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PROTEIN SUBUNIT DISTRIBUTION IN WHEAT FLOURS AND THEIR RELATION TO BAKING QUALITY

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The protein composition of the following wheat cultivars was studied: Yubileinaya 50, GKF-2, Rana 1, Sava and Partizanka. Our main purpose was to separate the gluten proteins of high and low molecular mass, establish their quantity and their proportions. The molecular mass distribution of high molecular mass proteins was determined by gel chromatography.

The molecular mass distribution studies have shown that in high quality flour the proportion of high and medium molecular mass gluten proteins to low molecular mass components (<50 000) approximates 1 : 1. The overweight of low molecular mass gluten components results in a poorer bread making and gluten quality. In the formation of the baking quality, the quality and quantity of the high molecular mass gluten fractions play a decisive role.

Keywords: Protein in wheat flours, baking quality, gel chromatography

Efforts to elucidate the correlation between protein composition and baking quality in wheat flour have been unsuccessful so far and produced many contradictory results. Some differences in quality and quantity of the gluten content of wheat cultivars of different dough forming capacity were established, however, their exact relation to dough quality is not known and the prognostication of bread quality on the basis of protein fractions is not possible.

Research results hitherto established prove the assumption that the quantity and quality of the high molecular mass gluten complex (glutenin) determines the dough forming capacity of flour (POMERANZ et al., 1970; HUEBNER, 1970).

The gluten proteins of high molecular mass form the group of flour proteins the most difficult to separate and dissolve. Glutenin extracts contain generally other proteins, too, and the inconsequencies observed in the composition and structural examination of glutenin preparations are due to this fact (BUSHUK & WRIGLEY, 1971; EWART, 1968, 1972; BIETZ et al., 1973).

The most important characteristics of glutenin structure, related to its functional role, are molecular mass and the composition of the polypeptide chain (BIETZ & WALL, 1972; HUEBNER & WALL, 1974; HUEBNER et al., 1974; ORTH & BUSHUK, 1973). ORTH and BUSCHUK (1972) published a study on the correlation between the protein fractions obtained according to Osborne and the baking quality. The comparison of 26 spring wheat cultivars has shown bread

volume to be inversely proportional to the quantity of glutenin dissolved in 0.1 *M* acetic acid.

MECHAM and COLE (1972), COLE and MECHAM (1973), respectively, found that the quantity of the undissolved residual protein of protein extraction according to Osborne from wheat flour, the so called "gel protein" is related to the baking quality of the flour. They chromatographed the dissolved gel protein on an agarose column and found the molecular mass distribution to agree with that of the glutenin fraction.

1. Materials and methods

1.1. Materials

The wheat cultivars used in the experiments were obtained from the Cereal Research Institute, Szeged. The flours milled from 5 aestivum wheat cultivars were used. These are: Yubileinaya 50, Rana 1, Sava, Partizanka and GKF-2. These cultivars were grown in 1980 in Fülöpszállás utilizing 330 kg per hectar chemical fertilizer in the proportion N : P : K (1.5 : 1 : 1). The moisture content of the wheat samples was set at 15% and then they were milled on a laboratory mill (Quadromat Senior Brabender) to 60% extraction. After screening the particle size of the flour was below 150 μ.

1.2. Methods

1.2.1. Determination of the baking quality of the flour samples. The baking quality was established by the standard methods of the milling, baking and pasta industries (KARÁCSONYI, 1970).

Table 1

Moisture and protein content of the flour samples

Wheat cultivar	Moisture content (%)	Dry matter (%)	Protein[a] (N×5.7)	Protein[b] (N×5.7)
Yubileinaya 50	14.82	85.18	14.32	12.31
GKF-2	14.46	85.54	12.78	10.93
Rana 1	14.52	85.49	13.16	11.31
Sava	14.17	85.83	11.82	10.14
Partizanka	14.70	84.30	13.45	11.56
Average deviation from the mean	0.05	–	0.18	0.18

[a] = as percentage of dry matter content
[b] = as percentage related to 14% moisture content

The total protein content according to Kjeldahl, the quantity of wet and dry gluten, the value of gluten spread (Table 1), was determined.

The moisture absorption capacity, the time needed for dough development and stabilization and the extent of dough softening were determined by valorigraphy (Table 2).

Test baking was carried out to determine the volume and the form quotient (Table 3).

1.2.2. Analysis by acetic acid fractionation of the protein composition. First the salt-soluble protein fractions (albumin, globulin) were extracted with 1.0 *M* sodium chloride solution. Then the gluten was washed starch-free. The extraction of gluten protein was carried out with a 0.1 *M* acetic acid solution, 70% ethyl alcohol and 0.2% potassium hydroxyde solution (PALLAGI, 1980). The quantity of the extracted protein was established by the photometric method with biuret reaction (KOVÁCS, 1976). The procedure is shown in Fig. 1.

Table 2

Gluten characteristics in the flour samples

Wheat cultivar	Wet gluten (%)	Dry gluten (%)	Spread of gluten (mm)
Yubileinaya 50	36.5	12.50	3.0
GKF-2	35.05	11.89	2.5
Rana 1	31.00	10.90	3.0
Sava	30.65	10.50	7.2
Partizanka	34.20	11.90	3.0
Average deviation from the mean	0.20	0.20	0.15

Table 3

Results of the valorigraph tests

Wheat cultivar	Water absorption capacity (%)	Dough formation time (min)	Stability of dough (min)	Softening of dough (VU)
Yubileinaya 50	66.4	2.0	3.5	35
GKF-2	65.0	1.5	4.0	65
Rana 1	64.0	1.5	1.0	60
Sava	63.0	2.0	3.0	60
Partizanka	64.8	2.5	2.0	30
Average deviation from the mean	0.31	–	–	3.2

1.2.3. Analysis by gel chromatography of the high molecular mass gluten fraction. A chromatographic column of 1.8 × 60 cm and an eluant of 4 *M* guanidine hydrochloride were used. The column packing was Sepharose 4B agarose

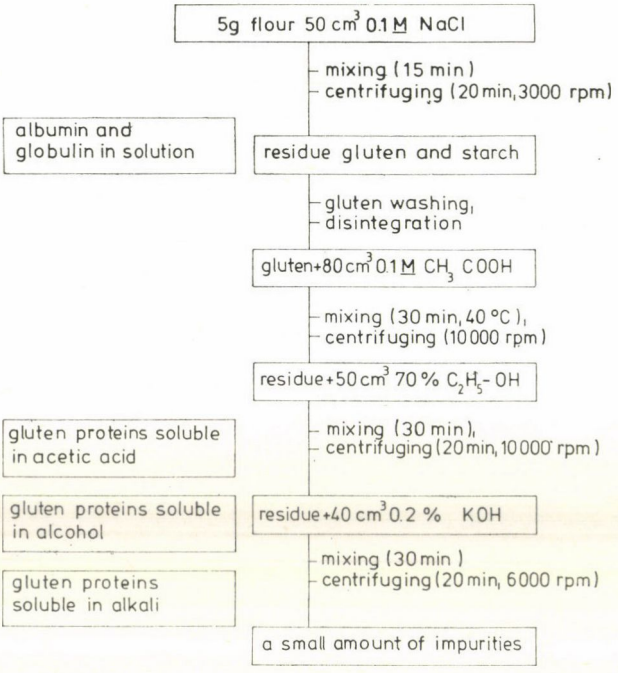

Fig. 1. Fractionation of wheat flours with 0.1 *M* CH_3COOH

gel. The rate of elution was 12 cm³ per h. For a partition 30 mg protein was applied to the column in 3 cm³ eluant. Three cm³ fractions were collected in an automatic fraction collector. Measurements were carried out at 280 nm with a Pye-Unicam SP-8-100 UV spectrophotometer.

2. Results

The results of measurements were summed up in tables. The results shown are the averages of three parallel measurements. The deviations are given to each table. The deviations belonging to different cultivars did not differ to a significant degree as established by the homogeneity test of Cochran, thus only average values are given (RASCH et al., 1973).

3. Conclusions

Baking quality is a complex concept including all the properties of flour which enable the preparation of bakery products of good quality.

The protein content of the flour is very important from the aspect of both baking quality as well as nutritive value. The highest protein content (14.2%) was found in cultivar Yubileinaya 50. Flours of Partizanka, Rana 1, GKF-2 have a protein content lower by 1.5%. Significantly lower is the protein content of the fodder wheat cultivar Sava (11.8%).

The flour of Yubileinaya 50 had the highest gluten content (36.65%). Cultivars Partizanka and GKF-2 had also a high gluten content (35.05 and 34.2%, resp.). The quantity of the gluten content is not proportional to the quality of the dough, decisive is the quality of gluten.

The spread of gluten permits of drawing conclusions as to the physical characteristics of the dough. With the exception of the flour from Sava cultivar which was of medium spread (7.2 mm), all the other flours showed a low spread (lower than 6 mm). The valorigrams have shown that the water absorption capacity of all the flour samples was satisfactory being between 63 and 67%. The stability of the dough characterizes the form and gas retaining capacity. The doughs of cultivars Yubileinaya 50 and GKF-2 were of high stability (3.5 and 4.0 min, resp.). The lowest stability had the dough made of Rana 1 (1 min).

Softening of the doughs is given in valorigraph units (VU). Softening of the dough shows the rearrangement of the gluten structure during mixing and the reduction in the energy requirement of mixing.

The lowest softening values were measured in the doughs of Partizanka (30 VU) and Yubileinaya 50 (35 VU). The higher softening values obtained in the dough of the other three flours (60–65 VU) show a not very elastic structure, rapidly splitting up.

The highest bread volume was achieved with Yubileinaya 50 and Partizanka flours (365 and 310 cm^3, respectively). The volume obtained with the other three flours was lower by about 20–30%.

Based on the flour analyses and the dough tests and considering all the partial factors the baking quality of the flours studied was put in the following order:

Yubileinaya 50
Partizanka
GKF-2
Rana 1
Sava

Most of the low molecular mass gluten proteins were dissolved in 0.1 *M* acetic acid. The results of the ethyl alcohol extraction have shown, however,

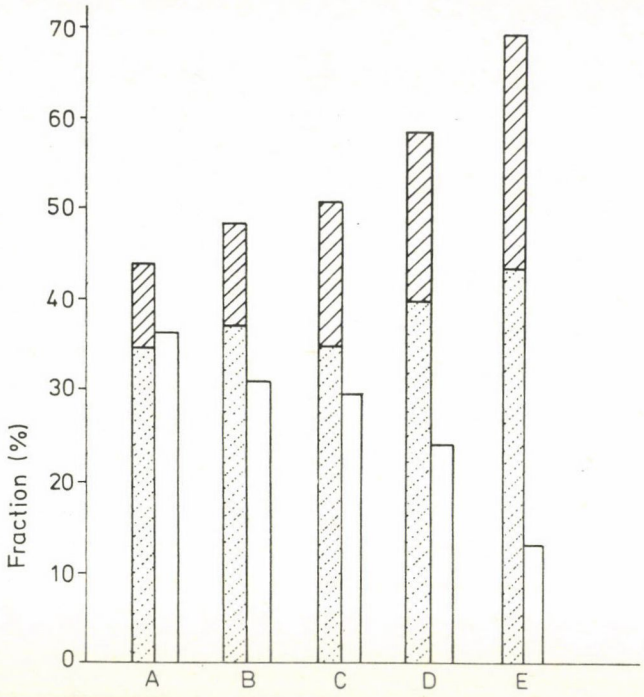

Fig. 2. Distribution of gluten protein fractions obtained by acetic acid fractionation as percentage of the protein content of flour samples. A = Yubileinaya, B = Partizanka, C = GKF-2, D = Rana 1, E = Sava, ▒ Soluble in 0.1 *M* CH_3COOH, /// Soluble in 70% C_2H_5-OH, □ Insoluble in 0.1 *M* CH_3COOH

that some higher molecular mass or aggregated gliadin fractions remained undissolved with the high molecular mass gluten components.

The baking quality of the flours investigated was found to be proportional to the quantity of the protein fraction insoluble in 0.1 *M* acetic acid. The quantity of the protein fractions obtained by acetic acid fractionation is shown as the percentage of the total protein content in Fig. 2.

A summary of the proteins soluble in 0.1 *M* acetic acid and in 70% ethyl alcohol is illustrated in the figure. In flours of good baking quality the fraction of gluten protein soluble in 0.1 *M* acetic acid does not exceed 40% of the total protein content and the joint amount of proteins soluble in either acetic acid or ethyl alcohol is below 55%. The elution diagrams of the gluten proteins insoluble in 0.1 *M* acetic acid are shown in Fig. 3.

For the sake of lucidity the elution diagrams of only two samples of characteristically differing baking quality are shown.

Partition by gel chromatography resulted in three protein fractions of different molecular mass. Their average molecular mass calculated on the basis of the elution diagrams can be seen in Fig. 3.

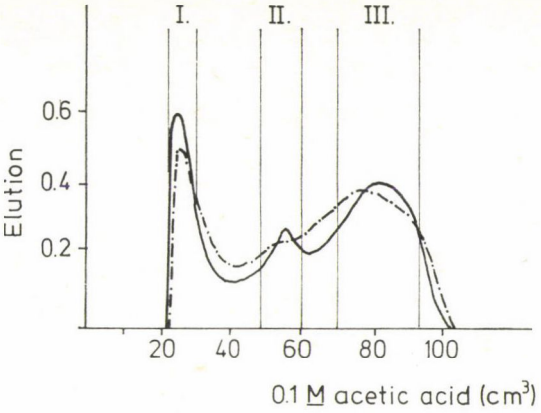

Fig. 3. Elution diagrams of the gluten protein fractions insoluble in 0.1 *M* CH_3COOH. I.: 2 400 000—1 400 000, II.: 280 000—130 000, III.: 50 000—23 000, —— Yubileinaya, ····· Sava

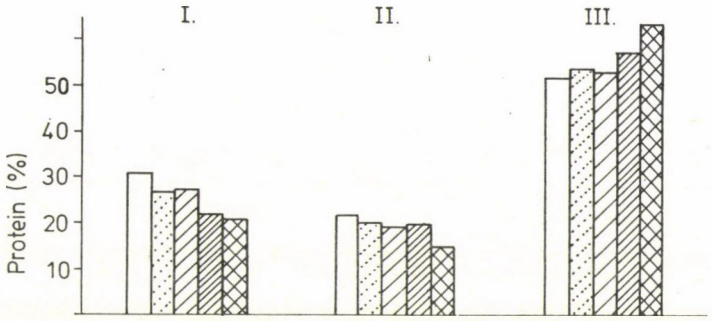

Fig. 4. Percentage distribution of the gluten protein fractions insoluble in 0.1 *M* CH_3COOH of different wheat cultivars in the important molecular mass ranges. Yubileinaya 50 □, Partizanka ▦, GFK-2 ▨, Sava ▨, Rana 1 ▨, I.: 2 400 000—1 400 000, II.: 280 000—130 000, III.: 50 000—23 000

In the flour samples studied the character of the elution diagrams corresponded. The difference among them appeared in the quantity of the protein fractions I to III in flours of different baking quality.

The percentage distribution of protein fractions in the three groups of different molecular mass, related to the total protein content, based on the area under the elution peaks, is as follows:

	I	II	III
Yubileinaya 50	37.0	20.0	43.0
Partizanka	34.0	18.0	48.0
GKF-2	34.5	17.0	48.5
Rana 1	20.0	17.5	62.5
Sava	19.0	14.0	67.0

Table 4

Results of the baking tests

Wheat cultivar	Form quotient	Volume of bread (cm^3)	Cross section (cm^2)
Yubileinaya 50	1.42	365	36.0
GKF-2	1.83	285	30.0
Rana 1	1.27	243	28.0
Sava	1.76	265	28.0
Partizanka	1.56	310	33.0
Average deviation from the mean	0.20	3.5	0.51

Table 5

Quantity of protein fractions obtained by acetic acid fractionation as percentage of total protein in the flour

Wheat cultivar	Protein soluble in salt (%)	Protein soluble in 0.1 *M* CH_3COOH (%)	Protein soluble in 70% C_2H_5—OH (%)	Protein soluble in 0.2 *M* KOH (%)
Yubileinaya 50	14.30	34.70	9.20	36.40
GFK-2	14.60	39.30	11.20	28.50
Rana 1	14.30	40.00	18.90	24.20
Sava	14.70	43.70	25.90	13.00
Partizanka	13.95	35.40	15.50	30.90
Average deviation from the mean	0.34	0.30	0.28	0.30

Results in the table are the averages of 8 parallel measurements

In flours of good baking quality the proportion of the fraction of highest molecular mass (I) was around 30%, while in flours of poorer quality was around 20%.

The percentage distribution of protein fractions related to the total protein content, in the molecular mass range I—III, based on the areas below the elution peaks, is illustrated in Fig. 4.

As it is seen in the column diagram, in flours of good baking quality fraction I is present in a larger amount. In flours of poorer quality the quantity of fractions of lower molecular mass (<50 000) increases substantially.

The study of the molecular mass distribution of high molecular mass gluten proteins has shown that the condition of good baking quality is that

the proportion of high and medium molecular mass fractions to components below 100 000 molecular mass should be at least 1 : 1, or higher.

In order to attain a better knowledge of the relationship we intend to carry out the investigation of a large series of flour samples.

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QUANTITATIVE DETERMINATION OF MUSCLE PROTEIN IN MEAT PRODUCTS BY MEASURING CREATINE CONTENT

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The total protein content in meat products is made up of proteins of various origins. Creatine is a compound found exclusively in muscle tissue, and by measuring the amount of creatine spectrophotometrically it is possible to deduce the content of the biologically most valuable muscle tissue (meat protein), even in the presence of other proteins (connective tissue, Na-caseinate, soya protein). In an alkaline medium creatine gives a red colour with the reagents α-naphthol and diacetyl. Mathematical calculations show that an analysis based on the measument of the complex colour intensity is sufficiently accurate to be used for determining the muscle tissue content (WONG, 1971). The experimental results show that the error calculated at the 95% level of probability from the mean extinction value and expressed in terms of the N content does not exceed 0.5%. Other protein sources do not contain perceptible amounts of creatine.

In the course of earlier experiments it was found that the creatine content measured spectrophotometrically was proportional to the N content of the muscle tissue determined using the Kjeldahl method. The analyses were carried out on pork, beef and pork-beef mixtures with various ratios, with and without heat treatment (GÁBOR et al., 1980).

The aim of the present series of experiments was to test the suitability of this method for certain meat products, namely for a type of sausage known as Párizsi.

Keywords: Muscle protein, creatine in meat, spectrophotometry

1. Materials and methods

1.1. Reagents

Trichloracetic acid (TCA): 10 g dissolved in 100 cm^3 distilled water;
Sodium hydroxide, sodium carbonate buffer: 60 g NaOH + 160 g anhydrous Na_2CO_3 dissolved in 1000 cm^3 distilled water;
Diacetyl: 0.1 cm^3 dissolved in 100 cm^3 distilled water, freshly prepared;
α-naphthol solution: 1 g dissolved in 100 cm^3 buffer solution, freshly prepared;
50 mg% creatine solution dissolved in 10% trichloracetic acid;
Sulphuric acid, analytically pure,
Taschiro indicator;
Boric acid, 4%;
Catalytic agents: K_2SO_4, $CuSO_4$ and metallic selenium;
H_2SO_4, 0.1 *N*;

NaOH, 0.1 *N*;
NaOH solution: 33 g dissolved in 100 cm^3 distilled water;
Quartz sand.

1.2. Materials

Pork,
Beef,
Soya concentrate GL-750 70% (Central Soya Co., USA).

