV-647 mineral and vitamin supplement (premix). Supplement was deprived in group 2 when the animals reached a body weight of 75 kg. No supplement (premix) was given to animals in group. The levels of conjugated dienes in the pork liver samples are shown in Table 1. The level of conjugated dienes was significantly lower in liver than in the other examined tissues. By the use of variance analysis it is clear, that total premix deprivation induced a significantly less intensive free radical formation and lipid peroxidation in spare ribs and chops as compared to the controls. It was also found that in spare ribs with high fat content the lipid peroxidation is significantly more intensive than in chops. Statistical data showed a strong correlation between the two effects – the fat content and the premix supplementation – which influence the free radical reactions. The results may be attributed to the catalytic effect of trace minerals in the premix.

Table 1

Effect of premix deprivation on conjugated dienes in porks and pig liver

	Conjugated dienes A_{233}		
		Premix deprivation	
Tissue	Control	Partial	Total
Spare ribs	25.5 ± 8.9[a]	20.3 ± 13.6	12.7 ± 7.2[a']
Chop	9.2 ± 3.2[b]	6.1 ± 2.0	4.0 ± 2.2[b']
Ham	4.3 ± 2.1	6.6 ± 3.6	4.6 ± 2.6
Liver	1.5 ± 0.2*	1.6 ± 0.2*	1.6 ± 0.2*

a – a' and b – b': significant difference using analysis of variance

*: significant difference to every pork sample in the same group; $P \leq 0.001$

2.2. A new pig genotype of Hungarian Big White (75%) and mangalica (pigs with curly bristles, 25%) was bred and then pigs were kept in a small farming system, with all possibilities of a natural lifestyle. They did not receive either antibiotics or yield increasing hormones. The pigs were slaughtered at bodyweight of approximately 160 kgs. Control pigs of genotype Hungarian Big White were kept in a large-scale farming system and slaughtered at 100 – 120 kgs of bodyweight. The two groups (12 animal each) received practically the same feed.

Table 2 shows the MDA contents and SOD activities, referring to the parameters of lipid peroxidation in the animal tissues. MDA levels were significantly lower in the pork of the new genotype compared to the traditionally bred pigs. In the liver, ham and chop samples of the new genotype the SOD activity was higher than in the control, referring a more active enzyme defense system. Our results pointed out, that in the tissues of the new pig genotype a remarkable resistance was found against the free radical formation.

Table 2

Malondialdehyde concentration and superoxide dismutase activity in the various body parts of pigs from a new genotype and from animals bred on large-scale farming
(n = 12)

	Malondialdehyde (MDA) nM/100 g		Superoxide dismutase (U/mg prot.)	
	New genotype	Control	New genotype	Control
Spare rib	6.4 ± 1.7***	13.6 ± 3.9	7.4 ± 1.5	6.5 ± 2.5
Chop	4.4 ± 2.0***	10.8 ± 2.2	8.2 ± 2.2*	5.5 ± 1.5
Ham	6.6 ± 2.7***	12.0 ± 2.5	11.1 ± 3.1***	6.0 ± 0.6
Liver	49.8 ± 12	58.6 ± 13	16.9 ± 2.0	13.0 ± 1.0

* significant at $P \leq 1\%$ probability level,
*** Very highly significant at $P \leq 0.1\%$ probability level

2.3. Investigating the effect of food composition we found that in fresh pork sample MDA and the conjugated dienes gave fairly good correlation with the fat content (Fig. 2). This means that the formation of free radicals and lipid peroxides starts already in fresh meat and its extent is proportional with the amount of fats.

2.4. Storage conditions may decrease the activity of the enzymic defense system. Figure 3 shows the changes of the glutathione-peroxidase activity in pork samples stored in various circumstances. Storing experiments were carried out one side at 4 °C for 2 and 4 days and other side at −18 °C, deep frozen state for 3 months. The activity significantly decreased in pork after storing for 2 and 4 days at 4 °C. Details for the conditions are given in Fig. 3.

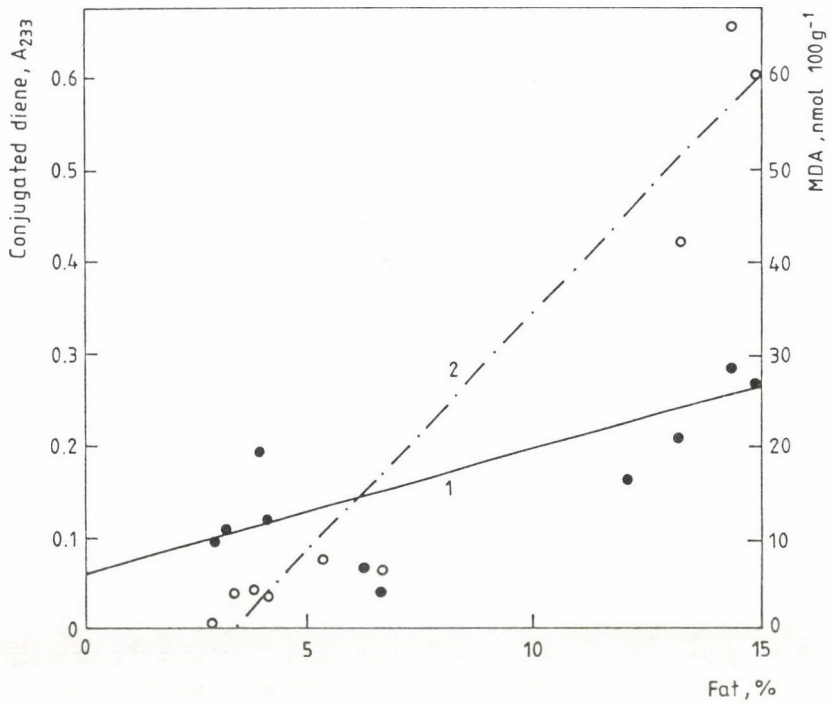

Fig. 2. Changes of MDA and conjugated diene levels in fresh meats as a function of fat content. 1: MDA ($y = 1.199x + 6.10$; $r = 0.74$); 2: conjugated diene ($y = 0.046x - 0.168$; $r = 0.90$). The value of correlation coefficient (r) between MDA and conjugated dienes = 0.84

2.5. Chemiluminescence technique has a wider acceptance because of its high speed and sensitivity. Scavenging of superoxide and peroxide radicals has an overall information both for the scavenger compounds (antioxidants) and enzymic defence system.