1.3. Spectrophotometric measurement of creatine content

10.00 g material was homogenized with 50.00 cm^3 TCA, then centrifuged for 20 min at 3000 rpm. The liquid phase was transferred to a 200 cm^3 volumetric flask and the process was repeated twice. The combined extracts were made up to 200 cm^3 with TCA solution. After standing for 20 min the solution was filtered. Using a pipette, 5.00 cm^3 alkaline buffer, 3.00 cm^3 α-naphthol solution and 2.00 cm^3 diacetyl reagent were added to 1.00 cm^3 filtrate. The mixture was made up to 25 cm^3 with distilled water.

The photometric compensating solution had the same composition, except that the extract was replaced by 1 cm^3 TCA reagent.

After being left to stand for 20 min the measurements were carried out in a 1 cm^3 cuvette at room temperature, at a wavelength of 520 nm.

The photometric measurements were made using a VSU-2P spectrophotometer.

1.4. Construction of a calibration curve for determining the correlation between extinction and creatine content

A series of solutions with creatine concentrations of 50, 75, 100, 115, 150, 200 and 250 $\mu g\ cm^{-3}$ was prepared from the 50 mg% stock creatine solution by dilution with TCA. To 1.00 cm^3 of the diluted solution, 5.00 cm^3 alkaline buffer solution, 3.00 cm^3 α-naphthol solution and 2.00 cm^3 diacetyl reagent were added and the mixture was made up to 25.00 cm^3 with distilled water.

In the reference solution the creatine solution was replaced by TCA solution.

The spectrophotometric measurements were carried out as described in para. 1.3.

1.5. Measurement of total nitrogen content

The measurements were carried out using the Kjeldahl determination, as required by the HUNGARIAN STANDARD (1978).

1.6. Determination of dry matter content

The determination was carried out as laid down in the HUNGARIAN STANDARD (1974).

1.7. Determination of fat content

The determination was carried out as laid down in the HUNGARIAN STANDARD (1972).

1.8. Preparation and testing of standard series of samples

Round steak of beef and shoulder of pork from which the tendons, surface connective tissue and fat had been removed were minced separately twice using a mincer with 3 and 2 mm holes. They were mixed in the proportions required for Párizsi sausage and were homogenized in a mixer. A standard series of samples was prepared by replacing 20, 40, 60 or 80% of this meat mixture with a soya preparation made by soaking a 1 : 2 ratio of soya concentrate in water. The series also included an unadulterated meat mixture and a sample containing 100% soaked soya concentrate. Half of the 200 g samples were stored in an airtight container at 4 °C. The sausage-manufacturing process was modelled in the laboratory in the manner described below. The other half of the samples were stuffed into sausage skins.

Smoking was modelled by heating the samples to 105 °C in a drying cabinet for 1.5 hours without a smoke solution, after which they were cooked for half an hour in water at 72 °C. After cooling to room temperature the samples were minced and stored under the same conditions as those which were not heat-treated.

These samples were used for the measurements of dry matter content and total nitrogen content (Kjeldahl) required for the calculations, and for the photometric measurements.

1.9. Preparation and testing of samples of Párizsi sausage

Samples of Párizsi sausage and of Párizsi sausage mixture were obtained from the Budapest Meat Company (Budapesti Húsipari Vállalat), who certified that the samples were all from the same lot and had been manufactured according to the requirements of the pertinent standard for Párizsi sausage.

Samples were stored at +4 °C. All the tests were carried out on 15 parallel samples, both for the Párizsi sausage (heat-treated product) and for the sausage mixture (non-heat-treated sample).

The spectrophotometric measurements were carried out as described in para. 1.3.

The dry matter content and total nitrogen content (after Kjeldahl) of the samples were determined.

2. Results

2.1. Results of measurements

The correlation between the creatine content and the extinction values, measured as described in para. 1.4, is shown in Fig. 1.

The data for the non-heat-treated and heat-treated standard samples are illustrated in Tables 1 and 2.

The histograms (Figs. 2 and 3) constructed from the data in the tables give a good illustration of the linearity between the creatine content calculated on the basis of extinction and the meat-nitrogen values for both non-heat-treated and heat-treated samples.

According to the diagram (Fig. 4) constructed from the tabular data, there is a linear correlation between the spectrophotometrically measured creatine content of the standard samples and the calculated meat-nitrogen content (Kjeldahl) in both non-heat-treated and heat-treated samples.

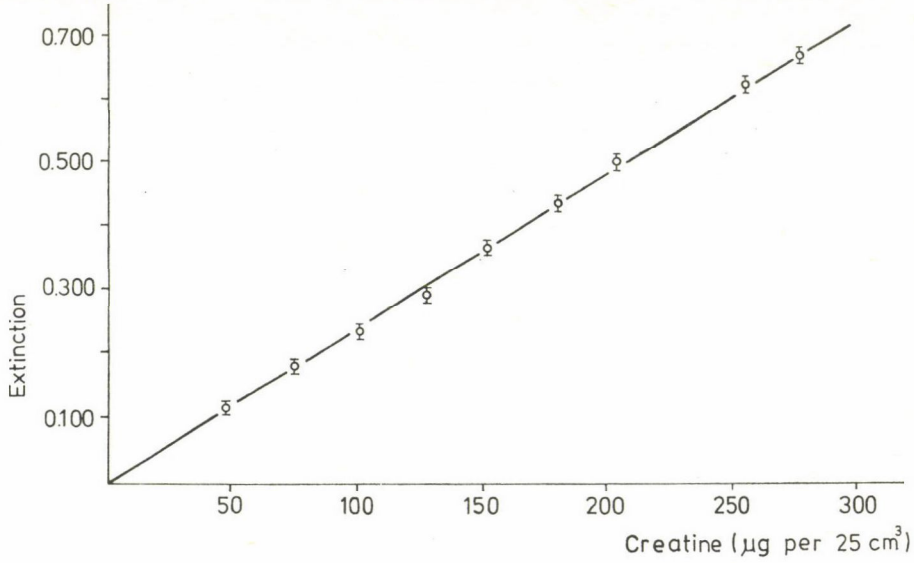

Fig. 1. Calibration curve for creatine: $y = 0.0025x - 0.019$. Correlation coefficient (r) = 0.998

Table 1

Analytical data for non-heat-treated standard samples

Composition of sample (%)		Dry matter (%)	Total nitrogen (%) in terms of		Meat protein N mg per g in terms of[a]		Extinction measured for creatine content (%)	Standard deviation (±s)	Creatine mg per g material
M	S		total matter	dry matter	total matter	dry matter			
100	—	25.64	35.28	137.59	35.28	137.59	0.699	0.019	5.65
80	20	26.56	35.65	134.22	28.24	110.08	0.570	0.015	4.63
60	40	27.54	35.27	128.06	21.17	82.56	0.425	0.022	3.49
40	60	28.54	35.29	123.65	14.11	55.04	0.276	0.024	2.32
20	80	29.62	34.87	117.72	7.06	27.52	0.139	0.017	1.25
—	100	30.13	34.41	114.21	—	—	0.001	0.010	0.00

M = Meat mixture
S = Soya concentrate — water (1 : 2)
[a] = Calculated value

Table 2

Analytical data for heat-treated standard samples

Composition of sample (%)		Dry matter (%)	Total nitrogen (%) in terms of		Meat protein N mg per g in terms of[a]		Extinction measured for creatine content ($\bar{x}$)	Standard deviation (±s)	Creatine mg per g material
M	S		total matter	dry matter	total matter	dry matter			
100	—	30.58	42.43	138.75	42.43	138.75	0.708	0.025	5.71
80	20	33.59	45.24	134.68	33.94	111.00	0.575	0.022	4.67
60	40	37.11	48.28	130.45	25.46	83.25	0.435	0.022	3.57
40	60	36.15	45.31	125.33	16.97	55.50	0.284	0.018	2.38
20	80	37.18	44.16	118.77	8.49	27.75	0.148	0.019	1.32
—	100	34.88	40.05	114.82	—	—	0.006	0.010	0.00

M = Meat mixture
S = Soya concentrate — water (1 : 2)
[a] = Calculated value

The difference between the slopes of the curves stems from the different moisture contents of the heat-treated and non-heat treated samples. If the values calculated for dry matter are plotted, the curves coincide (Fig. 5).

2.2. Calculation of the creatine content from the extinction values

Literary data indicate that there is practically no difference between the creatine content of beef and pork muscles (KIERMEYER, 1968). The mean value for the creatine content of muscles in various parts of the body is

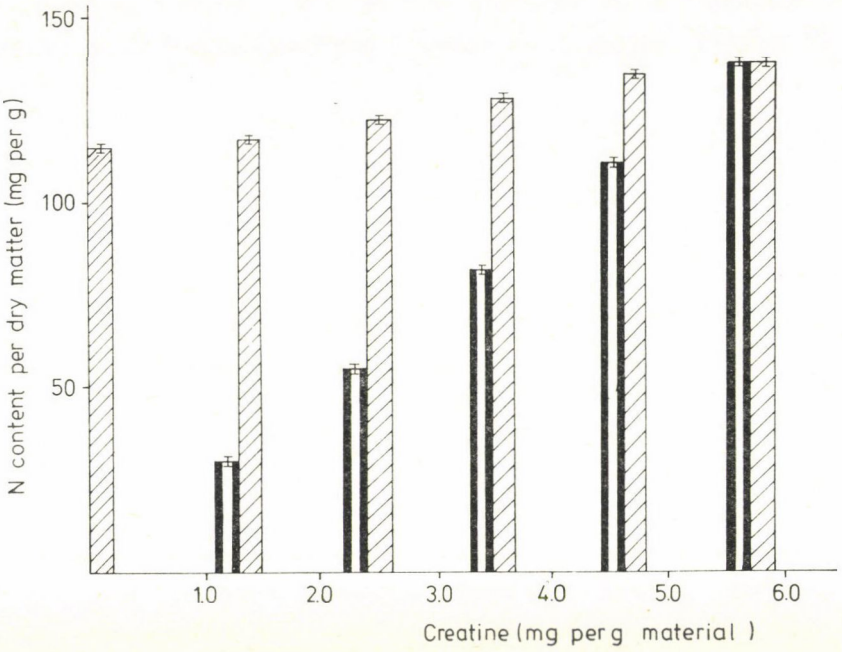

Fig. 2. Correlation between creatine content and meat N content in non-heat-treated standard samples. ▨ total nitrogen, ▬ meat nitrogen

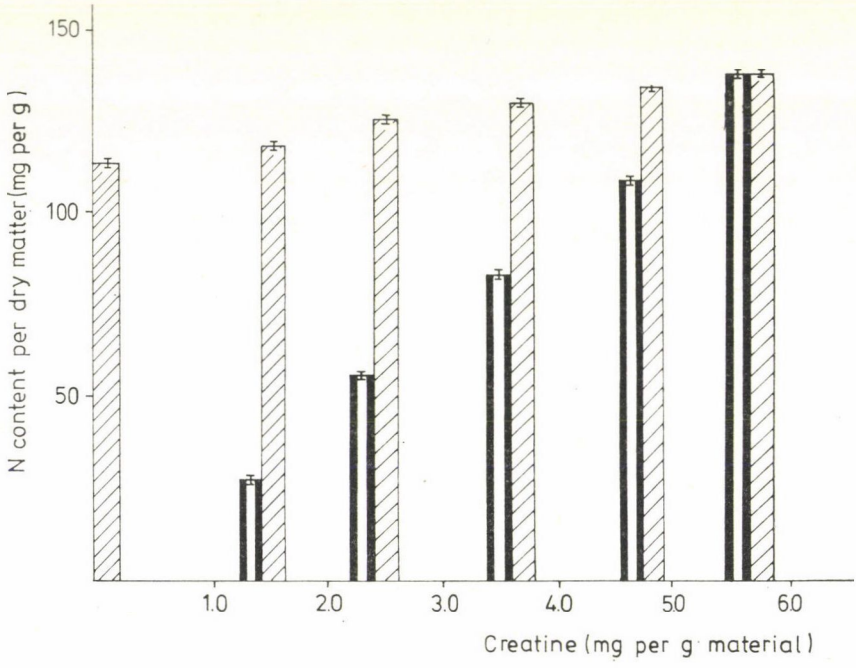

Fig. 3. Correlation between creatine content and meat N content in heat-treated standard samples. ▨ total nitrogen, ▬ meat nitrogen

3.68 mg creatine per g muscle. The mean total N content for the same tissues is 33.2 mg N per g muscle (KHAN & COWEN, 1977).

Thus, 11.08% of the total N content of muscles is made up of creatine. This correlation is used in the calculations.

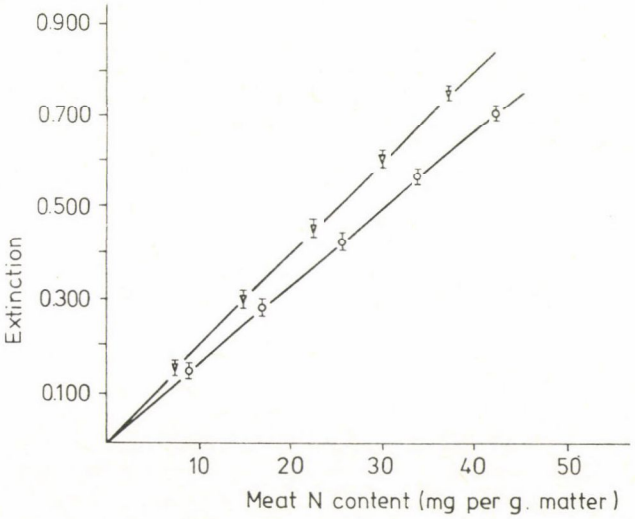

Fig. 4. Correlation between meat N mg values and measured extinction in non-heat-treated and heat-treated standard samples. —▽—: non-treated ($y = 0.020x - 0.003$; $r = 0.999$), —○—: treated ($y = 0.017x - 0.005$; $r = 0.998$)

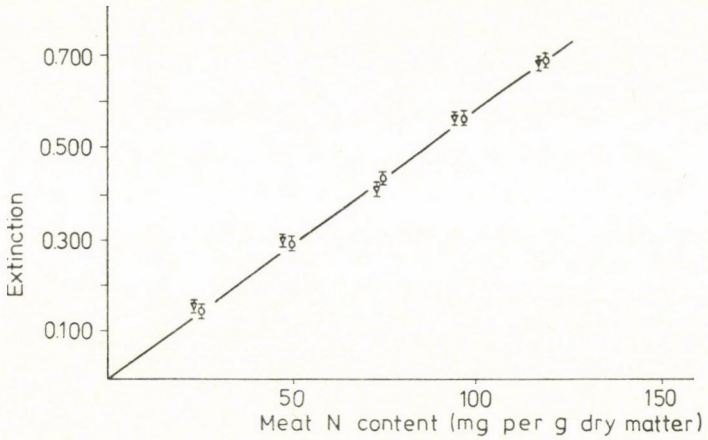

Fig. 5. Correlation between meat N mg values and measured extinction in non-heat-treated and heat-treated standard samples expressed in terms of dry matter. —▽—: non-treated, —○—: treated ($y = 0.005x - 0.002$; $r = 0.998$)

Table 3

Comparison of nitrogen and protein content of non-heat treated Párizsi sausages based on Kjeldahl method and creatine content

Sample No.	Kjeldahl method		Measurements based on creatine content		
	N content (N g per 100 g)	Protein content (g per 100 g)	E	N content (N g per 100 g)	Protein content (g per 100 g)
1	2.095	13.09	0.264	2.043	12.76
2	2.060	12.87	0.251	1.949	12.18
3	2.047	12.79	0.267	2.065	12.90
4	2.062	12.88	0.276	2.129	13.31
5	2.100	13.12	0.260	2.014	12.58
6	2.084	13.02	0.260	2.014	12.58
7	2.049	12.80	0.270	2.087	13.04
8	2.085	13.03	0.254	1.971	12.31
9	2.070	12.93	0.260	2.014	12.58
10	2.058	12.86	0.250	1.942	12.14
11	2.082	13.01	0.249	1.938	12.11
12	2.051	12.81	0.262	2.028	12.67
13	2.085	13.03	0.240	1.869	11.68
14	2.077	12.99	0.248	1.927	12.04
15	2.060	12.87	0.266	2.072	12.95
Mean value ($\bar{x}$)	2.071	12.94	0.258	2.003	12.52
Standard deviation ($\pm$ s)	0.0165	0.104	0.067	0.068	0.426

The regression equation of the correlation between the creatine content and the extinction (Fig. 1) is:

$$Y = 0.0025x - 0.019,$$

where

Y = extinction (E),

x = creatine quantity (μg).

Taking into account the amounts and dilutions specified, the creatine content (g per 100 g product):

$$x = \frac{E + 0.019}{0.0025} \cdot \frac{200 \cdot 10}{10^6} = 0.8\ (E + 0.019),$$

where

stock solution volume = 200 cm^3

volume used = 1 cm^3

material added = 10 g.

Table 4

Comparison of nitrogen and protein content of non-heat treated Párizsi sausages based on Kjeldahl method and creatine content

Sample No.	Kjeldahl method		Measurements based on creatine content		
	N content (N g per 100 g)	Protein content (g per 100 g)	E	N content (N g per 100 g)	Protein content (g per 100 g)
1	2.166	13.53	0.273	2.108	13.17
2	2.186	13.66	0.262	2.028	12.67
3	2.183	13.64	0.279	2.152	13.45
4	2.209	13.80	0.257	1.994	12.46
5	2.185	13.65	0.261	2.021	12.63
6	2.203	13.77	0.263	2.035	12.71
7	2.164	13.52	0.269	2.070	12.93
8	2.226	13.91	0.260	2.014	12.58
9	2.222	13.88	0.240	1.869	11.68
10	2.203	13.76	0.270	2.077	12.98
11	2.232	13.95	0.246	1.912	11.95
12	2.180	13.62	0.246	1.912	11.95
13	2.200	13.77	0.249	1.934	12.08
14	2.178	13.61	0.249	1.934	12.08
15	2.187	13.66	0.270	2.077	12.98
Mean value ($\bar{x}$)	2.195	131.7	0.260	2.009	12.55
Standard deviation ($\pm$ s)	0.020	0.128	0.011	0.079	0.495

Nitrogen content, N (g per 100 g product):

$$\frac{\text{creatine content} \times 100}{11.08} = 7.22\ (\mathrm{E} + 0.019)\,.$$

Muscle protein content, N (g per 100 g product):

$$6.25 \times \mathrm{N} = 45.13\ (\mathrm{E} + 0.019).$$

Using this correlation, direct information can be obtained from the extinction value, measured as described above, on the percentage muscle protein content of the samples examined.

2.3. Samples of Párizsi sausage

The analytical data obtained for Párizsi sausage samples are summarized in Tables 3 and 4, from which the following conclusions can be drawn. For both non-heat-treated and heat-treated samples the mean protein content

values calculated on the basis of creatine measurements are lower than the values obtained by the Kjeldahl method. This can be explained by the fact that the Kjeldahl method is able to measure not only muscle protein, but also protein from connective tissue and other sources, which may be used as additives in the manufacture of the product.

3. Conclusions

In the first part of the experiment it was demonstrated that a calibration curve closely approximating linearity can be prepared by spectrophotometric measurements on solutions with known creatine contents.

The series of experiments carried out on standard samples proved that the creatine content measured spectrophotometrically was proportional to the amount of muscle protein.

Analytical data obtained from samples of Párizsi sausage showed that the meat protein can be calculated from creatine measurements, thus giving important information on the protein composition of the product. The creatine-protein conversion factor (11.08) introduced into the calculations limits the accuracy of the determination to 4%, when expressed in terms of protein as total matter.

The creatine calibration curve must be constructed in order to eliminate the error arising from the inaccuracy of the spectrophotometer.

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EXAMINATION OF THE COMPOSITION AND EFFICIENCY OF CERTAIN SURFACE-ACTIVE ADDITIVES (CPL AND CSL PREPARATIONS) USED IN THE BAKING INDUSTRY

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The increasing use in Hungary of surface-active additives in the manufacture of bakery products necessitates the elaboration of adequate analytical methods for evaluating these preparations and for testing the products made with them. Such methods are also required in order to establish the correlation between the composition and efficiency of the additives.

Ten CPL and CSL preparations were examined, some of which are already on the market, the remainder being the products of experimental laboratory processes. The metal content was determined by atomic absorption, the fatty acids and lactic acid by gas chromatography and the fatty acyl lactic acid derivatives by intensive liquid chromatography, in the form of p-bromophenacyl derivatives.

The $CaCl_2$ contents of the samples varied between 0.95–10.03%, the free lactic acid between 1.5–11.7%, the free fatty acid between 5.1–46.8%, the fatty acyl lactic acid derivatives between 29.0–44.7% and the fatty acyl polylactic acid derivatives between 12.5–56.8%.

Considering the effect exerted on the rheological properties of the dough and the analysis of the composition, it seems likely that the efficiency of the preparations is fundamentally determined by the fatty acyl lactic acid derivatives. An excessive amount of free fatty acids or fatty acyl polylactic acid derivatives reduces the efficiency.

Keywords: Additives of baking industry, fatty acid acyl group of milk acid, metal components

In the course of baking technologies the water added to the flour is basically absorbed by two principal components of the flour: the starch and the gluten proteins. The rheological characteristics of the dough thus formed are influenced by the water content of the system, the quality and quantity of the gluten protein skeleton and by the intensity of the interactions between the gluten skeleton and the starch, or possibly the lipids (Lásztity, 1972; Moór, 1977). If further substances, which may enter into an interaction with some component of the system, are introduced into this system, this will change the characteristics of the dough. Palmityl and stearyl lactates are able to interact with both gluten protein and starch, i.e. with both principal components of the dough (Mercier et al., 1980). This effect is particularly evident in flours of poor baking quality. The effect of anion-active emulsifiers in improving the consistency of the dough and increasing its stability has

long been known (MOÓR, 1978, 1980; THEWLIS, 1981; COLE, 1973; HAMPL & TVRZNIK, 1977; KOZMINA, 1978; KUNTZE, 1977; AUERMAN, 1972).

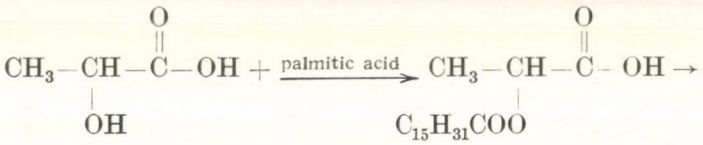
The effect on the quality of the product is seen mainly in an increase in the quality of the bread crumbs and in an increase in the volume. These additives result in saving the fat used to increase the volume (MOÓR, 1979, 1979a).

The improved quality of the bread crumbs is retained during storage, i.e. the ageing of the bread is retarded due to the effect of the emulsifier. This is directly correlated with the interaction arising between the starch and the emulsifier (ROTSCH, 1967; SCHNEEWEISS et al., 1975; WILHOFT, 1973; DE STEFANIS et al., 1977; SEISEL, 1970).

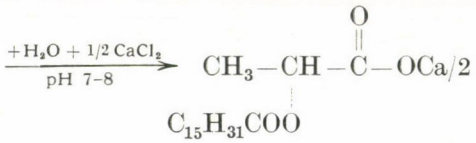
These observations justify the general introduction of emulsifiers in Hungary, since Hungarian experiments have also unanimously proved the favourable effect of emulsifiers (TELEGDY-KOVÁTS & LÁSZTITY, 1962, 1966).

In Hungary tests have been made not only on monoglycerides (GMS), but also on mono- and diglyceride mixtures (GMDS), diacetyl tartaric acid ester (DATA), calcium stearyl lactate (CSL), calcium palmityl lactate (CPL) and sodium stearyl and palmityl lactates (SSL, SPL), some of which have been used to quite a large extent (MOÓR, 1977).

Calcium palmityl lactate (CPL) is produced via the following reactions:



lactic acid palmityl lactic acid



calcium palmityl lactate

The palmitic acid is heated with lactic acid at a high temperature. The water produced during the reaction is distilled off and the reaction product is converted into the calcium salt. The precipitated crystalline product is filtered and dried.

In connection with other possible manufacturing processes it should be mentioned that STANLEY (1972) reacted lactic acid salt with fatty acid and extracted the product with dilute sulphuric acid. BASHKAYEVA (1974) reacted anhydrous lactic acid with an equivalent amount of stearyl chloride in solvent, in a nitrogen atmosphere at 120 °C. The product was stearyl lactic acid with a yield of 93–97% and a purity of 92.5–95%.

ELLIGER (1979) reacted lactic acid benzyl ester with stearyl chloride to produce pure stearyl lactate.

In the reaction described above various side reactions and the presence of unused reaction components must be expected. It is a well-known fact that in the course of the condensation reaction, polylactic acid may be formed through the interaction of a number of lactic acid molecules, and this may be acylated with fatty acid; lactic acid acylated with fatty acid may also acylate further lactic acid molecules.

Under industrial production conditions the reaction product may also be contaminated with metallic components particularly during salt formation. The examinations were thus designed primarily to demonstrate and determine these components. Knowing the compositions of the various preparations, an attempt was made to draw conclusions on the correlation between composition and technological efficiency.

1. Materials and methods

1.1. Materials

Information on the samples examined is presented in Table 1.

1.2. Methods

1.2.1. Determination of inorganic components.

1.2.1.1. Metallic components. — Of the inorganic components, in samples converted into calcium salts, the Ca content, the Na, K, Fe and Pb contaminations and, among the anions, the chloride content were analysed. The examinations were carried out on a nitric acid extract of the samples by means of atomic absorption, emission and argentometric potentiometric titration.

Preparation of the samples by extraction with nitric acid: 1 g sample was digested with 20 cm^3 1 mol dm^{-3} nitric acid over a water bath for 1 h, then made up to 50 cm^3, and, after cooling, filtered through filter paper. The metallic components were determined with the aid of a Zeiss AAS 1 atomic absorption photometer.

Conditions under which the determination was carried out: Solutions containing 20 mg dm^{-3} K, Na and Ca ions and 10 mg dm^{-3} Fe and Pb ions, and dilutions of these, were used as standard solutions. The characteristic parameters for the determination are summarized in Table 2.

A standard deviation of 1.5% was obtained for the macrocomponents and 2% for the microcomponents.

1.2.1.2. Determination of chloride content. — The chloride content of the samples was determined from the nitric acid extract by means of potentio-

Table 1

The samples examined

Symbol or name of sample	Content of sample; remarks
Admul CSL 2002	Foreign product
Admul CSL 2008	
Oleofinx CSL 1290	
PL 7901[a]	Production batches of palmityl lactic acid
PL 7902[a]	
PL 7903[a]	
CPL 2[a]	Ca salt of palmityl lactate
CPL 3[a]	
CPL 6[a]	Ca salt of stearyl lactate
CPL 7[a]	

[a] Experimental laboratory production batches

Table 2

Characteristic parameters of the determination

Component	Wavelength (nm)	Slit (mm)	Degree of sensitivity	
			Photo-multiplier	Amplifier
Ca	422.7	0.003	2	4
Fe	248.4	0.006	2	4
Pb	217.0	0.006	2	4
Na	589.05	0.004	1	1
K	770.1	0.020	4	2

metric titration using 1.0 mol dm^{-3} NaCl solution. 10 cm^3 0.1 mol dm^{-3} silver nitrate solution was added to 25 cm^3 nitric acid extract and the unused silver ion was determined with 1 mol dm^{-3} sodium chloride solution in an automatic titrimeter containing a chloride-ion sensitive electrode and a saturated calomel electrode connected by a potassium nitrate salt bridge. The equivalent value of the 10 cm^3 0.1 mol dm^{-3} silver nitrate was established in a separate experiment. On the basis of the titration curve, the point of equivalence was determined at 350 mV. The accuracy of the determination was $\pm 2\%$.

1.2.2. Determination of organic components. It was assumed that the original components (fatty acid and lactic acid), the end product (fatty acyl lactic acid) and condensation products formed from these and containing

several lactic acid molecules would be present, and methods were elaborated to examine them.

Preparation of the samples. When analysing CPL the first step is to liberate the acids from their calcium salts. This can be accomplished in various ways: with the aid of an ion exchanger, after JURRIENS and RECOURT (1966), or by acidic treatment, after SZÉLES (1980).

The acids were liberated using hydrochloric acid. One g of the sample under analysis was measured into an Erlenmeyer flask together with 75 cm^3 80% aqueous ethanol and sufficient of the 1 mol dm^{-3} hydrochloric acid solution to liberate the acids, judged by the known calcium content. After mixing for 1 h, the fatty acid esters were extracted from the mixture with ether.

The free lactic acid content of the sample remained in the aqueous solution and could be determined. The solution was neutralized and evaporated to dryness over a water bath. The quantity of free lactic acid was determined from the residue.

1.2.2.1. Gas-chromatographic determination of fatty acids and lactic acid. — Hundred mg of the sample under examination was measured into a round-bottomed flask with a ground glass stopper and 5 cm^3 methanol containing 10% BF_3 were added. For the determination of free lactic acid the evaporation residue was washed into the flask with this reagent.

The solution was boiled for 30 min under a reflux condenser and after cooling, 1–2 mm^3 of the solution was injected directly onto the gas chromatography column. A calibration curve was plotted using sample solutions with known quantities of fatty acid and lactic acid. The quantities of material were calculated with the aid of the calibration curve.

The conditions under which the gas-chromatographic separation was carried out were as follows:

Equipment:	Chrom 41 gas chromatograph (made in Czechoslovakia)
Separating column:	2400 × 4 mm
Separating phase:	10% polyethylene glycol succinate on a Chromosorb W HP carrier (100–120 mesh particle size)
Temperature:	Evaporator: 250 °C Thermostat: programmed, 100 °C for 6 min, rising to 180 °C at a rate of 10 °C min^{-1}, then isotherm
Carrier gas:	Nitrogen, 40 cm^3 min^{-1} at a pressure of 0.5 bar
Detector:	Flame ionization H_2 feed-in: 35 cm^3 min^{-1} air feed-in: 500 cm^3 min^{-1}
Recorder:	2000 mV
Sensitivity:	1/100
Paper velocity:	0.5 cm per min

Under these conditions the lactic acid methyl ester has a retention time of 250 sec, the palmitic acid methyl ester 1098 and the stearic acid methyl ester 1266 sec.

The quantitative evaluation was carried out using a Digint 35 μ integrator (manufactured by Chinoin), which printed the retention times and the numerical value proportional to the area under the curve. The standard deviation for the lactic acid determination was 4–5% and for the fatty acid determination 3.0–3.5%.

1.2.2.2. Liquid-chromatographic analysis of fatty acyl derivatives. — Liquid chromatography is an efficient large-scale separation process, which can be used to separate even complex samples.

After testing various procedures the fatty acid and fatty acyl lactic acid components of the samples were successfully separated in the form of p-bromophenacyl derivatives.

Preparation of p-bromophenacyl ester derivatives. From an ether solution of the sample under examination a quantity equivalent to approximately 1 mg fatty acid or its derivative was measured into a 5 cm^3 reaction vessel containing 5 mg K_2CO_3 using a Hamilton precision syringe and was evaporated to dryness by blowing dry nitrogen through it. 0.5 cm^3 acetonitrile and 100 mm^3 p-bromophenacyl-8 (0.1 mmol cm^{-3} p-bromophenacyl bromide and 0.005 mmol cm^{-3} crown ether in acetonitrile, manufactured by Pierce) were added to the sample. The reaction vessel was closed with a Teflon-coated lid and heated for 30 min in a Kutesz aluminium block test-tube thermostat at 80 °C. The sample was then cooled to room temperature. The reaction which takes place is illustrated in Fig. 1.

The crown ether acts as a catalyst in the reaction, since it binds potassium ions and catalyses the reaction between phenacyl bromide and acid. The reaction takes place with a high degree of efficiency even under mild conditions

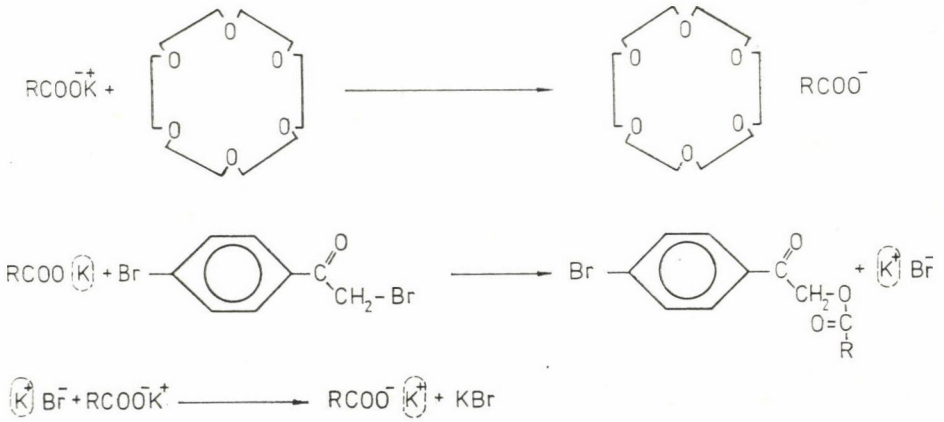


Fig. 1. Preparation of p-bromophenacyl ester derivatives

and the ester formed shows very intense absorption at 250–260 nm, which is the normal range for liquid chromatography detectors.

Since the molar extinction coefficient, almost independently of the acidic part, is 35 000 absorbance units (mol dm^{-3} cm^{-1}), a 1–50 μg amount of the acids can be determined. The molar extinction coefficient of the original acids is in the region of 14–23, i.e. only an amount 1000 times greater could be determined.

Conditions under which p-bromophenacyl derivatives are separated. A Waters liquid chromatograph was used to separate the p-bromophenacyl derivatives of the acids. The equipment consisted of a 6000 A high-pressure pump, a U6K sample charging apparatus, a separating column and an M-440 UV photometer. The latter was equipped with a 254 nm wavelength filter.

For the separations a μ Bondapack "Fatty acid" column (300×4 mm), manufactured by Waters, was used.

Conditions under which separation on the fatty acid column was carried out:

Eluent: tetrahydrofuran–acetonitrile–water (25 : 45 : 30)
Flow rate: 2 cm^3 min^{-1}
P: 85 bar
Detector sensitivity: 0.05 absorbance units per 20 mV
Recorder: RadelkisOH 814/1 compensograph with a paper velocity of 30 cm per h.

1.2.2.3. Analysis of derivatives using thin-layer chromatography. — An attempt was made to elaborate a rapid method for the analysis of the polycondensates formed during the production of palmityl lactic acid, so that these could be demonstrated and quantitatively estimated.

SASS-KISS and VÁCZY (1979) reported on the thin-layer chromatographic separation of Admul 1914 Ca SL (calcium stearyl lactate) on a Kieselgel G layer by petroleum ether–ether and acetic acid (50 : 50 : 1). An alkaline alcoholic solution of bromophenol blue was used as the developing agent.

In the course of examinations the separation was found to be adequate on a Silufol layer (made in Czechoslovakia) with a 7 : 3 hexane–ether mixture.

Developing was carried out using a 0.1% alcoholic bromophenol blue solution to which ammonium hydroxide was added dropwise until it turned blue.

On the chromatogram the palmitic acid moved to the highest point, $R_f = 0.85$, while the lactic acid did not move at all, $R_f = 0$.

The fatty acid esters separate approximately into three groups with R_f values of 0.54, 0.38 and 0.20, respectively.

The sprayed chromatogram can be rapidly evaluated densitometrically with a blue filter, since the blue background does not absorb, while the yellow spots produced by the acids do.

Either with visual estimation or densitometric evaluation, layer chromatography is suitable for the separation of fatty acyl lactic acid esters from free fatty acid and lactic acid, and for that of condensates.

The procedure is not suitable for the separation of different fatty acids, since neither the fatty acids nor their derivatives separate, but form a single spot. This is an advantage if the aim is simply to determine the course of the reaction and to demonstrate free fatty acid and free lactic acid.

1.2.3. Dough rheological examination of the special effect of the preparations. The changes taking place in the rheological characteristics of the dough were followed by penetrometric measurements. The measurements were carried out on a Labor-MIM penetrometer. Doughs made from BL 55 flour in a Laborograph manual kneading machine with a constant water addition of 58% were examined after standing for 30 min at 30 °C and the degree of penetration was determined in PE units (1 PE = 0.1 mm). The standard deviation was 3–5%.

2. Results and discussion

2.1. Analytical results for inorganic components

The analytical results obtained for the inorganic components of the samples are presented in Tables 3 and 4.

It can be seen from the results that there are wide differences between the preparations with regard to the $CaCl_2$ content. A particularly high $CaCl_2$ content is found in the experimental laboratory preparations. This is probably due to the imperfection of the technological process for the formation of the Ca salt. A thorough investigation and testing of this technological step and an increase in the efficiency are essential for the quality of the product.

Table 3

Chloride content of the samples and the calcium chloride content calculated from it

Sample	Chloride (mg g^{-1})	Calcium chloride (% w/w)
CPL 2	48.2	7.54
CPL 3	64.1	10.03
CPL 6	41.6	6.51
CPL 7	45.0	7.04
Admul 2002	6.1	0.95
Admul 2008	12.2	1.91
Oleofinx	8.1	1.27

The samples were not significantly contaminated with alkali metals, the degree of contamination generally being less than 1% (w/w). Comparing experimental CPL samples with trade samples, the latter were found to contain higher quantities. This is presumably introduced into the sample when the pH is adjusted for salt formation.

Heavy metal contamination probably occurs in the course of manufacturing, possibly as calcium chloride contaminants. The degree of heavy metal contamination is not significant, but it would be advisable to discover how the contamination occurs, in order to prevent it.

2.2. Results of determination of organic components

2.2.1. Fatty acid composition. Among the fatty acid components of the samples examined by gas chromatography, palmitic acid and stearic acid were found, as shown in Table 5.

In the same series of analyses the free and total lactic acid contents of the samples were determined, as summarized in Table 6.

The results show that there are certain differences in the free lactic acid contents of the individual preparations, but these differences are not characteristic; the marketed preparations also have varying free lactic acid values. The free lactic acid content of PL preparations is lower than that of CSL preparations.

There are characteristic differences in the bound lactic acid quantity, which was calculated as the difference between the total and free lactic acid contents. The low values found for samples CPL 2 and CPL 3 show that these really do contain very little genuine ester. Although this determination does not give any idea of the distribution of the esters, it is a good indication of the quantity.

Table 4

Inorganic contaminating components in the samples

Sample	Na ($mg\ g^{-1}$)	K ($mg\ g^{-1}$)	Fe ($\mu g\ g^{-1}$)	Pb ($\mu g\ g^{-1}$)
CPL 2	0.55	0.73	12	18
CPL 3	1.93	1.02	60	18
CPL 6	0.14	1.95	67	18
CPL 7	0.83	0.94	46	33
Admul 2002	1.59	0.87	3	2
Admul 2008	6.14	1.35	5	2
Oleofinx	0.76	0.91	2	2

Table 5

Fatty acid components of the samples examined (mol %)

Sample	Palmitic acid	Stearic acid
PL 7901	99	trace
PL 7902	99	trace
PL 6903	99	trace
CPL 2	99	trace
CPL 3	99	trace
CPL 6	39	61
CPL 7	35	65
Admul 2002	49	51
Admul 2008	45	55
Oleofinx	35	65

Table 6

Free lactic acid content of samples (mol %)

Sample	Free lactic acid	Bound lactic acid	Total lactic acid
PL 7901	2.3	41.3	43.5
PL 7902	2.2	41.5	43.7
PL 7903	1.5	41.1	42.6
CPL 2	5.5	14.2	19.7
CPL 3	11.7	6.8	18.5
CPL 6	8.5	30.9	39.4
CPL 7	4.8	16.0	20.8
Admul 2002	8.4	19.7	28.1
Admul 2008	2.3	34.7	38.0
Oleofinx	10.0	19.6	29.6

2.2.2. Results of liquid-chromatographic analysis of fatty acyl derivatives. p-bromophenacyl derivatives have numerous advantages. The molar extinction coefficient is chiefly associated with the p-bromophenacyl part and is independent of the quality of the acidic part. Thus, for the majority of derivatives tested, it has a value of 18 700 absorbance units (mol dm^{-3} cm^{-1}).