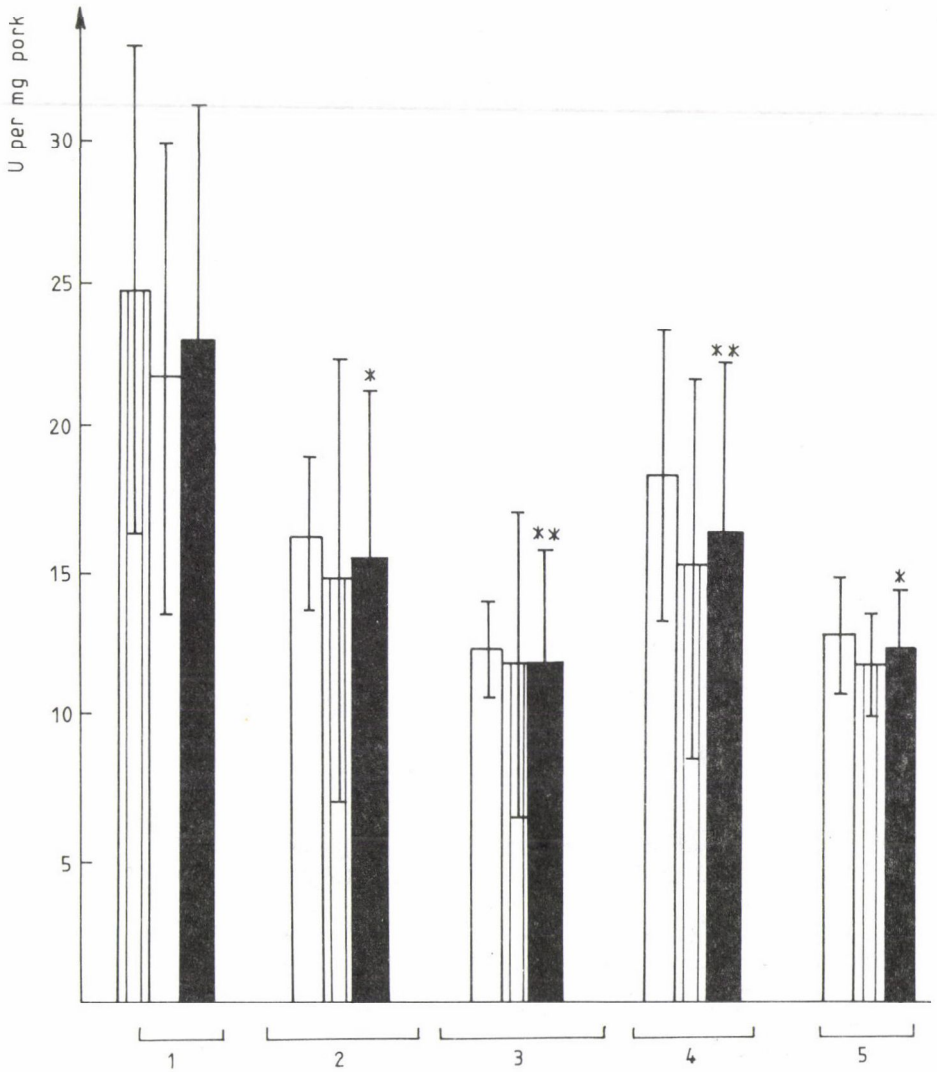

Fig. 3. Effect of storing and deep-freezing on the glutathione-peroxidase activity in pork. *: $P \leq 0.05$; **: $P \leq 0.005$ (compared to the fresh pork); □ fat > 13%, n = 4; ◫ fat < 13%, n = 5; ▪ total samples, n = 9. 1: Fresh; 2: stored at 4 °C for 2 days; 3: stored at 4 °C for 4 days; 4: deep-frozen, defrosting at 4 °C; 5: deep-frozen, defrosting in microwave oven

Figure 4 introduces the scavenger capacity of various animal tissues. Ox-liver homogenate has a higher scavenger capacity than the fatty goose-liver. Stored beef homogenate exerted a less scavenging effect as compared to the fresh tissue.

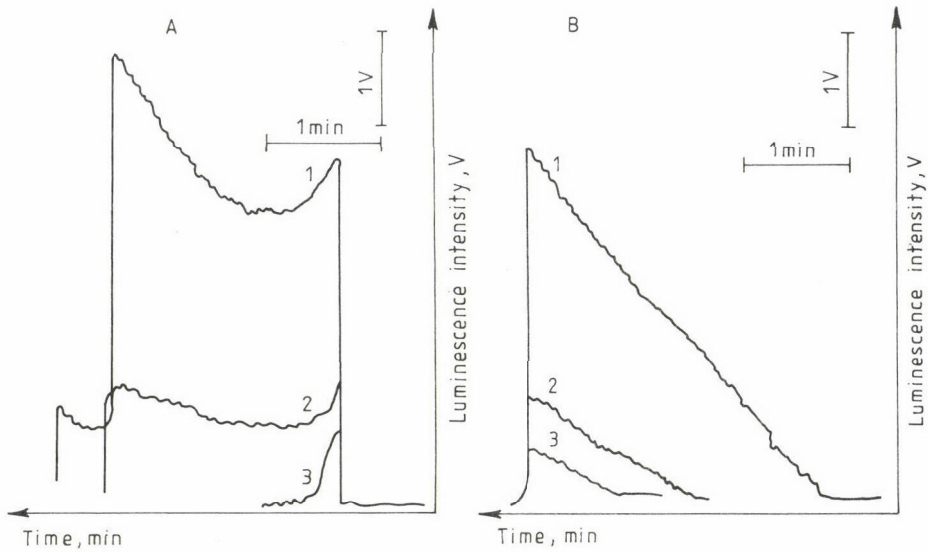

Fig. 4. Chemiluminescence intensity of the scavenger capacity in liver: A: scavenging peroxide radical; 1 = 30 µl 10^{-4} mol H_2O_2; 2 = +20 µl 3% goose-liver homogenate; 3 = +20 µl 3% ox-liver homogenate; B: scavenging superoxide radicals; 1 = 50 µl 10^{-3} mol adrenaline; 2 = +20 µl 3% stored beef homogenate; 3 = +20 µl 3% fresh beef homogenate

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SELENIUM CONTENT OF SOME HIGHER FUNGI

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The selenium content of different and higher macrofungi species was analysed. Although most of the species have no measurable selenium content, in a few cases high selenium level was found. On the basis of our results the Boletus species (*B. edulis, B. luridus*) can be regarded as selenium "accumulators", the highest concentration being about 3.0 mg per 100 g.

Species possessing high selenium content could be used as "medicinal" fungi. Since the human diet has a suboptimal selenium concentration in general, the ingestion of selenium "accumulating" fungi might serve as natural selenium supplements.