After the separation it is unnecessary to construct a calibration curve for the quantitative evaluation, since the ratios of the areas under the peaks give the mol % composition.

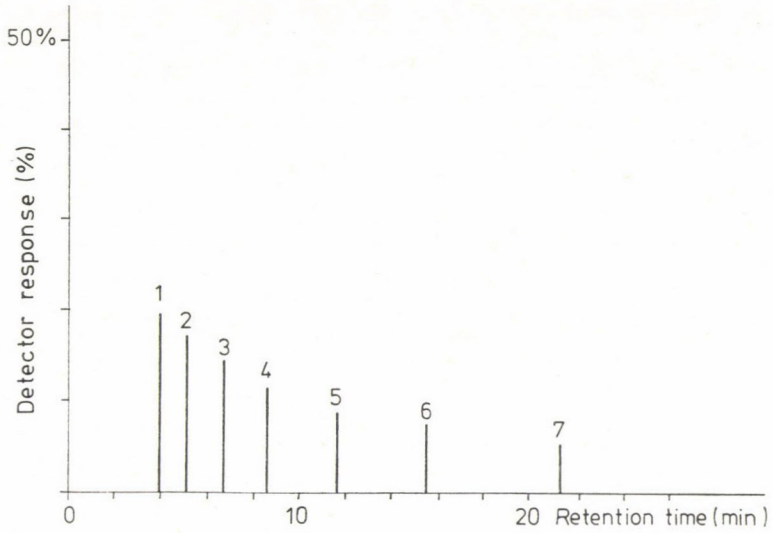


Fig. 2. Stylized chromatogram of fatty acid derivatives. 1 = Capric acid; 2 = Lauric acid; 3 = Myristic acid; 4 = Palmitic acid; 5 = Stearic acid; 6 = Arachidic acid; 7 = Behenic acid

The correlation between the retention ratio of p-bromophenacyl derivatives of fatty acids with different numbers of carbon atoms and the number of carbon atoms was investigated. The separation can be seen on the stylized chromatogram in Fig. 2.

As expected, the logarithm of the retention ratio was in linear correlation with the number of carbon atoms in the fatty acid (see Fig. 3) and the diagram constructed can be used to identify unknown fatty acids in unknown samples.

The logarithms of the individual condensation products also show a close linear correlation with the number of lactic acid units. This fact was used in the identification.

The identification of the compounds belonging to each peak was confirmed by extracting the derivatives and determining the palmitic acid–lactic acid ratio by gas chromatography.

A few characteristic results for each type are presented in Tables 7–9 while the values of the characteristic components are summarized in Table 10.

The chromatographic analyses confirmed that there is no significant difference between the PL samples.

The results show that there are considerable differences between the samples in the total quantity of fatty acyl derivatives and in the proportion of monoacyl and polyacyl derivatives.

Fatty acyl lactic acid was present in the highest amount in the Admul sample and in the lowest amount in sample PL 7903.

Greater and more characteristic differences can be observed between the samples for free fatty acids.

The PL samples and the CPL 2 and CPL 3 samples derived from them were prepared using pure palmitic acid, so these only contained palmitic acid. CPL 6 and CPL 7, which were prepared using stearic acid of technical quality, contain considerable amounts of palmitic acid and their composition resembles those of trade preparations.

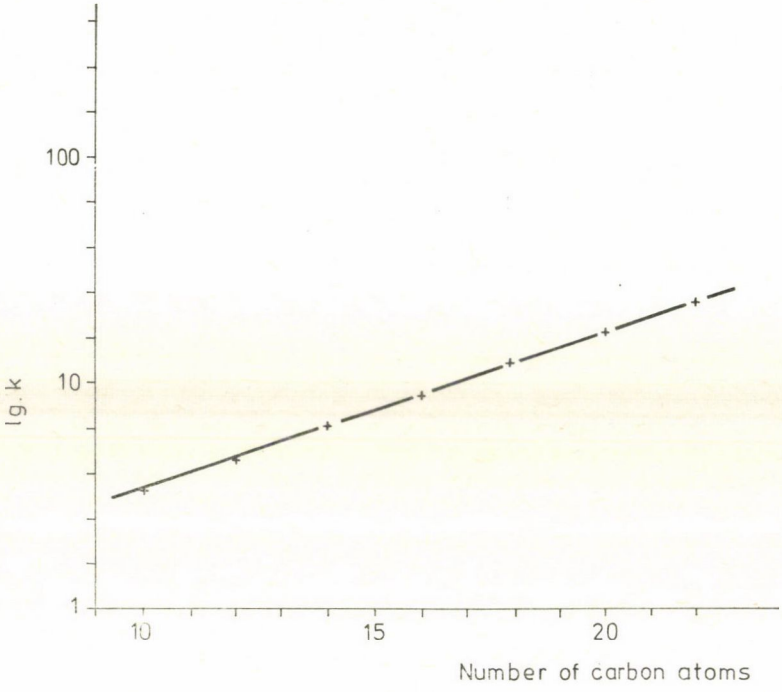


Fig. 3. Correlation between the number of carbon atoms in the fatty acids and the logarithm of the retention ratio of their phenacyl esters. $\lg k = -0.16 + 0.0684\ C$

Table 7

Fatty acid–lactic acid condensation components of sample PL 7901

Retention time (min)	Retention ratio (k)	Quantity (mol %)	Identification of the component
9.6	8.56	14.6	Palmitic acid
10.6	9.56	44.7	Palmityl monoester
11.7	10.65	19.5	Palmityl diester
13.3	12.25	9.9	Palmityl triester
14.0	12.94	7.4	Palmityl tetraester
15.4	14.34	3.8	Palmityl pentaester

Table 8

Fatty acid – lactic acid condensation components of sample CPL 2

Retention time (min)	Retention ratio (k)	Quantity (mol %)	Identification of the component
8.3	8.56	41.3	Palmitic acid
9.2	9.60	39.7	Palmityl lactic acid
10.5	11.09	12.0	Palmityl diester
12.2	13.05	5.5	Stearic acid
13.9	15.01	1.7	Palmityl tetraester
15.4	16.74	0.9	Palmityl pentaester

Table 9

Fatty acid – lactic acid condensation components of sample Oleofinx CSL 1290

Retention time (min)	Retention ratio (k)	Quantity (mol %)	Identification of the component
6.4	5.79	0.5	Myristic acid
6.8	6.22	0.3	Myristyl lactic acid
3.0	8.56	4.5	Palmitic acid
9.8	9.40	5.2	Palmityl lactic acid
12.0	11.74	21.0	Stearic acid
13.4	13.23	30.6	Stearyl lactic acid
14.0	13.86	27.7	Stearyl diester
14.8	14.71	10.2	Stearyl triester

Table 10

Tabular summary of the quantities of fatty acid acyl lactic acid condensation products in mol %

Sample	Free fatty acid	Fatty acid acyl lactic acid	Fatty acid acyl polylactic acid
PL 7901	14.6	44.7	40.7
PL 7902	9.5	37.2	53.3
PL 7903	5.1	29.0	65.9
CPL 2	46.8	39.7	13.5
CPL 3	35.9	32.5	31.6
CPL 6	46.2	39.5	14.3
CPL 7	47.6	38.6	13.8
Admul 2002	43.1	54.7	2.2
Admul 2008	42.1	46.2	11.7
Oleofinx	26.0	36.1	37.9

Table 11

Characteristic results of the dough rheological tests

Sample	Surface-active additive (%)		
	0.0	0.3	0.5
	Consistency (PV)		
CPL 2	122	96	97
CPL 3	122	101	98
CPL 6	122	80	78
CPL 7	122	82	80
Admul 2002	122	78	72

2.3. Results of penetrometric analysis of dough

For the analysis doughs were made with water, with the addition of 0, 0.3 and 0.5% surface-active material. The characteristic results of the penetrometric analyses are illustrated in Table 11.

It can be clearly seen that the expected effect was exerted on the dough: in each case the penetration value decreased, i.e. the consistency of the dough became harder. Although the nature of the effect was identical, there were considerable differences between the extent of the effect for the various preparations. Among the experimental CPL preparations the effects of CPL 6 and CPL 7 were most marked. It should be noted, that the changes observed were significant in every case (for the other preparations, too).

3. Conclusions

On examining the composition of the CPL samples and comparing trade and experimental preparations it was found that each of them contained a mixture or at least two fatty acids, so they were presumably made from mixtures or from fatty acid of technical purity. The amount of free fatty acid was higher than that of the monoester in every case. Thus a certain amount of free palmitic acid does not involve a deterioration in quality. The importance of the fatty acid mixture lies in the fact that the melting point of the product is lower than that of the two pure derivatives. In general only the monoester is present, the amount of polyesters being negligible.

The following proposals can be made on the basis of the examinations with regard to the composition of trade products judged to be adequate and on the basis of theoretical considerations for the CPL products:

A) The quantity of free lactic acid should not exceed 5–8%.

B) The quantity of free fatty acid should not exceed that of fatty acyl lactic acid.

C) The product should contain 30–60 mol % of at least two (16 and 18 carbon atom) saturated fatty acids.

D) The chloride content should not exceed 10–12 mg g^{-1} product.

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INVESTIGATION INTO THE INTERACTION OF DIFFERENT PROPERTIES IN THE COURSE OF SENSORY EVALUATION

III. INTERACTION BETWEEN COLOUR, TEXTURE AND FLAVOUR

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The previous papers in this series dealt with the effect of colour on the evaluation of flavour (URBÁNYI, 1982) and that of texture on the evaluation of flavour (URBÁNYI, 1983). In the course of these experiments only the two given characters were examined in each case. Considering the fact that food quality is determined simultaneously by a number of characters, it is expedient to study the combined interaction of these characters.

In the opinion of KRAMER (1975) sensory characters are not independent of each other, but may overlap and influence each other.

This connection between the sensory characteristics of food can be graphically illustrated in such a way that individual characters are placed around the edge of a circle, from which the connections and overlapping can be read off (KRAMER, 1968).

On studying the role of colour, FRANCIS (1977) found that colour was an important factor among the sensory characters, since, if the colour was not attractive, the consumer would not accept the flavour or texture either.

Taking these findings into consideration, further studies were made on the simultaneous interaction between colour, texture and flavour. The results of these experiments are presented in this paper.

Keywords: Sensory evaluation, interaction of sensory properties

1. Materials and methods

1.1. Materials

The experimental material in one of the experiments was potato, two varieties of which were chosen, with colours which could be easily distinguished. One was yellow, while the other had a pinkish tinge.

The other product tested was quick-frozen strawberries, prepared from two different varieties, each at three different stages of ripeness. Thus, within each variety there were samples which differed in flavour, colour and texture. But since all three characters depend on the degree of ripeness, they are not independent of each other.

The raw materials used in the experiments were bought on the open market, so it was impossible to identify the varieties.

1.2. Methods

The evaluations were carried out, as in the previous papers, using the scoring method described by AMERINE and co-workers (1965), which was developed by Peryam and Shapiro. This 9-point scale applies the following terminology:

9 — excellent
8 — very good
7 — good
6 — below good, above fair
5 — fair
4 — below fair, above poor
3 — poor
2 — very poor
1 — extremely poor.

Ten-man committees made the evaluations, in the course of which re-tasting was allowed.

The results of the evaluations were analysed using Kramers's method, as described in the first paper in this series (URBÁNYI, 1982).

2. Results

In the first phase of the experiments samples were prepared from two differently coloured varieties of potato, in such a way that there were differences in colour, flavour and texture. Then the differences in texture were eliminated by mashing and the colour and flavour were evaluated. Finally, in the last phase of the experiment, the colour differences were eliminated by colouring the samples, so that only the flavour was evaluated.

Textural differences within the variables were produced by varying the cooking time. Both varieties were divided into two parts, one of which was cooked for 20 min, the other for 30 min. In the first phase the potatoes cut in lumps were evaluated.

The following four samples were given to the panelists:

— Pink tinged potato cooked for 20 min
— Pink tinged potato cooked for 30 min
— Yellow potato cooked for 20 min
— Yellow potato cooked for 30 min

The Kramer analyses of the results of the evaluations are presented in Table 1.

The evaluation of whole potatoes indicated that the judges distinguished easily between the colours of the two varieties. Both the samples prepared

Table 1

Kramer analysis of evaluation scores of potato samples of different varieties
(Number of panel members: 10)

Samples	Rank sum		
	Colour	Flavour	Texture
	Whole samples		
1	17.0	20.0	27.0
2	17.0	14.5**	17.5
3	34.5*	33.5*	32.0
4	31.5	32.0	23.5
	Mashed samples		
1	15.0*	21.0	
2	15.5*	23.5	
3	35.0*	32.5	
4	34.5*	23.0	
	Mashed, coloured samples		
1		21.0	
2		25.5	
3		24.5	
4		29.0	

* Significant at $P \geq 0.05$ probability level (17 – 33)
** Highly significant at $P \geq 0.01$ probability level (15 – 35)

Symbols:
1 Pink potatoes cooked for 20 min
2 Pink potatoes cooked for 30 min
3 Yellow potatoes cooked for 20 min
4 Yellow potatoes cooked for 30 min

from the pink variety were judged to be better than the yellow samples, and no difference was established between them. Colour differences between the yellow samples were due to the non-uniform colour of the individual potatoes.

With regard to flavour, too, samples prepared from the pink potatoes were given higher points. The flavour of pink potatoes cooked for a longer period was judged to be significantly better at the 99% level of probability, while the yellow samples cooked for a shorter period were judged to be poorer at the 95% level of probability.

The evaluation of texture shows that for both varieties, samples cooked for a longer period were judged to be better. In addition, the texture of samples with better flavour and a more attractive colour was awarded higher points.

In order to study the correlations between the individual characters, linear regressional analysis was carried out on the points awarded by the panelists. The correlation coefficient obtained for colour and flavour ($r = 0.98$) indicates a close correlation. The corresponding value for flavour and texture was 0.67, while that for colour and texture was 0.56.

When the difference in texture was eliminated, there was a reduction in the difference in flavour, which was no longer significant.

For mashed samples the value of the correlation coefficient calculated for colour and flavour decreased considerably to 0.67.

Finally, when all the samples were uniformly coloured yellow, the differences in colour disappeared. The data for flavour evaluation in this case showed a further reduction in the differences between the flavours. While in the first phase, when there were differences in all three characters, the rank sums ranged from 14.5 to 33.5, in the second phase the range was 21.0–32.5, and in the third phase, when differences between both colour and texture had been eliminated, the range was only 21.0–29.0.

The correlation found between the points awarded for flavour in the various phases of the experiment was also investigated. The correlation coefficient for evaluations of flavour in the whole and the mashed state was 0.65, that between whole and mashed coloured samples was 0.56, and that between coloured and non-coloured mashed samples was only 0.09. Since no close correlation could be pointed out between the evaluation of flavour despite the fact that there was no real difference between the flavours of identical samples in the various phases of the evaluation, it can be concluded that the results of flavour evaluation were influenced by the other characters.

For the colour, which could be distinguished independently from the other characters, the correlation coefficient calculated between the whole and pulped samples was 1.00, proving the lack of dependence of colour judgment on the other characters.

Similar experiments were carried out with quick-frozen strawberries. Samples of two varieties, each at three stages of ripening, were evaluated. It should be noted that in this case all three characters depend on the stage of ripeness and are thus connected to each other. The samples were as follows:

— Variety I, half ripe (approx. 50%)
— Variety I, medium ripe (approx. 75%)
— Variety I, ripe
— Variety II, half ripe (approx. 50%)
— Variety II, medium ripe (approx. 75%)
— Variety II, ripe.

This grouping gave a series of samples which all differed from each other in colour, flavour and texture. All three characters were evaluated.

In order to eliminate differences in the texture, these same samples were pulped, after which only the colour and flavour of the samples were evaluated. Finally, the samples were given a uniform colour with red colouring, so that only the flavour was evaluated.

Table 2. contains the Kramer analysis of the evaluations.

Table 2

Kramer analysis of the scores for strawberry samples
(Number of panel members: 10)

Samples	Rank sum		
	Colour	Flavour	Texture
	Whole samples		
1	46.5	43.5	29.0
2	20.5*	19.5**	20.0*
3	16.0**	18.0**	29.5
4	60.0**	58.5**	30.5
5	41.0	38.5	50.0
6	26.0	32.0	51.0
	Pulped samples		
1	42.0	43.5	
2	19.0**	22.0	
3	14.5**	20.5*	
4	60.0**	58.0**	
5	45.5	38.0	
6	29.0	28.0	
	Pulped, coloured samples		
1		41.5	
2		24.0	
3		15.5**	
4		57.5**	
5		41.0	
6		30.5	

* Significant at $P \geq 0.05$ probability level (22 – 48)
** Highly significant at $P \geq 0.01$ probability level (20 – 50)

Symbols:
1 Variety I, half ripe
2 Variety I, medium ripe
3 Variety I, ripe
4 Variety II, half ripe
5 Variety II, medium ripe
6 Variety II, ripe

When evaluating whole strawberries the judgements were identical for colour and taste and there was only one difference in ranking for the texture. The correlation coefficient $r = 0.98$ between the colour and the flavour indicates a close correlation. No significant correlation was observed between colour and texture or flavour and texture. A similar result was obtained for the evaluation of pulped samples. A comparison of the two evaluations shows that when there are significant differences (in many cases significant at the 99% level of probability) between the colour and the flavour, the influence of the texture is not manifested. This observation is confirmed by the fact that here, too, a close correlation ($r = 0.93$) was obtained for the points awarded for colour and flavour.

The evaluation of the flavour of coloured samples led to identical results, i.e. when the differences were great, the influence of the colour lost its effect or acted to strengthen the differences in flavour. The agreement in the evaluations of colour and flavour are related to the real differences connected with the degree of ripeness.

The following correlation coefficients were obtained for the flavour evaluations of strawberry samples in various states:

whole strawberries, pulped strawberries: $r = 0.93$

whole strawberries, pulped and coloured: $r = 0.96$

pulped strawberries, pulped and coloured: $r = 0.92$

The close correlations confirm that when there are considerable differences in flavour the influence of the other characters is not manifested.

The $r = 0.98$ value found between the colours of whole and pulped strawberries is in agreement with that observed for potatoes.

3. Conclusions

Experiments carried out in order to examine the interaction between colour, flavour and texture show that if there are no great differences between the various characteristics of the samples the influence of the other characters is felt in the points awarded for each character. The evaluation of the colour, however, which is easier to distinguish and can be judged independently of the other characters, is not influenced by the other two characters.

When there are considerable differences between the samples, the evaluations are not influenced in the manner described above. Thus, in making sensory evaluations, particular attention should be paid to this phenomenon when there are no great differences between the samples.

In summary it can be stated that the various sensory characters affect the evaluation of the other characters to an extent depending on the degree of difference between the samples.

Taking this effect into consideration, if an objective evaluation of only slightly differing samples is to be made, particularly for the flavour, which cannot be judged instrumentally, it is advisable to evaluate samples where the colour and texture have been standardized without altering the flavour.

A similar conclusion was drawn by BOHREN and JORDAN (1953), who used coal-tar dyes to mask colour differences between dried egg samples, and by HELM and TROLLE (1946), who used flavourless dyes to mask differences in colour when evaluating beer.

In order to mask differences in texture and eliminate their effect, KEFFORD and CHRISTIE (1960) also recommended carrying out the evaluations on homogenized purees, rather than in the original state.

The experiments were carried out on samples of potato and strawberries. For both experimental materials the first evaluations were made on samples which differed in flavour, colour and texture. In the following stage the differences in texture were eliminated by pulping, so that only colour and flavour were evaluated, while in the final stage differences in colour were also masked and the flavour alone was evaluated.

Correlations were studied between the various characteristics of samples examined in the same state and between the flavour evaluations of identical samples judged in different states.

On the basis of the experiments the following conclusions can be drawn.

If the differences between the various characters of the samples are slight, the points awarded for each character are influenced by the other characters, but the evaluation of colour, which can be more easily distinguished and can be evaluated independently of the other characters, is not influenced by the other two characters.

When there are considerable differences between the individual characters the influence of the other characters cannot be demonstrated. Thus, when making sensory evaluations, particular attention should be paid to this phenomenon if there are only slight differences between the samples.

In summary it can be stated that the individual sensory characters affect the evaluation of the other characters to an extent depending on the degree of difference between the samples.

Taking this effect into consideration if an objective evaluation of only slightly different samples is to be made, particularly for the flavour, which cannot be judged instrumentally, it is advisable to evaluate samples where the colour and texture have been standardized without altering the flavour.

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GAMMA RADIATION TECHNOLOGICAL FEASIBILITY OF INCREASING SHELF-LIFE OF TABLE GRAPES

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Gamma radiation (1 and 2 kGy) and different sources of sulfur dioxide (0.25% SO_2, 4 g $K_2S_2O_5$, 4 g $K_2S_2O_5$ + 4 g $C_6H_8O_7$ per kg grape) and moist air heat (55 °C for 15 minutes) were used to increase shelf-life of Black Alicante table grapes stored at 10 °C and 95% relative humidity. The grape clusters were placed on foam polystyrene trays and packed with polyvinyl-chloride film.

Gamma radiation at 2 kGy completely prevented decay of Black Alicante table grapes stored for 40 days at 10 °C without any discernible change in the organoleptic attributes of the berries.

Moist air heat, 0.25% SO_2 and 1 kGy gamma radiation used alone neither prevented nor delayed moulding, where as potassium meta-bisulfite mixed or not mixed with citric acid delayed moulding for 15 and 35 days, respectively.

1 kGy gamma radiation dose in combination with 0.25% SO_2 controlled moulding for the entire storage period of 40 days.

Potassium meta-bisulfite mixed with citric acid or in combination with 2 kGy induced adverse organoleptic changes in the berries.

Keywords: Shelf-life, gamma irradiation of fruits, chemical treatment of fruits

Wastage in grapes during storage or transport is mainly caused by gray mould (*Botrytis cinerea* Pers), detachment of berries from the clusters and desiccation (BEATTIE & OUTBRED, 1970).

Various methods of preservation of table grapes have been used individually without much success.

The shelf-life of table grapes can be increased to a certain extent by low temperature (0 °C) and high relative humidity storage condition, but it is a very expensive method.

In many countries of the world, sulfur dioxide fumigation at weekly or 10 days intervals at low storage temperature (−1 to 1 °C) is a common practice for the long term storage of table grapes (BEATTIE & OUTBRED, 1970).

Sulfur dioxide was first used in 1925 for increasing shelf-life of table grapes (COMBRINK & GRINSBERG, 1972). Since that time the technique of sulfur dioxide application to grapes during storage have not yet been resolved.

HARVEY (1955), COUEY and UOTA (1961), MCCLELLAN and co-workers

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(1973) believe that under laboratory conditions, fumigation kills fungus spores or mycelium on the surface of grapes, but cannot kill fungus mycelium that has already invaded tissues of the berries. The gray mould fungus infects the grape flower through the stylar end, and then becomes latent in the necrotic stigma and style tissues of the berry.

NELSON and RICHARDSON (1967) and GENTRY and NELSON (1968) found that at higher temperature a high concentration of SO_2 was required to prevent *Botrytis* rot in grapes and increased concentration of SO_2 caused severe bleaching of grapes. NELSON and AHMEDULLAH (1970) concluded that for the decay protection for four months, yet with bleaching within acceptable limits, generators are needed which release SO_2 at a more constant rate than those, now available. NELSON and co-workers (1971) and NELSON and AHMEDULLAH (1972) found that packing methods of grapes and the thickness of polyethylene liner play a major role in the prevention of moulds and bleaching of grapes by SO_2. HEDBERG(1977) recommends two-stage sulfur dioxide and low temperature for long storage of table grapes. CODOUNIS (1978) concluded from his studies that with two-stage SO_2 generator most of the SO_2 given off by meta-bisulfite is oxidizing to sulfate and only a small part of it is useful in grape storage and the absorption by grapes is minimal.

Based on the foregoing discussion the use of SO_2 for grape storage has the limitations of inability to reach the inside of berries (COMBRINK & GRINSBERG, 1972; COUEY & UOTA, 1961, and MCCLELLAN et al., 1973) at the center of compact clusters (COMBRINK & GRINSBERG, 1972; HEDBERG, 1979; NELSON et al., 1970), irregular distribution within the package (COMBRINK & GRINSBERG, 1972), necessity for repeated treatments, frequent injury to the grapes, corrosion of metals, impairing marketability and not practical in transport and retailmarkets (COMBRINK & GRINSBERG, 1972; HARVEY, 1955; NELSON & AHMEDULLAH, 1970).

NELSON and co-workers (1959) using electron accelerator as a source of radiation found marked reduction in *Botrytis cinerea* spread on grapes at 1×10^5 and 2×10^5 rep doses (0.91 to 1.83 kGy) without any quality changes. However, in their studies, doses of 4×10^5 rep (3.64 to 7.28 kGy) caused browning of the berries and off-flavour of grapes. BERAHA and co-workers (1961) found that at 1.83 kGy Tokay grapes inoculated with *Botrytis cinerea* Pers. remained mould-free for four days at 24 °C, whereas for 10 days storage they required 500.000 rep doses (4.57 kGy) and the time of appearance and intensity of rotting were inversely proportional to the dose. They have shown that at 4.57 and 9.11 kGy moulding was completely prevented but these doses give off-flavour and cause bleaching of the grape. MAXIE and co-workers (1964) found gamma irradiation to cause damage at 1–3 kGy to Emperor or Tokay grapes and concluded that irradiation cannot be substituted by sulfur dioxide and is not feasible for grape storage.

BARKAI-GOLAN and co-workers (1969) found synergestic effect between radiation (0.25–1 kGy) and heating (45–50 °C) in reducing the viability of *Penicillium digitatum* spores. They also found inverse relationship between percentage of viable spores and an increase in radiation dose or temperature.

The purpose of this study was to investigate the joint effect of heat, sulfur dioxide and gamma radiation and to develop a two-stage SO_2 generator system under elevated temperature for increasing shelf-life of table grapes.

1. Materials and methods

Black Alicante table grape (*Vitis vinifera* L.) were obtained from a storehouse in Westland, The Netherlands, on October 30, 1980. The study included such treatments as gamma irradiation (0, 1 and 2 kGy), 0.25% v/v sulfur dioxide, potassium meta-bisulfite (4 g to 1 kg grapes), potassium meta-bisulfite plus citric acid (1 : 1 ratio); moist air heating (55 °C for 15 minutes) and the combination of gamma radiation with all the other treatments. All treatments had two replications and in each replication about half a kilogram of grapes was used.

For the gamma *radiation treatment* a grape cluster was weighed, and then placed in a polystyrene foam box ($25 \times 16 \times 2$ cm) and wrapped in polyvinylchloride film and the film was sealed with sealing machine. The prepacked grapes were then irradiated at 1 and 2 kGy with a ^{60}Co gamma source of 50 000 Ci capacity. For the measurement of dose distribution and dose uniformity ratio the Clear Perspex dosemeter system was used. The dose rate was 2.5 kGy h^{-1} and the dose uniformity ratio was 1.4. Following irradiation the grapes were stored at 10 °C and 95% relative humidity.

For the *sulfur dioxide treatment*, grapes were weighed and packed in the same way as was described for the radiation treatment. After packing, the volume of the packed boxes was determined and 0.25% v/v sulfur dioxide (5.6 cc SO_2) was injected into each of the boxes and they were then transferred to the storage room. Thereafter, the sulfur dioxide injection was repeated twice per week till the end of the experiment.

For the *potassium meta-bisulfite* ($K_2S_2O_5$) *treatment*, two grams of this compound were placed in each of the paper sacks (SO_2 generator or diffuser), size 9×7 cm, and one such sack containing $K_2S_2O_5$ was placed in each of the polystyrene foam boxes containing weighed grapes and wrapped in polyvinylchloride film and stored at 10 °C and 95% relative humidity.

The *potassium meta-bisulfite was combined with citric acid treatment* at the ratio of about 1 : 1. Two grams of $K_2S_2O_5$ were mixed with two grams of crystalline citric acid ($C_6H_8O_7$) for better sulfur dioxide releasing and placed in each of the paper sacks (SO_2 generator-diffuser). One paper sack was

then placed in the polystyrene foam boxes containing weighed grapes. They were wrapped in polyvinylchloride film and stored under the previously mentioned conditions.

For *heat treatment*, grapes were weighed and then placed in air heated to 55 °C ± 1 °C and at about 90% relative humidity for 15 minutes. Following heating they were packed in polystyrene foam boxes and wrapped in polyvinylchloride film. Some were transferred to the storage room and some were irradiated at 2 kGy two hours after heating.

For the *combined treatments*, the SO_2 treated prepacked grapes were irradiated at 1 and 2 kGy one half hour after the SO_2 injection and then stored. Grapes treated with potassium meta-bisulfite and the conbination of meta-bisulfite and citric acid were irradiated 24 hours after these treatments.

Grapes were then stored at 10 °C and 90% relative humidity.

During storage the number of moulded berries in each treatment and each replication were recorded. In addition to that the samples were frequently checked for visible colour changes of stem and berries and the exudation of the berries. After 40 days storage at 10 °C temperature and 95% relative humidity the experiment was terminated.

The percentage of moulded berries during the 40 days storage period was calculated based on the determined total number of berries per replicate. Percentage total loss (dessication and moulding) was also determined by weighing.

At the end of the experiment before opening the packages their CO_2 and O_2 content was measured with Orsat apparatus. After opening the packages the total weight of grapes was recorded. The number and weight of moulded and intact berries was also determined.

For the organoleptic quality studies following the 40 days storage period, the intact berries were cut off from their clusters and stored for 7 more days at 0 °C and 95% relative humidity. The berry samples stored 47 days were tested by 8 judges for their colour, texture (firmness) and taste by the following scoring system:

Quality terms	Scores
Excellent	10
Very good	9
Good	8
Fairly good	7
Adequate	6
Acceptability limit	5
Somewhat poor	4
Poor	3
Very poor	2
Extremely poor	1

For statistical analysis, the scores of each jugde were transformed into rank numbers and from these the rank totals (rank sums) were calculated (KRAMER, 1956).

For instrumental measurements each cluster was arbitrarily divided into two parts: shoulder (stem end of the cluster) and end.

From each of the two parts of a cluster 3 berries were randomly selected and the firmness at both sides at about the middle of each berry was measured by a simple nondestructive system. By means of a plunger a pressure of 300 grams is placed upon the fruit via a balance system during 15 seconds. Penetration of the plunger in the flesh of the fruit is read off on a scale of 0–100. The diameter of the plunger can be varied according to the size of the fruit.

Colour changes of the berries were measured with a reflectometer (Carl Zeiss model RA3) attached to the spectrophotometer (Carl Zeiss model M4QIII) at 620 nm wavelength and 0.18 mm slit width. For the colour change measurements, 8 berries were randomly selected from each of the two parts of the cluster and the percent reflection was measured both at the stem and blossom end of each berry.

Analysis of variance (CLARKE, 1980) was used for the statistical analysis of % total loss, % reflection and firmness.

2. Results

2.1. Percentage visible moulding of berries

Results of percentage visible moulded berries during 40 days storage period at 5-day intervals are shown in Figs. 1, 2 and 3.

The percentage of visible moulded berries of Black Alicante table grapes, after 40 days storage period, are shown in Fig. 4.

2.2. Percentage total loss

Percent total loss (% desiccation weight loss + % decay loss) of Black Alicante table grapes stored for 40 days at 10 °C and 95% relative humidity is shown in Table 1.

Results in Table 1 indicate that gamma radiation at a dose of 2 kGy alone or/and in all combinations except in combination with potassium meta-bisulfite has reduced the percent total loss significantly at the $P \geq 0.05$ level.

There was no significant difference at $P \geq 0.05$ in the percent total loss between the control, heat alone, 1 kGy, 1 kGy in combination with either potassium meta-bisulfite or potassium meta-bisulfite mixed with citric acid.

Sulfur dioxid, potassium meta-bisulfite and potassium meta-bisulfite mixed with citric acid treatments applied individually decreased the percent

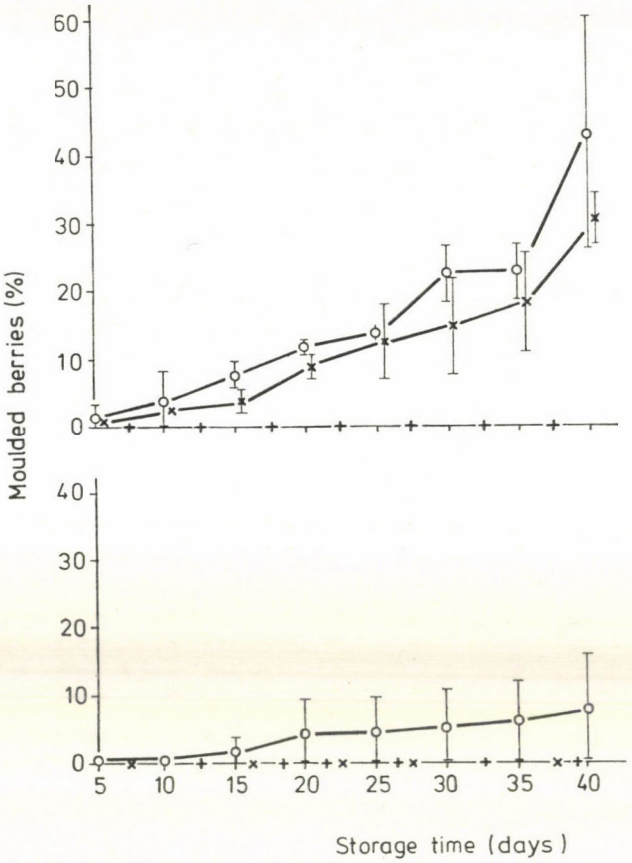

Fig. 1. Effect of radiation (1, 2 kGy) and combined treatments (SO_2: 0.25%) on visible moulding of Black Alicante grape during 40 days storage at 10 °C. $\overline{\overline{\mathrm{x}}}$ = Average of two replicates with the highest and lowest values. —○—: 0 kGy, —x—: 1 kGy; —+—: 2 kGy

total loss significantly at the $P \geq 0.05$ level as compared to the control treatment.

Percent weight loss due to % desiccation was very small ranging from 2.5 to 3.42% for all the treatments except heat alone and radiation at a dose of 1 kGy in combination with potassium meta-bisulfite which caused 5.8 and 6.3% desiccation weight loss, respectively (Table 1).

This reduction in percent desiccation over the 40 days storage period was due to the use of polyvinylchloride foil for wrapping the grapes in the polyester foam tray. Polyvinylchloride film also kept the grapes and their stem fresh over the mentioned storage period.

Percent weight of sound berries is shown in Fig. 5.

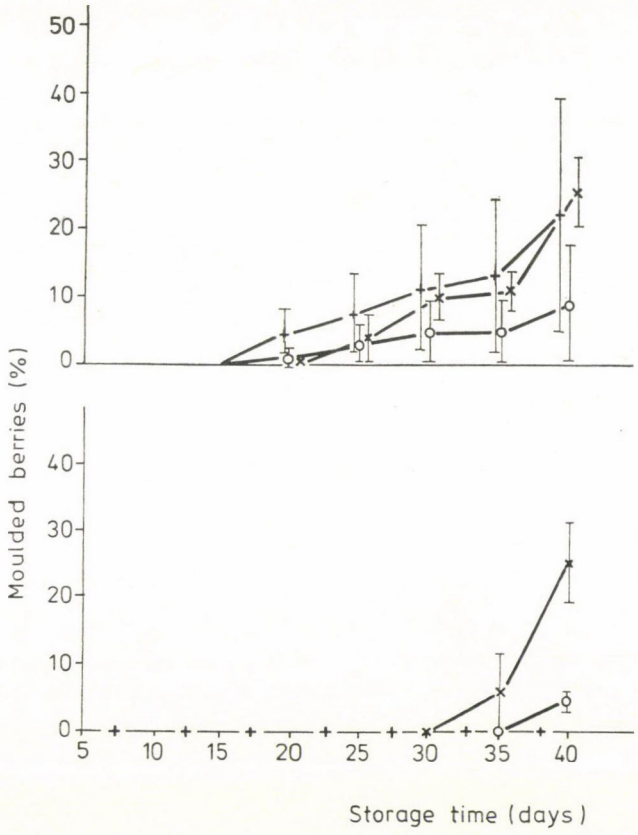

Fig. 2. Effect of radiation (1, 2 kGy) and combined treatments ($K_2S_2O_5$: 4 g per kg; $K_2S_2O_5$ + $C_6H_8O_7$: 4 + 4 g per kg) on visible moulding of Black Alicante grape during 40 days storage at 10 °C. $\bar{x}$, $\bar{x}$, $\bar{x}$ = Average of two replicates with the highest and lowest values. —o—: 0 kGy; —x—: 1 kGy; —+—: 2 kGy

2.3. O_2 and CO_2 concentration inside the packages

In addition to the reduction in percent desiccation weight loss and increased keeping quality of grapes obtained by the use of unvented polyvinylchloride foil, a controlled atmosphere storage system was also developed (Table 2).

The unperforated polyvinylchloride overwrapping decreased the percent oxygen and increased the CO_2 content in the packages of grapes up to 2.2% (Table 2).

Table 2 shows that after 40 days storage period, the lowest and highest O_2 content in the packages was 9.6 and 16.3, respectively.