Keywords: selenium, accumulation, higher fungi, Boletus

Selenium is one of the microelements in tissues of plants, animals and human organisms (GIRLING, 1984; EHLIG et al., 1968). The effects of selenium deficiency have been registered in different groups of animals (chickens, calves, lambs). The symptoms of selenium-poisoning ("alkaline disease") were characterized in young horses, cows, pigs and lambs (WIESNER, 1967). Accumulation of selenium was found in certain taxonomical plant groups, i.e. in species of Fabaceae (DAVIES, 1972a) and Asteraceae (DAVIES, 1972b). The selenium concentrations in different grass species in Hungary were analysed (HARASZTI & VETTER, 1986). According to different authors (STIJVE, 1977; STIJVE & BESSON, 1976) the selenium concentration of wild, edible mushroom species ranges between 0.01 and 20.0 ppm (rare: 40 ppm), but the average selenium concentration is about 0.2 to 0.4 ppm (on dry weight basis). A few taxons of higher fungi, i.e. certain Agaricus and Boletus species (STIJVE, 1977; QUINCHE, 1980; QUINCHE, 1983) however have higher selenium content.

In the human organism the role of selenium is particularly questionable. This element is required for example for growth and normal proliferation. Selenium treatment helps in the vitamin E deficiency. The exact mode of action of selenium is unknown although an increase in the activities of arylsulphatase and β-glucoronidase was shown in selenium deficiency (PAIS, 1980). According to certain authors, the synthesis of ubiquinone (Coenzyme Q) is dependent on selenium; and this element

plays a role in the respiration chain and in the conversion of NAD-NADH. Certain selenium-compounds have anticarcinogen effect (PAIS, 1992).

Selenium has a positive role in the physiological-biochemical processes of the heart and the blood system. Selenium treatment can decrease the occurrence of different heart diseases. Human food of plant origin has relatively low selenium level, especially in case of cereals (0.25–1 ppm). There arises a very interesting and important question: can we find and use other foods as selenium sources?

The aim of this work was to measure the selenium concentration in different common mushroom species and to select these species (or other taxonomical units) with high selenium content. The present paper belongs to a series dealing with the minerals of higher mushrooms (VETTER, 1989; 1990a; 1990b).

1. Materials and methods

Mushroom samples (about 100) were gathered from different sites of Hungary. The fruit bodies (in a few cases caps and stipes were separated) were dried, ground and the samples digested in closed teflon bombs (HNO_3:H_2O_2 = 1:1, for 30 min at 121 °C). The selenium concentration of filtered and diluted materials was determined with ICP apparatus in four replications. The concentration of samples was characterized with standard deviation (SD) and coefficient of variation (CV). Selenium concentrations higher than 0.010 mg per 100 g (0.1 ppm) dry mass (DM) are given in Table 1.

2. Results and discussion

About 50 fungal species (and samples) were analysed, but only 18 samples contained measurable quantity (more than 0.1 ppm) of selenium (Table 1). The first 5 species belong to the wood destroying nutrition type and in general have a low selenium content. Low selenium level was measured in the cultivated common mushroom, *Pleurotus ostreatus* (0.21 mg per 100 g, DM), too. Other analysed species and samples – belonging to the orders Agaricales and Russulales – have variable selenium content. Significantly high selenium concentrations (accumulations) were measured in the Boletus species investigated. The measured concentrations varied

Table 1

The selenium concentration of certain mushroom species
(mg per 100 g DM)

Species and place of sampling	Arithmetical mean (mg per 100 g DM)	Standard deviation (SD)	Coefficient of variation (CV)
Family Aphyllophoraceae			
Leatiporus sulphureus (Bull.: Fr.) Murrill. /Sikáros, m. Pilis, poplar/	0.076	0.010	13.1
Family Polyporaceae			
Polyporus badius (Pers.: S. F. Gray) Schw. /Normafa, m. Budai, oak wood/	0.167	0.013	7.7
Hypholoma capnoides (Fr.: Fr.) Kummer /Őserdő, m. Bükk, pine wood/	0.211	0.015	7.1
Pholiota aurivella (Batsch.: Fr.) Kummer /Őserdő, m. Bükk, beech wood/	0.088	0.007	7.9
Pleurotus ostreatus (Jacq. et Fr.) Kummer /Gemenc, on Populus nigra/	0.105	0.007	6.6
Family Tricholomataceae			
Lepista inversa (Scop.: Fr.) Pat. /Pilisszentkereszt, m. Pilis, pinewood/	0.069	0.080	11.6
Lepista nuda (Bull.: Fr.) Cke. /Hűvösvölgy, m. Budai, oak forest/	0.102	0.009	8.8
Family Boletaceae			
Boletus luridus Schff. /Bot. Gard. of Soroksár/	1.191	0.090	7.5

Table 1 (cont.)