Table 1

Effect of gamma radiation, combined treatments and packaging on percent decay, desiccation 95% relative

Treatments	0 kGy				
	Desiccation		Decay		Total loss
	1	2	1	2	
0	2.72	2.8	24.18	58.7	
Average	2.76		41.44		44.2[a]
0.25% SO_2	2.64	2.63	16.78	0	
Average	2.63		8.4		11.0[bc]
4 g $K_2S_2O_5$ kg^{-1}	2.56	2.42	16.98	0	
Average	2.5		8.5		11.0[bc]
4 g $K_2S_2O_5$ kg^{-1} + 4 g $C_6H_8O_7$ kg^{-1}	3.1	3.0	5.57	2.93	
Average	3.05		4.25		7.25[bc]
Heat 55 °C 15 min	2.92	8.70	19.45	54.31	
Average	5.8		36.9		42.7[a]

Treatments which have the same single letter are not significantly different at the 0.05

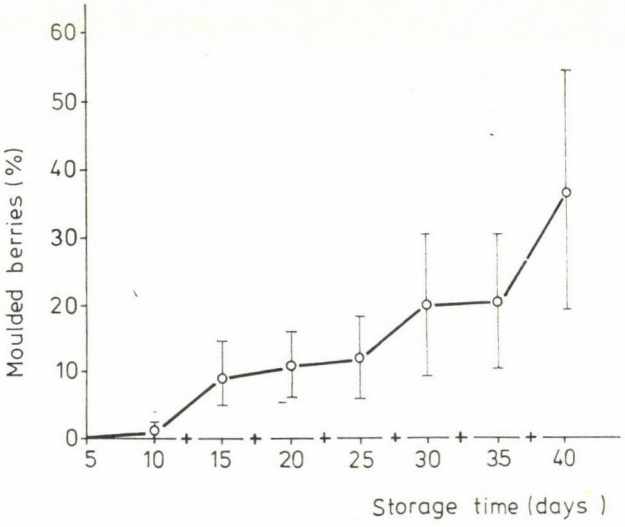

Fig. 3. Effect of radiation (2 kGy) and combined treatments (heat: 55 °C) on visible moulding Black Alicante grape during 40 days storage at 10 °C. $\bar{\bar{\gamma}}$ = Average of two replicates with the highest and lowest values. —o—: 0 kGy; —+—: 2 kGy

and total loss of Black Alicante table grapes during 40 days storage period at 10 °C and humidity

1 kGy					2 kGy				
Desiccation		Decay		Total loss	Desiccation		Decay		Total loss
1	2	1	2		1	2	1	2	
2.89	3.17	30.7	28.48		2.53	4.31	0	0	
3		29.6		32.6^{ab}	3.42		0		3.42^{c}
2.97	3.13	0	0		2.61	2.88	0	0	0
3		0		3.0^{c}	2.75		0		2.75^{c}
9.95	2.59	15.47	29.5		2.33	4.25	3.84	38.27	
6.3		22.5		28.8^{abc}	3.3		21		24.3^{abc}
3	3.14	30.27	17.76		2.51	2.65	0	0	
3.1		24		27.1^{abc}	2.6		0		2.6^{c}
					2.9	3.1	0	0	
					3		0		3.0^{c}

probability level. 1 = stem end; 2 = blossom end

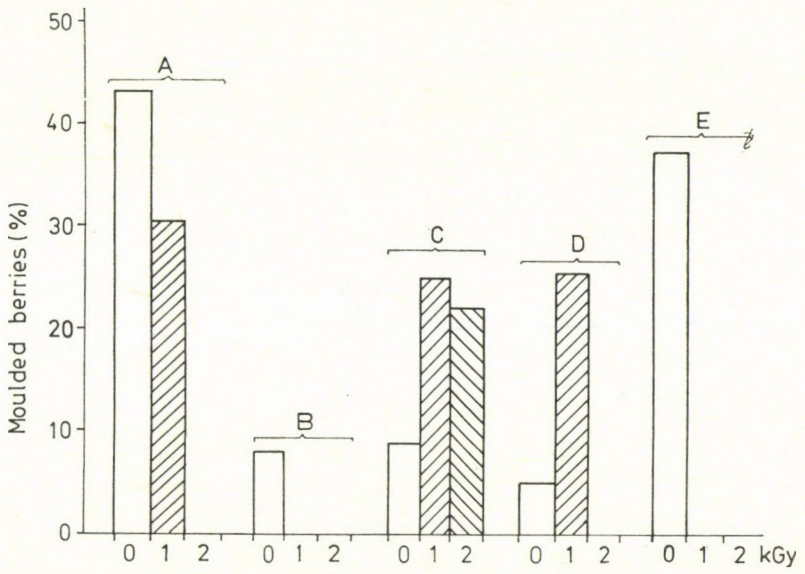

Fig. 4. Effect of gamma radiation and combined treatments on the visible moulding of Black Alicante table grape stored for 40 days at 10 °C. A = control; B = 0.25% SO_2; C = $K_2S_2O_5$; D = $K_2S_2O_5$ + citric acid; E = heat treatment (at 55 °C)

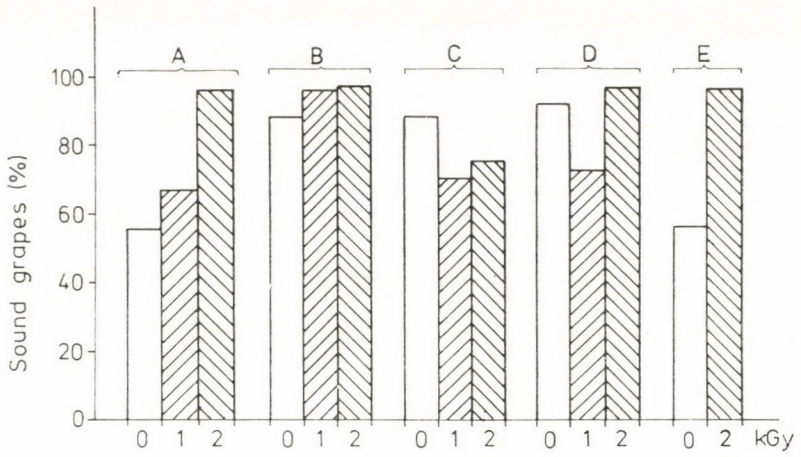

Fig. 5. Effect of gamma radiation and combined treatments on the keeping quality of Black Alicante table grape during 40 days storage at 10 °C. (Weight of sound grapes as percent of the initial weights of the samples.) A = control; B = 0.25% SO_2; C = $K_2S_2O_5$; D = $K_2S_2O_5$ + citric acid; E = heat treatment (at 55 °C)

2.4. Instrumentally measured qualities of grapes

The results of the percent reflection of colour changes at the stem and blossom ends of the berries can be seen in Fig. 6. A low percent reflection indicates that the grape berries had dark colour, a higher percent reflection indicates a lighter colour (discolouration). It can be seen in Fig. 6 that the stem end of the berries gives higher percent reflection than the blossom end in all treatments, including the control. This occurs because the stem of the berries is shaded by close contact of the berries in a cluster during the growth period and does not receive as much sunlight as their blossom end. However,

Table 2

Effect of polyvinylchloride liner packaging system on the percent content of CO_2 and O_2 of the Black Alicante table grape packages after 40 days storage at 10 °C and 95% relative humidity
(Averages of 2 batches)

Treatments	0 kGy		1 kGy		2 kGy	
	CO_2	O_2	CO_2	O_2	CO_2	O_2
0	2.10	14.10	2.10	12.40	1.25	13.95
0.25% SO_2	0.25	15.0	0.5	11.35	0.70	12.80
4 g $K_2S_2O_5$ kg^{-1}	1.10	16.30	1.00	14.60	0.94	14.40
4 g $K_2S_2O_5$ kg^{-1} + 4 g $C_6H_8O_7$ kg^{-1}	0.45	15.85	0.70	18.50	0.20	12.60
Heat 55 °C 15 min	2.20	9.60	—	—	1.10	12.40

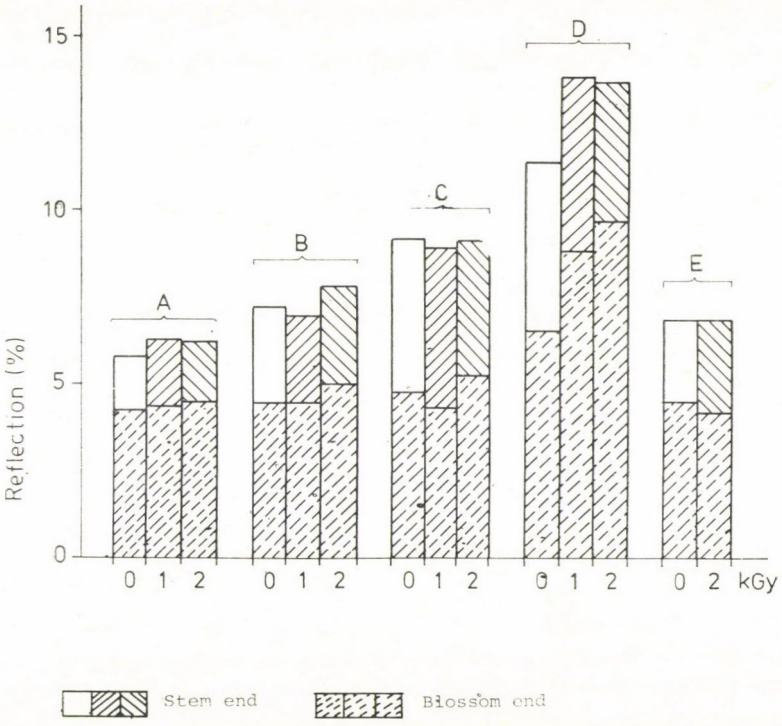

Fig. 6. Effect of gamma radiation and combined treatments on percent reflection of Black Alicante table grape berries measured with reflexometer attached to a spectrophotometer, after 40 days storage at 10 °C. A = control; B = 0.25% SO_2; C = $K_2S_2O_5$; D = $K_2S_2O_5$ + citric acid; E = heat treatment (at 55 °C)

all treatments except gamma radiation at any doses applied, increased percent reflection of the stem end of the berries in comparison with the control treatment (Fig. 6).

Potassium meta-bisulfite and potassium meta-bisulfite mixed with citric acid in themselves or in combination with gamma irradiation increased the reflection at the stem end of the berries more than any other treatment (Fig. 6). Potassium meta-bisulfite either in itself or in combination with irradiation increased the percent reflection of the berries more at the stem end than the blossom end, as compared to the control. Percent reflections of the berries in both parts (stem and blossom end) are also shown in Table 3.

Results in Table 3 show that gamma irradiation up to the highest dose (2 kGy) used alone did not affect changes in reflection of both the stem and blossom ends of the berries as compared with the control. Whereas, potassium meta-bisulfite mixed with citric acid and in combination with either 1 or 2 kGy, were the only treatments which increased the per cent reflection of

Table 3

Effect of gamma radiation and combined treatment on percent reflection of Black Alicante table grape berries measured with reflexometer attached to spectrophotometer at 620 nm after 40 days storage at 10 °C

Treatments	0 kGy				1 kGy				2 kGy			
	1		2		1		2		1		2	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
0	5.8 defghk	0.025	4.3 k	0.135	6.3 defg	0.07	4.3 k	0.025	6.2 defgh	0.105	4.5 ghk	0.1
0.25% SO_2	6.7 def	0.26	4.5 ghk	0.15	6.9 de	0.08	4.4 hk	0.05	7.8 cd	0.37	5.0 fghk	0.06
2 g $K_2S_2O_5$	9.2 c	1.84	4.7 ghk	0.81	8.9 c	0.45	4.3 k	0.04	9.0 c	0.82	5.2 efghk	0.14
2 g $K_2S_2O_5$ + 2 g $C_6H_8O_7$	11.3 b	0.18	6.0 defgh	1.18	13.7 a	1.05	8.8 c	1.5	13.5 a	0.55	9.6 bc	0.67
Heat 55 °C 15 min	6.9 de	0.74	4.4 hk	0.38					6.8 def	0.28	4.54 ghk	0.23

$\bar{x}$ = Mean of 12 replicates, ±s = Standard deviation of the mean. Treatments which have the same single letter are not significantly different at the 5% probability level. 1 = stem end; 2 = blossom end

Table 4

Effect of gamma radiation and combined treatment on the firmness of berries of Black Alicante table grapes measured with penetrometer after 40 days storage at 10 °C and 95% relative humidity

Treatments	0 kGy		1 kGy		2 kGy	
	1	2	1	2	1	2
0	17.9^a	20.4^a	22.4^a	22.0^a	19.5^a	21.1^a
0.25% SO^2	18.3^a	21.0^a	21.8^a	21.8^a	20.8^a	21.7^a
4 g $K_2S_2O_5$ kg^{-1}	18.8^a	19.6^a	19.3^a	19.0^a	19.2^a	19.6^a
4 g $K_2S_2O_5$ kg^{-1} + 4 g $C_6H_8O_7$ kg^{-1}	17.4^a	19.5^a	16.7^a	17.8^a	18.8^a	20.9^a
Heat 55 °C 15 min	18.1^a	18.1^a			19.6^a	22.0^a

Treatments which have the same single letter are not significantly different at the 5% probability level. 1 = steam end; 2 = blossom end

the blossom end of the berries significantly ($P \geq 0.05$) as compared with the blossom end of the berries of the control treatment (Table 3).

Potassium meta-bisulfite mixed with citric acid used alone increased the percent reflection of the stem end of the berries significantly in comparison with the stem end of the berries of the control treatment (Table 3).

The results of the firmness measurements in the berries from both ends of the clusters are shown in Table 4.

The higher the reading of the arbitrary numbers the softer is the fruit and the lower the reading the firmer is the fruit.

The results in Table 4 indicate a slight softness of the berries from the blossom end of the clusters for 9 out of 14 treatments as compared to the berries from the stem end or shoulder of the clusters. However, there was no significant difference in the firmness of the berries between any two treatments at $P > 0.05$.

2.5. Organoleptic testing of the quality of grapes

The average scores and rank totals for the colour, texture (firmness) and the taste of the grapes can be seen in Fig. 7. It is shown in Fig. 2 that there was a marked change in the colour of the berries of grapes treated with potassium meta-bisulfite mixed or not mixed with citric acid in combination with 1 and 2 kGy. These treatments were significantly rejected by the panel members in comparison with that of the control.

The panel members preferred significantly at $P \geq 0.05$ the colour of grapes from the control treatment and the 2 kGy in combination with heated moist air treatment.

The score means and rank sums for the firmness of grape berries in Fig. 7 indicate that the panel members noticed a significant softening in such treatments as potassium meta-bisulfite mixed or not mixed with citric acid in combination with 2 kGy and potassium meta-bisulfite mixed with citric acid without irradiation.

The results in Fig. 7 show that there was no significant change in the taste of irradiated grapes. However, the panel members noticed a significant off-taste in the berries treated with potassium meta-bisulfite mixed or not mixed with citric acid in combination with 2 kGy and potassium meta-bisulfite mixed with citric acid without irradiation (Fig. 7).

2.6. Overall comparison of the preservative effects of the single and combined treatments

The means of the average scores for such organoleptic qualities as colour, firmness and taste of the grape berries stored for 40 days at 10 °C were calculated and plotted against percent total loss.

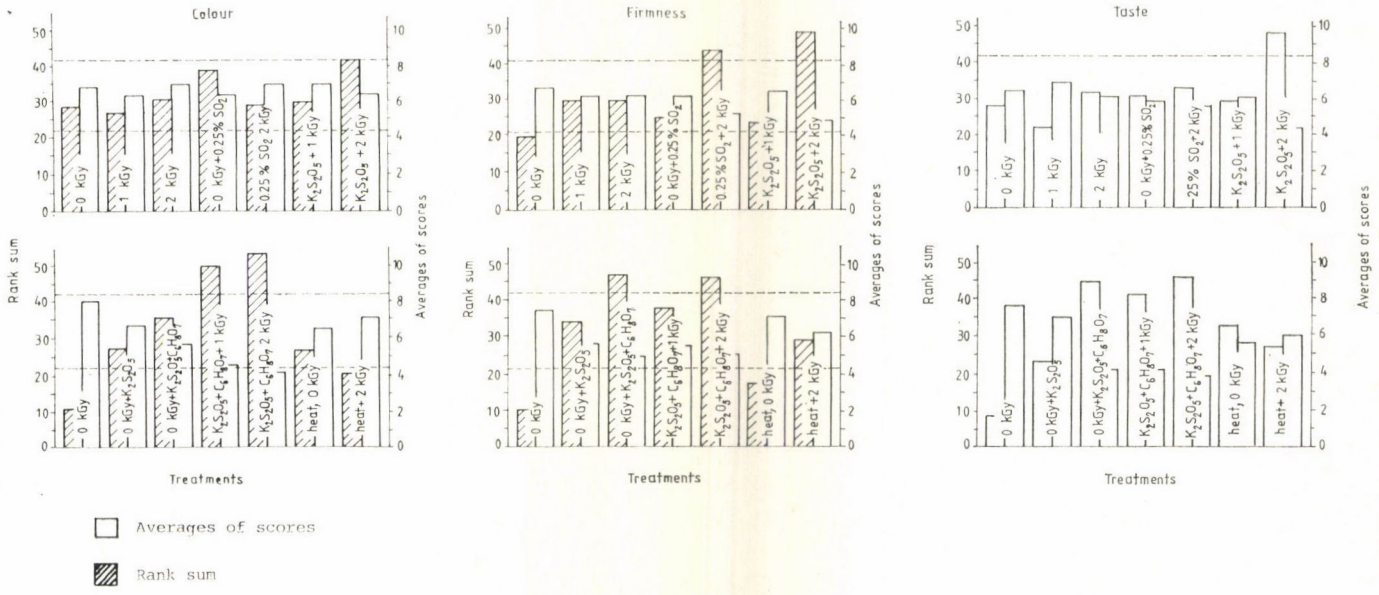

Fig. 7. Results of sensory tests of the colour, firmness and taste of grape berries 47 days after irradiation and combined treatments. Values between the dotted lines are not significantly different from each other at P = 0.05 probability level

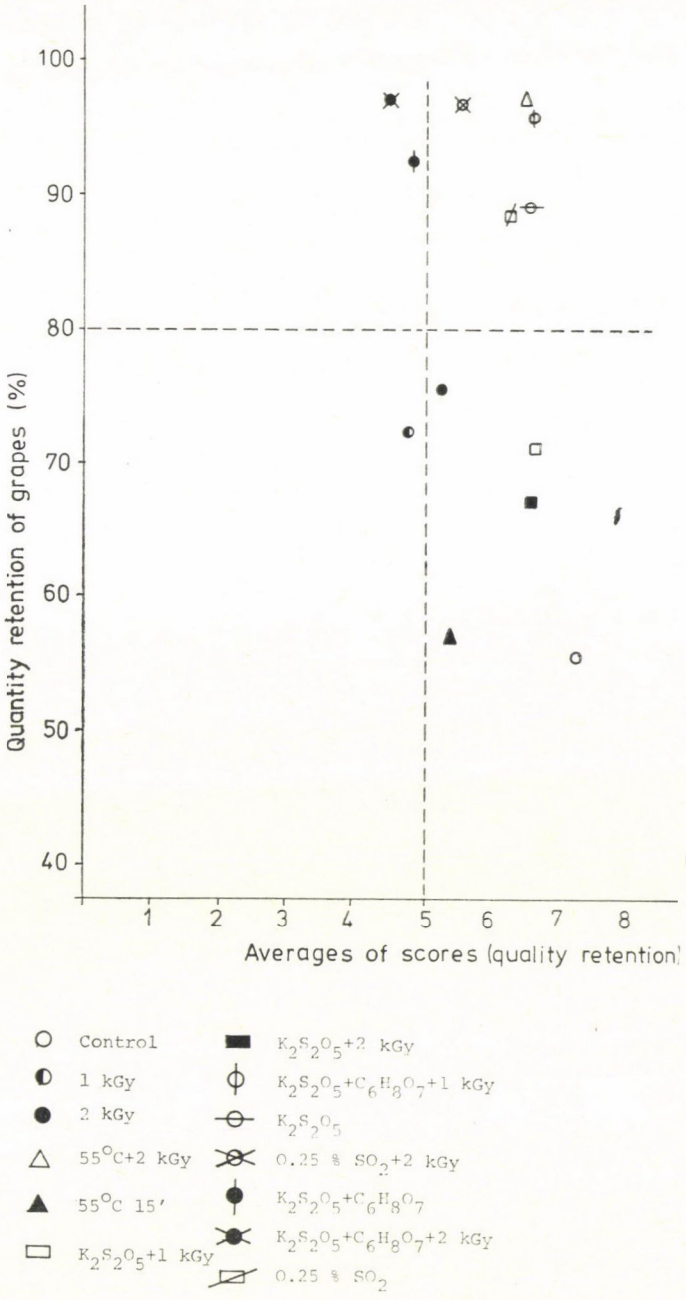

Fig. 8. Effect of gamma radiation, SO_2 sources, heat and combined treatments on the quantity and quality retention of Black Alicante grapes stored at 10 °C for 40 days. A 80% quantity retention and a score mean of 5 for quality retention were considered the border lines for the marketability and quality acceptability of grapes

A mean 5 for the average scores of the organoleptic qualities was considered the lower limit of acceptability and 80% quantity retention was considered the lower limit for marketability of the grapes. The means of the average scores and percent total loss is summarized in Fig. 8.

3. Discussion and conclusion

MAXIE and co-workers (1964) concluded that gamma radiation at doses of 2 and 3 kGy has little or no practical possibilities as a substitute for sulfur dioxide fumigation of grapes. They also concluded that at the above mentioned dosages of gamma irradiation, the organoleptic qualities of the Emperor and Tokay grapes were not acceptable.

The results of this study are in conflict with their conclusion. In the experiments of this study, gamma irradiation alone at a dose of 2 kGy, prevented moulding completely for the entire 40 days storage period at 10 °C. Furthermore, there were no organoleptic changes in the quality (firmness, taste, percent reflection colour and percent total soluble solid) of Black Alicante table grape berries irradiated at 2 kGy.

HEDBERG (1977, 1979), CODOUNIS (1978), COMBRINK and GRINSBERG (1972) consider the use of sulfur dioxide as a main factor in controlling decay in table grapes. Whereas, HARVEY (1955), COUEY and UOTA (1961), MCCLELLAN and co-workers (1973) concluded that sulfur dioxide can only kill fungus spores and mycelia on the surface of the grapes, but cannot kill fungus mycelia that has already invaded tissues of the berries.

The data of our investigation show that the biweekly fumigation with 0.25% SO_2 did not prevent moulding of Black Alicante table grapes, whereas potassium bisulfite mixed or not mixed with citric acid only delayed moulding for 35 and 15 days, respectively. In addition to that potassium bisulfite mixed or not mixed with citric acid and used in itself induced a significant discoloration of the berries measured as percent reflection at the $P \geq 0.05$ level as compared to the control.

It is true that SO_2 can only kill the decay organisms on the surface of the berries and cannot kill their mycelia and spores in the tissue of the berries. But just because of that it is more logical to believe that gamma radiation has more possibilities particularly in case of prepacked grapes, to control decay in table grapes. Also if it is true that at higher temperature a higher concentration of SO_2 is required to control moulds on grapes, then it is easy to conclude that such high concentration of SO_2 causes objectionable changes in the organoleptic qualities of the grapes. The results of this investigation demonstrate that none of the sulfur dioxide sources effectively controlled moulding of the grapes at storage temperature of 10 °C but induced discolouration of the berry.

Therefore, sulfur dioxide may prevent decay at low storage (−1 to 1 °C) temperature, but it does not prevent decay at a higher temperature.

Based on the foregoing discussion, the authors conclude that at 10 °C storage temperature and transport, gamma irradiation at a dose of 2 kGy of prepacked grapes is a better method of increasing the shelf-life of Black Alicante table grapes.

*

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caused by evaporation of moisture; and this results into loss of weight and the fruit starts either to rot or becomes dry. These problems and other related ones have contributed towards a scientific investigation which may prove useful in reducing them.

The use of ^{60}Co gamma rays in various food products has been of some importance as a new technological method of preservation lately. Its effects on fungal development and other pathogenic organisms have been studied carefully. Doses of 1 kGy or more have been found to prolong the storage life of citrus fruits but the use of higher doses has been a problem because they induced peel damage and loss of ascorbic acid (BERAHA et al., 1959a; SHRIKHANDE & KAEWUBON, 1974). A dose of 0.5 kGy has also been found to show some signs of pitting on the peel (KAHAN et al., 1968).

Softening of the peel has also be seen as a disadvantage resulting from high radiation doses (CLARKE, 1959).

This softening phenomenon by ionizing radiation has been clearly shown by SOMOGYI and ROMANI (1963).

Weight losses are also slightly increased by irradiation as suggested by KAHAN et al. (1965).

Apart from the already discussed problems of high irradiation dose, there is a promotion of stem-end rot which has been associated with high doses. This fact is related to the textural changes which high doses cause on the peel thereby allowing the dormant *Alternaria citri* fungi to become active. This has been explained by BERAHA et al. (1959a, b) who reported on an increased susceptibility of irradiated lemons to *Cladosporium* and *Alternaria*. They suggested that this problem was due to textural changes in the peel which predisposes the fruit to infection by facultative species, the other reason can also be that high doses cause the decomposition of some protecting agents against fungi as explained by EL-SAYED (1978).

However, using low doses has proved possible if a double or a triple combination treatment with either chemicals or mild heat is applied.

Reports from BARKAI–GOLAN et al. (1969) have shown that double combination treatments of mild heat and irradiation interacted synergistically to inactivate the *Penicillium digitatum* spores inoculated in freshly picked shamouti oranges and grape fruits. Their results have further shown the possibility of extending the fruit storage life by combined treatments as compared to the individual treatments alone. A triple combination treatment of irradiation, biphenyl and mild heat has been reported to have a more effective result of controlling *Penicillium digitatum* inoculated in the fruits (BARKAI–GOLAN et al., 1976).

In the following we report possibilities of using double or triple combination treatments with the aim of reducing the high doses in preservation of lime fruits.

1. Materials and methods

1.1. Test material

Limes of Mexican variety and Persian variety were used. The Mexican variety limes were air-freighted to ITAL, Wageningen from Kenya while the Persian variety limes were bought from a tropical fruit importer at Amsterdam (Windig B. V.). Both fruits were in a green mature state and were stored at a controlled storage room of 10 °C and 90% relative humidity awaiting preventive treatments.

1.2. Inoculated limes

For microbiological experiments, *Penicillium digitatum* spores were collected from a lime fruit which was bought from a fruit and vegetable shop. The *Penicillium digitatum* infected lime was placed in 20 °C and 90% relative humidity conditions for spore growth. The lime was kept under these conditions for one week to allow sporulation. The spores were removed after one week into a sterile Czapek Dox Agar medium for growth. After the spores had formed, they were removed and suspended into 10 cm^3 of sterile distilled water. The spores were counted using a haemacytometer and the suspension was found to contain 10^5 spores per cm^3.

Using a Pipetman micropipette, the lime fruits were inoculated with 10 μl of the spore suspension which was equivalent to 10^3 spores of *Penicillium digitatum*. The inoculation was made into two bored holes opposite to each other made on the flavedo parts of the fruits by use of cork borer. The depth of these holes was between 1.5 mm and 2 mm.

After inoculation, the limes were left to rest for 24 hours at 20 °C and 90% relative humidity prior to irradiation, mild heat and potassium meta-bisulfite solution treatments.

For physiological experiments the fruits were not inoculated.

1.3. Heat, potassium meta-bisulfite and radiation treatments

The Mexican variety limes were treated as follows: mild heat of 20 °C (control), 45 °C and 55 °C for 5 minutes, dipping into potassium meta-bisulfite ($K_2S_2O_5$) of 0.1%, 0.5% and 1% concentration, resp., and irradiation doses of 0.25 kGy and 0.5 kGy, resp.

The Persian variety limes received a mild heat treatment of 20 °C and 45 °C, resp., dipping into a $K_2S_2O_5$ solution of 0.1% concentration and the same irradiation doses. The time interval between mild heat or $K_2S_2O_5$ treatments and irradiation was within one hour for both varieties.

riper than group GER22, their predicted values do differ by 8 percent. But group GER21 seems to be only slightly riper than group GER30, their predicted values are 62 and 60 percent, respectively, in good accordance with the visual impression.

Table 3

Data of the Germersdorf variety samples (1981), predicted on the basis of BIG1

Harvesting date	Label of the group	Assessed ripeness values (%)	Difference of the predicted and assessed ripeness of groups (%)	Standard deviation of predicted ripeness within a group (%)	Extent in predicted ripeness within a group (%)
12–06–1981	GER 10	60	— 7.4	1.4	5.9
	GER 11	70	—13.0	3.1	10.7
	GER 12	80	—13.4	3.1	10.5
	GER 13	90	—15.2	5.4	22.7
15–06–1981	GER 20	65	— 9.3	1.7	5.6
	GER 21	70	— 8.5	2.6	9.2
	GER 22	85	—14.0	3.2	8.7
	GER 23	90	—11.7	4.4	13.8
17–06–1981	GER 30	70	—10.5	2.8	8.5
	GER 31	80	—12.9	3.5	13.2
	GER 32	85	— 6.0	4.1	14.5
	GER 33	90	— 0.7	8.5	26.8
19–06–1981	GER 40	70	—12.3	2.5	12.2
	GER 41	80	—13.5	2.9	11.3
	GER 42	85	— 9.6	3.3	11.4
	GER 43	95	—11.4	4.3	18.5
	GER 44	100	—10.4	4.7	15.7
22–06–1981	GER 50	80	—12.3	3.0	12.1
	GER 51	90	—15.9	4.5	17.7
	GER 52	100	— 9.8	6.4	21.7

Table 4

Statistical characteristics of different predictions of Germersdorf variety cherries in 1981

Label of the equation	Standard error of prediction	Multiple correlation coefficient	Bias
BIG1	3.70	0.955	—10.88
BIG2	3.06	0.968	—15.89
BIG3	3.81	0.957	—11.19
BIG4	3.32	0.962	—13.45

tunately in 1981 we could not get samples of the same cherry variety as we got in 1980. In spite of this we did try to use our equations to predict the ripeness of samples.

For that the $\log \frac{1}{T}$ values of unassessed samples were measured at the wavelengths being in the chosen calibration equation and the predicted values were calculated. The samples were arranged on a desk according to the predicted values and a photo in color was taken to illustrate how the regression equation works. Also the assessed samples harvested in 1981 were predicted by each calibration equation. The assessed values and predicted values of groups were then compared by regression analysis. Standard error of predictions, correlation coefficients and biases were calculated. The standard error of predicted ripeness within groups were also calculated by one of the prediction equations.

The spectra of assessed samples were taken and stored on disks for further investigations.

2. Results

The four calibration equations generated on Bigarreau-Burlat cherry samples in 1980 are the following:

$$R_{\mathrm{BIG1}} = 41.74 + 22.85\ V(610) - 19.70\ V(660)$$
$$R_{\mathrm{BIG2}} = 54.01 + 52.25\ V(624)/V(668)$$
$$R_{\mathrm{BIG3}} = 39.56 + 19.19\ V(606) - 15.47\ V(673)$$
$$R_{\mathrm{BIG4}} = 168.66 - 71.98\ V(729)/V(616)$$

Equations indexed BIG3 and BIG4 are the optimized ones. Statistical characteristics of the calibrations are summarized in Table 1. It can be seen that there are no essential differences among the four equations.

In Table 2 the most interesting data of the samples are summarized. Each row refers to a group of samples harvested on the same day and representing the same assessed ripeness by 40 individuals. Each such group has a label for computing reasons. We give the harvesting dates, the labels, the assessed ripeness values, the differences of assessed and estimated values (DAE) gained from calibration equations and the STDPIM values. The STDPIM values characterize the homogeneity of groups and the uncertainty of optical measurements arising partly from the positioning uncertainty of samples and partly from electronic noise.

For prediction the ripeness of samples harvested in 1981, we have tried to use the calibration equation indexed BIG1. Fig. 3 shows the photo taken from Germersdorf variety cherries, the ripeness of which was predicted by the

ping in 45 °C water, showed 90% of rot after 10 days storage. The percentage rot in samples treated with hot water dip of 45 °C with 0.25 and 0.5 kGy, resp., was 50% and 30%, resp., at the 10th day of storage.

2.1.2. Combined heat and potassium meta-bisulfite treatment. The aim of this experiment was to see the effect, the mild heat and chemical combination treatment had and if possible be applied to situations where ionizing radiations are unavailable. For the Mexican variety stored at 10 °C the results (Fig. 3) showed that the fruits dipped at 20 °C for 5 minutes combined with 0.1% $K_2S_2O_5$ solution showed signs of decay after 20 days storage,

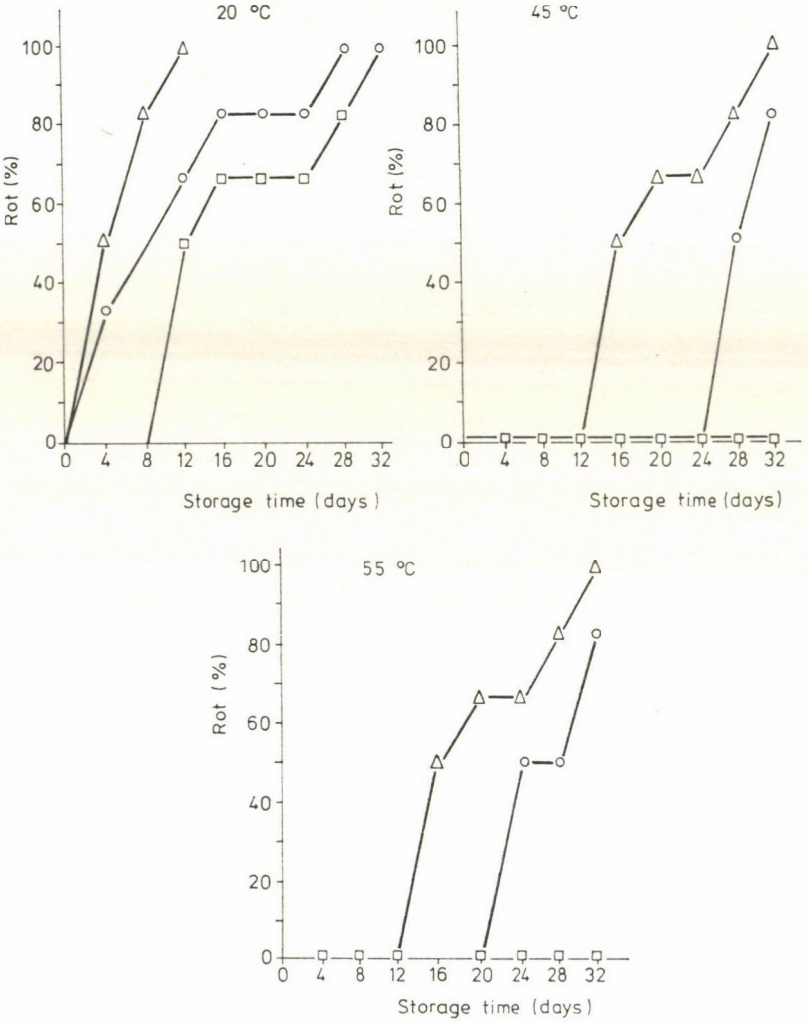

Fig. 1. Effect of warm water dipping and/or gamma radiation on the development of *Penicillium digitatum* inoculated in Mexican variety limes stored at 10 °C and 90% R. H. Dipping time: 5 min; —△—: 0 kGy; —o—: 0.25 kGy; —□—: 0.5 kGy

while the ones treated by dipping at 55 °C for 5 minutes with 0.1% $K_2S_2O_5$ showed signs of decay after 24 days.

The 0.5% $K_2S_2O_5$ treatment alone showed mould growth after 20 days storage while combined with 45 °C and 55 °C dipping for 5 minutes showed no visible signs of rot after 32 days storage.

The same results were shown in 1% $K_2S_2O_5$ treated samples with 45 °C and 55 °C dipping for 5 minutes, respectively. The 1% $K_2S_2O_5$ treated samples and dipped in 20 °C water for 5 minutes showed mould growth after 24 days storage.

2.1.3. Combined radiation and potassium meta-bisulfite solution. In this experiment the main aim was to see whether there was any possibility of increasing the sensitivity of *Penicillium digitatum* spores by use of a combined treatment of irradiation and potassium meta-bisulfite.

For the Mexican variety limes the results (Fig. 4) did show that the fruits treated with 1% $K_2S_2O_5$ solution alone retarded the appearance of rot up to 20 days while the ones that were doubly combined with 0.25 and 0.5 kGy, resp., retarded the appearance of rot by 24 and at least 32 days at 10 °C storage temperature.

The 0.5% $K_2S_2O_5$ treatment alone retarded the appearance of rot by 16 days while the ones that were doubly combined with 0.25 and 0.5 kGy, resp., retarded the appearance of rot by 24 and at least 32 days at 10 °C.

The 0.1 $K_2S_2O_5$ treatment alone retarded the appearance of rot by 12 days while the ones that were doubly combined with 0.25 and 0.5 kGy, resp. retarded the appearance of rot by 20 and 24 days at 10 °C.

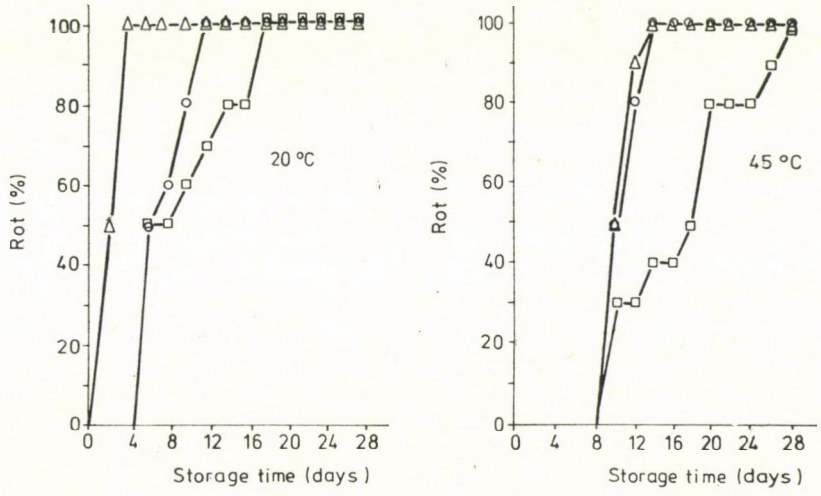

Fig. 2. Effect of warm water dipping and/or gamma radiation on the development of *Penicillium digitatum* inoculated in Persian variety limes stored at 15 °C and 80% R. H. Dipping time: 5 min; —△—: 0 kGy; —o—: 0.25 kGy; —□—: 0.5 kGy

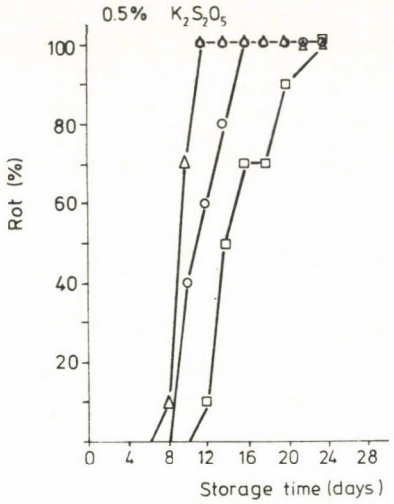

Fig. 5. Effect of potassium meta-bisulfite dip and gamma radiation on the development of *P. digitatum* inoculated in Persian variety limes stored at 15 °C and 80% R .H.— △ —: 0 kGy; —o—: 0.25 kGy; —□—: 0.5 kGy

Table 1

Effect of mild heat, potassium meta-bisulfite and/or gamma radiation on the weight loss of Persian lime variety