Species and place of sampling	Arithmetical mean (mg per 100 g DM)	Standard deviation (SD)	Coefficient of variation (CV)
Boletus luridus Schff. /Csillebérc, m. Budai, oak forest/	1.378	0.101	7.3
Boletus luridus Schff. /Csillebérc, m. Budai, oak forest/	0.697	0.020	2.8
Boletus edulis Bull. /Budakeszi, oak forest/	1.124	0.096	8.5
Boletus edulis Bull. /m. Pilis, oak forest/	3.018	0.151	5.0
Family Agaricaceae			
Agaricus arvensis Schff.: Fr. /Őserdő, m. Bükk, beech forest/	0.460	0.080	17.3
Clitopilus prunulus (Scop.: Fr.) Kummer /Őserdő, m. Bükk, beech forest/	0.490	0.010	2.0
Macrolepiota procera (cap) (Scop.: Fr.) Sing. /Zagyvapálfalva, m. Karancs, oak forest/	0.583	0.033	5.6
Macrolepiota rhacodes (Vitt.) Sing. /Lillafüred, m. Bükk, oak forest/	0.760	0.030	7.6
Family Russulaceae			
Lactarius controversus Pers.: Fr. /Bot. Gard. of Soroksár/	0.240	0.062	7.5
Lactarius acerrimus Britz. /Pilisszentkereszt, m. Pilis/	1.562	0.062	3.9

Abbreviation: m: mountain

from 0.69 to 3 mg per 100 g DM, being the highest value found in a sample of *Boletus*

edulis (3.018 mg per 100 g DM). The two Agaricus species had low, the Macrolepiota species had higher selenium content (in the latter: 0.58 to 0.76 mg per 100 g DM). It is interesting that among the analysed Lactarius species only *L. acerrimus* had a high selenium level (1.562 mg per 100 g DM).

This investigation confirms the accumulating ability of certain taxonomical groups. Practically only the genus Boletus shows this valuable property; the selenium level of Agaricus species is lower. It seems, that wood-destroying fungi are not able to accumulate selenium.

The high selenium level of these valuable, edible fungal species can play a positive role in the human diet, increasing the total selenium intake.

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BOOK REVIEWS

Low-calorie foods and food ingredients

R. KHAN (Ed.)

Blackie Academic & Professional, London, 1993. 183 pages

Fourteen contributors summarize in 8 chapters the current research and the actual needs about various topics of low-calorie foods and food ingredients.

In all industrialized countries (including Hungary), obesity is one of the main health problems, and this fact is reflected by the continuously increasing demands for low-calorie products to provide for healthy nutrition.

The first chapter deals with overconsumption regulated by physiological and environmental factors and with mechanisms mediating processes of satiation and satiety. Effects of the appetite supressants using carbohydrate and artificial sweeteners and fat products are discussed on the basis of differences between the two types of nutrients in the energy balance of the organism.

The second chapter outlines the following topics: regulatory bodies, definition and controls of low-calorie foods, satiety assessment of food additives. The rules of estimate in the "Acceptable Daily Intake" (ADI) and the regulation of food additives by the different national governments are summarized. Labelling of low-calorie foods is claimed to provide nutritional and medical information for the consumers.

The next two chapters discuss the low-calorie bulk sweeteners: sugar alcohols, fructo-oligosaccharides, and their nutritional properties. The metabolism and functional properties of dietary fibres, and their selected usage are also outlined.

In the fifth chapter the fat-replacer ingredients of foods are discussed. The risk of different illnesses due to that fat-rich nutrition is well known, and there is a wide range (about 200 products) of fat substituents and replacers on the market. Accordingly, selected examples are given, i.e. sucrose polyester ("Olestra"), low-calorie substitutes: carboxy/carboxylate esters ("Caprenin"), and modified starches (partially hydrolysed potato-starch products). The fat substitutes of protein origin are as follows: "Simplesse" is microparticulated egg white or milk protein, "Lita" is a zein-based product. Examples of fat replacers are: microcrystalline cellulose, pectin gels with calcium, and the fat-like appearance product: "Splendid". Finally, the markets for fat-reduced foods are summarized.

The sixth chapter addresses fat and calorie-modified bakery products.

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In the seventh chapter the high-intensity low-calorie sweeteners are covered: Saccharine, Cyclamate, Acesulfame-K. "Monellin" is a protein-isolatum; urea derivates such as aspartame, the guanidine originated sucrononic acid, and other agents are also discussed.

The final chapter summarizes the technical demands and the formulation of the low-calorie soft drinks, their sweetness bulling ingredients and the use of artificial sweeteners in them.

The reader can find updated references at the end of each chapter. Finally, a well-constructed index helps the users in looking for different topics. This book is of interest for everybody who deals with low-calorie foods and food ingredients.

Á. J. N. ZSINKA

Aseptic processing of foods

H. REUTER (Ed.)

Technomic Publishing Company, Inc., Lancaster, Basel, 1993. 313 pages

This volume of Technomic Series contains two parts. Part I: symposium on "Presterilization of products" held in November 1989 in Frankfurt/Main and Part II: symposium on "Aseptic packaging" held in May 1990 in Cologne. Twenty-eight invited speakers affiliated with European Food machinery and packaging companies as well as with research institutes gave a comprehensive survey on the state of art of this technology.

The presentations given in this symposium supplemented by an additional one are published in this book. The topics range from conventional and unconventional heat treatment systems for presterilization which are capable of performing extremely quick and efficient heating and cooling processes, product development in conformity with the sterilization processes, product development in conformity with the sterilization process, recent developments in aseptic packaging systems, gamma sterilization of packaging materials, properties of materials for aseptic packaging, hazard analysis as well as testing methods for aseptic installations.

The first part has four main chapters on 130 pages. The first chapter is Basic principles (Fundamentals of UHT and HTST sterilization of foodstuffs; Ohmic heating of particulate food products; Dielectric heating of foodstuffs and temperature distribution in the product). The second one is Process and equipment for UHT and HTST presterilization (Tubular heat exchangers systems for liquid foods with solid particles and criteria for structural behavior; Thermal stabilization of soups and sauces containing particles by double flow processing; Single-Flow Fraction Specific Thermal Processing ("Single-Flow FSTP") of liquid foods containing particulates; New system for the sterilization of particulate food products by ohmic heating; Pasteurization and sterilization of unpacked liquid food containing solid parts in a continuous process by means of microwaves. The third chapter is the shortest (5 pages) about the Steril conveyance of liquids, and the fourth one is the Products (Soups and sauces UHT processed and aseptically packed; Flavorings for UHT-treated and aseptically packed soups and sauces).

The second part of the book is Aseptic packaging (158 pages) and it surveys the flexible technique of aseptic packaging with regard to package selection since almost all shapes and wide range of materials may be used. The chapters are as follows: Processes for packaging materials sterilization and system requirements; Aseptic filling and packaging (Roll-fed carton packaging, Carton packaging from

sleeves, Vertical form-fill-seal machines for bags, Thermoform filling and sealing machines for plastic cups, Thermoform filling and sealing machines for plastic cups with steam sterilization, Aseptic handling of particular products, Manufacturing, filling and sealing of plastic bottles in the blow mould, Aseptic packaging in glass and plastic bottles, Aseptic packaging line for aerosol cans, Bulk aseptic packaging, the bag-in-box system, Sterile room techniques in the food industry); Packaging materials for aseptic packaging (Gamma sterilization of packaging, Thermoformable barrier sheets for shelf stable container in dairy applications, Glass for aseptic packaging) and the last is the Quality protection (Hazard analysis in aseptic good manufacturing practice, Testing of aseptic machines for their efficiency of sterilization of packaging materials by means of hydrogen peroxide). Most of the chapters are completed with references.

Aseptic processing with its increasingly extended range of application can be regarded as a real high food technology and as an example on how a new processing technique leads to a new product group, i.e. to the presterilized and aseptically packed food which is to the advantage of the consumer as well as of the food industry.

The book is well recommended to specialists on machinery, food technologists and researchers but it is also useful in the teaching on high school and university level.

I. VARSÁNYI

ANNOUNCEMENT

MEAT AND REFRIGERATION: NEW DEVELOPMENTS
Paris-Anthony – June 27-29, 1994

The International Institute of Refrigeration (IIF-IIR) will hold an international course on the applications of refrigeration in the food industry, within the framework of the European Consortium for Continuing Education in Advanced Meat Science and Technology (EC/CE/AMTST) and with the support of CEMAGREF, INRA and foreign partners.

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