Treatments	24th day		32nd day		40th day	
	Average ($\bar{x}_0$)	Difference ($\bar{x}_0-\bar{x}_1$)	Average ($\bar{x}$)	Difference (x_0-x_1)	Average ($\bar{x}$)	Difference ($\bar{x}_0-\bar{x}_1$)
20 °C + 0 kGy	2.27	0	2.08	0	2.23	0
20 °C + 0.25 kGy	3.01	—0.74*	2.40	—0.32	2.45	—0.22
20 °C + 0.50 kGy	3.30	—1.03*	2.93	—0.85*	3.21	—0.9 8*
45 °C + 0.5% $K_2S_2O_5$ + 0 kGy	2.55	—0.28	2.23	—0.15	2.35	—0.12
45 °C + 0.5% $K_2S_2O_5$ + 0.25 kGy	2.75	—0.48	2.43	—0.35	2.25	—0.02
45 °C + 0.5% $K_2S_2O_5$ + 0.50 kGy	3.22	—0.95*	2.90	—0.82*	2.97	—0.74*
0.5% $K_2S_2O_5$ + 0 kGy	2.27	0	2.15	—0.07	1.76	0.47
0.5% $K_2S_2O_5$ + 25 krad	3.26	—0.99*	2.92	—0.84*	2.59	—0.36
0.5% $K_2S_2O_5$ + 0.50 kGy	3.49	—1.22*	2.95	—0.87*	3.10	—0.87*
45 °C + 0 kGy	1.93	0.34	1.79	0.29	2.21	0.02
45 °C + 0.25 kGy	2.52	—0.25	2.55	—0.47	2.44	—0.21
45 °C + 0.50 kGy	2.57	—0.3	2.41	—0.33	2.44	—.021
L.S.D.	0.6963		0.6074		0.7132	

$\bar{x}_0$ = Mean of the control at a particular day
$\bar{x}_1$ = Mean of treated samples
* = differing from the control at 5% probability level ($P \geq 0.05$)
L.S.D. = Least significant difference at 5% probability level

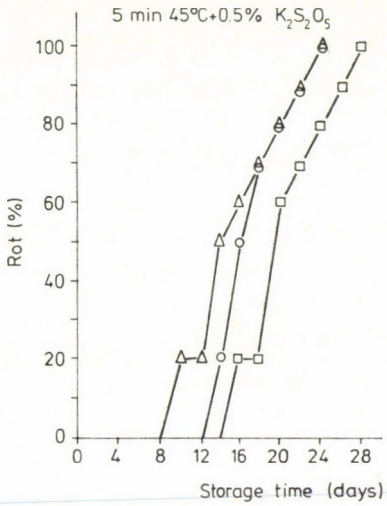

Fig. 6. Effect of mild heat, potassium meta-bisulfite and gamma radiation on the development of *P. digitatum* inoculated in Persian variety limes stored at 15 °C and 80% R. H. — △ —: 0 kGy; —o—: 0.25 kGy; — □ —: 0.5 kGy

Table 2

The results of statistical analysis of softness mean values at the end of the storage period in Mexican lime variety

Treatments	28th day	
	Average ($\bar{x}_0$)	Difference ($\bar{x}_0 - \bar{x}_1$)
20 °C + 0 kGy	1.70	—
20 °C + 0.25 kGy	1.80	—0.1
20 °C + 0.50 kGy	2.05	—0.35
45 °C + 0 kGy	2.05	—0.35
45 °C + 0.25 kGy	2.60	—0.90
45 °C + 0.50 kGy	3.0	—1.30*
55 °C + 0 kGy	2.70	—1.0*
55 °C + 0.25 kGy	4.60	—2.90*
55 °C + 0.50 kGy	5.1	—3.40*
L.S.D.	0.9249	

$\bar{x}_0$ = Mean of control at 28th day
$\bar{x}_1$ = Mean of treated samples
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Similar procedure was performed to get another optimized regression equation in the form of equation No. 2.

We found, that the regression surface over λ_1 and λ_2 plane was flat in quite a considerable region, implying that the regression equations containing different wavelength pairs within this region do not differ essentially. Therefore we chose two other wavelength pairs, one for a regression equation in the form of equation No. 1. and one in the form of equation No. 2. so we generated four regression equations altogether. Henceforth we call them calibration equations.

Substituting the $\log \frac{1}{T}$ values of each group into the calibration equations, we calculated the estimated ripeness values of groups, the difference of assessed and estimated ripeness values of groups, the standard error of calibration (SEC):

$$\mathrm{SEC} = \sqrt{\frac{\Sigma(Y - \hat{Y})^2}{n - 1 - p}}$$

where

Y is the assessed ripeness value of a group
$\hat{Y}$ is the estimated ripeness value of a group
n is the number of groups
p is the number of terms in the calibration equation,

the (multiple) correlation (MC)

$$\mathrm{MC} = \sqrt{\frac{\Sigma(Y - \hat{Y})^2}{\Sigma(Y - \bar{Y})^2}}$$

where $\bar{Y}$ is the average of assessed ripeness values of all groups and the figure of merit (FM)

$$\mathrm{FM} = \frac{Y_{\max} - Y_{\min}}{2(\mathrm{SEC})}$$

Substituting the $\log \frac{1}{T}$ values of individual fruits into the calibration equations we could "predict" the ripeness of individuals by the calibration equations. As this prediction is not perfectly independent from the calibration, to differentiate this prediction from a perfect one we call this prediction "a prediction of individuals by regression on means", shortly PIM.

We also compute the standard deviations of PIMs within the groups (STDPIM). (Formally this STDPIM is equivalent to the widely used measure of reproducibility. In our case it characterizes the inhomogeneity of groups and the repeatability of an individual fruit together with one number.)

Having studied the results, we chose one of the four calibration equations for predicting ripeness of unassessed fruit samples harvested in 1981. Unfor-

The ripeness values were assessed on the basis of visual impression of experts. These values depend on the optical properties of fruits basically influenced by pigment content. With the instrumental method described below we also measure optical properties similarly depending on the pigment content so the ripeness values given by this instrumental method should correlate with biological ripeness to the same extent as pigment content does.

The optical measurements of fruit samples were carried out with the Neotec Research Composition Analyzer type 6450. The geometry used for measurements is sketched in Fig. 1. Measurements were made in the 380–730 nm range. The cherries were placed on a diaphragm of 13 mm diameter one by one and we measured the spectrum of the $\log \frac{1}{T}$, where T is the ratio of the light reaching the detector through the diaphragm with and without a piece of fruit placed onto the diaphragm. This ratio has been computed automatically in an analogue manner in the above mentioned instrument and therefore in those spectral ranges where the energy travelling through the fruit and reaching the detector is very low and tends to zero, the error arising from

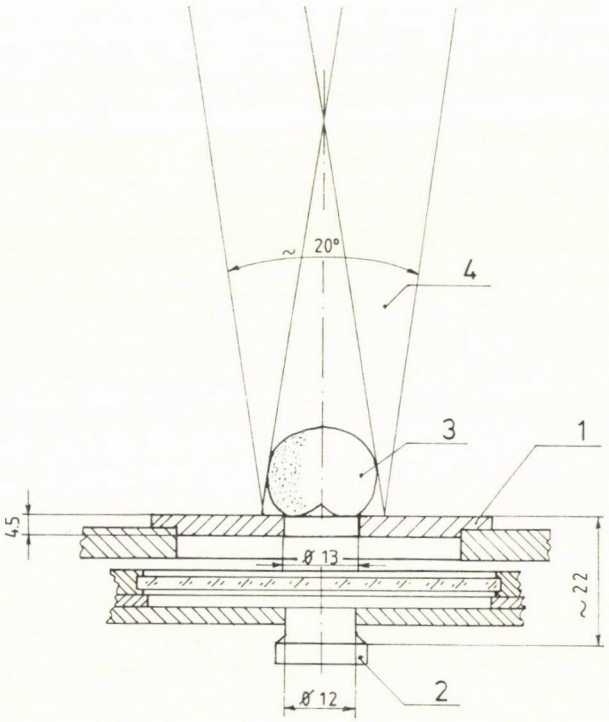

Fig. 1. Geometry used for the measurements. 1. diaphragm, 2. Si detector, 3. sample, 4. illuminating beam

Table 3

The results of statistical analysis of softness mean values on the 16th, 24th, 32nd and 40th day of storage in Persian lime variety

Treatments	16th day		24th day		32nd day		40th day	
	Average ($\bar{x}_0$)	Difference ($\bar{x}_0 - \bar{x}_1$)	Average ($\bar{x}_0$)	Difference ($\bar{x}_0 - \bar{x}_1$)	Average ($\bar{x}_0$)	Difference ($\bar{x}_0 - \bar{x}_1$)	Average ($\bar{x}_0$)	Difference ($\bar{x}_0 - \bar{x}_1$)
20 °C + 0 kGy	0.25	0	0.35	0	0.40	0	0.50	0
20 °C + 0.25 kGy	0.25	0	0.40	−0.05	0.40	0	0.40	0.1
20 °C + 0.50 kGy	0.35	−0.10	0.40	−0.05	0.25	0.15	0.35	0.15
45 °C + 0 kGy	0.25	0	0.50	−0.15	0.35	0.05	0.25	0.25
45 °C + 0.25 kGy	0.30	−0.05	0.35	0	0.40	0	0.40	0.10
45 °C + 0.50 kGy	0.5	−0.25*	0.30	0.05	0.70	−0.30*	0.95	−0.45*
45 °C + 0.5% $K_2S_2O_5$ + 0 kGy	0.25	0	0.35	0	0.30	0.1	0.30	0.20
45 °C + 0.5% $K_2S_2O_5$ + 0.25 kGy	0.35	0.1	0.85	−0.50*	0.45	−0.05	0.35	0.15
45 °C + 0.5% $K_2S_2O_5$ + 0.50 kGy	0.70	−0.45*	0.85	0.50*	0.60	−0.20	0.65	−0.15
0.5% $K_2S_2O_5$ + 0 kGy	0.35	−0.1	0.35	0	0.45	0	0.30	0.20
0.5% $K_2S_2O_5$ + 0.25 kGy	0.35	−0.1	0.60	−0.25	0.65	0.25	0.80	0.30
0.5% $K_2S_2O_5$ + 0.50 kGy	0.50	−0.25*	0.65	−0.30	0.70	0.30*	0.80	0.30
L.S.D.	0.20		0.33		0.30		0.40	

$\bar{x}_0$ = mean of the control at a particular date
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* = differing from the control at 5% probability level ($P \geq 0.05$)
L.S.D. = Least significant difference at 5% probability level

in longer mould-free periods by controlling *Penicillum digitatum* than the single treatment. Combining mild heat plus irradiation or potassium meta-bisulfite dip plus irradiation enhanced the mould free period synergistically. Synergistic effects of heat and gamma irradiation on the development of *Penicillium digitatum* were reported by BARKAI-GOLAN and co-workers (1969). The attenuation of resistance to radiation by means of biphenyl or sodium orthophenylphenate was found, too (BARKAI-GOLAN & KAHAN, 1967).

Conclusion of physiological observations shows that irradiation causes a temporary or a permanent softening. The slight increase of weight-loss with irradiation agrees with the observation of MONSELISE and KAHAN (1968).

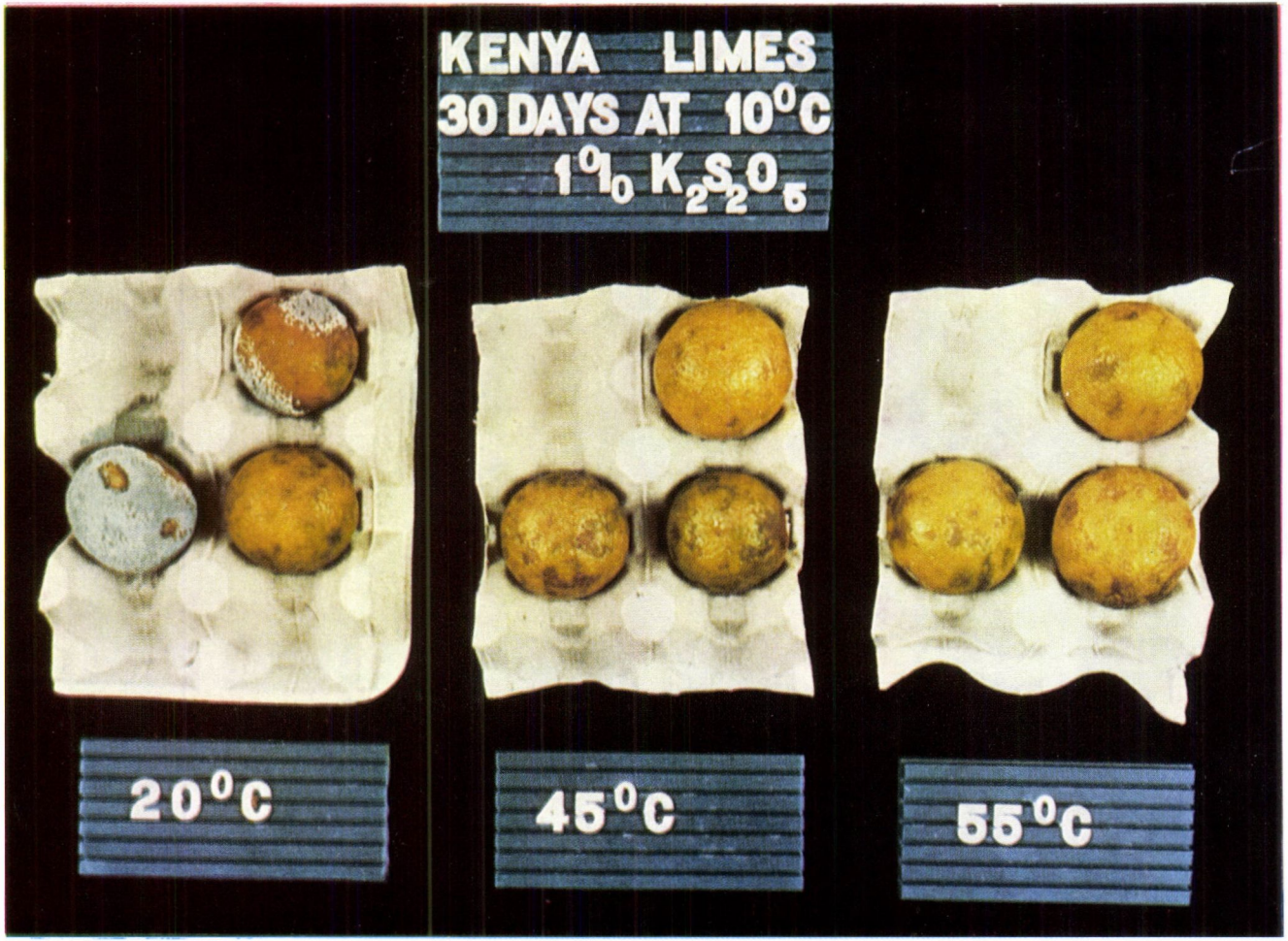

Fig. 10. Effect of heat treatment and meta-bisulfite on rot and skin colour of Mexican variety limes stored for 30 day at 10 °C and 90% R. H.

Delaying degreening of fruits and vegetables has been frequently reported in the literature. On the other hand, MAXIE and co-workers (1964) and SHRIKHANDE and KAEWUBON (1974) observed reduced chlorophyll retention in irradiated lemon fruits and attributed it to enhanced C_2H_2 production. The observations on percentage light reflection in our experiment seems to be neither a disadvantage nor an advantage. Lime fruit is sometimes consumed in a mature green state and sometimes in a yellow state.

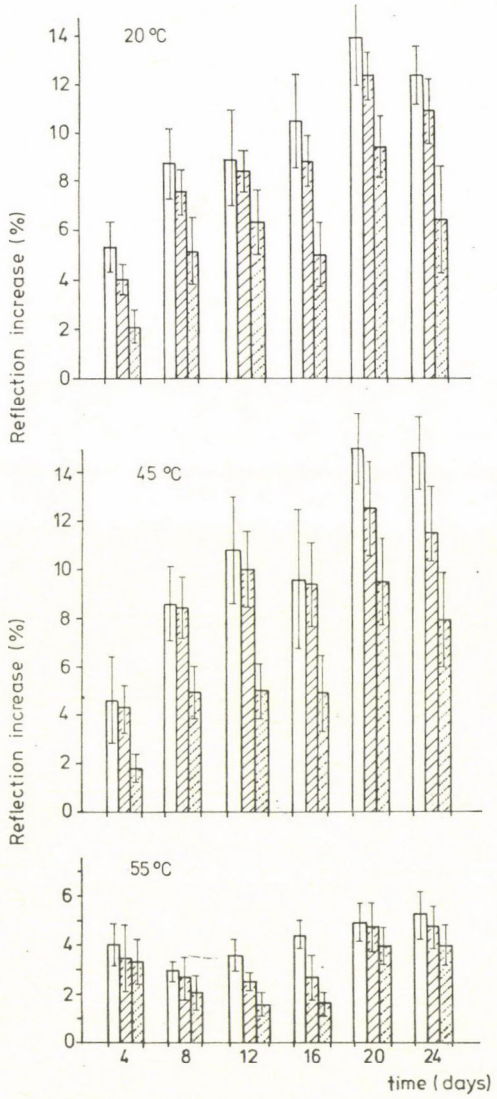

Fig. 9. Effect of mild heat and/or radiation on the percentage reflection increase during storage of Mexican variety limes stored at 10 °C and 90% R. H. Dipping time: 5 min. □: 0 kGy; ▨: 0.25 kGy; ▨: 0.5 kGy

KAFFKA and CZABAFFY (1981) published a report on preliminary studies performed in 1979. This report reviewed a list of literature dealing with methodology and instruments used in the quality determination of fruits in general. This present study aims at dealing with the instrumental determination of the quality of cherry using non-destructive optical technique. The study reported now was carried out in 1980 based on results obtained in 1979 and extended by some experiments on the samples picked in 1981.

In addition to the above we would like to deal also with some basic ideas and relevant terminology. As this paper is specially focused on cherry, attention must be drawn to two papers of importance dealing with scattering effect (LAW, 1973) and anthocyanin pigment development (YEATMAN et al., 1961) in cherries beside the literature cited in the mentioned preliminary study.

LAW (1973) has constructed a gonio-spectrophotometer and carried out extended investigations to elaborate an optical measuring method for detecting the presence or absence of pit in cherries.

YEATMAN and co-workers (1961) investigated the development of anthocyanin content in red tart cherries (*Prunus cerasus* L.) and their scald damage by optical methods. They have concluded, that a good correlation ($r = 0.83$) exists between the anthocyanin content and the difference of transmittance measured at 540 nm and 612 nm wavelengths and a decreasing transmittance measured at 620 nm belongs to an increasing anthocyanin content and an increasing ripeness.

Our final goal still remains — as set in the preliminary study — giving ready-to-use data for elaborating a single-purpose instrument for predicting quality parameters of cherries. Experimental results on other stone fruits will be reported in separate papers.

1. Materials and methods

The fruit samples for the investigations were received from RIFGOP, Budapest, and they had been assessed by the experts of the same institute.

In 1980 we received Bigarreau-Burlat variety cherries harvested on four different days representing three to five groups labelled with their average assessed ripeness values. Each group consisted of 40 pieces of fruit, and we had 16 such groups altogether, that is 640 pieces of fruit were investigated in 1980.

In 1981 we received Germersdorf variety cherries harvested on five different days representing three to five assessed ripeness values. Each group was represented by 20 pieces of fruit, and we had 20 such groups that is 400 pieces of fruit were investigated.

In 1981 we also received a certain quantity of unassessed fruit for experimental purposes.

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Acta Alimentaria, Vol. 13 (1), pp. 83—95 (1984)

ATTEMPTS TO ELABORATE A NON-DESTRUCTIVE OPTICAL METHOD FOR MEASURING CHERRY-RIPENESS

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Studies were made on Bigarreau–Burlat variety cherries in 1980 to establish equations for predicting the ripeness by non-destructive optical measurements and to get ready-to-use data for elaborating a single-purpose instrument predicting the ripeness of cherries.

A NEOTEC 6450 type Research Composition Analyzer was used to take the spectra of samples supplied by the Research Institute for Fruit Growing and Ornamental Plants, Budapest (RIFGOP) and assessed in ripeness by the experts of the same institute. The necessary computations were made also by the Research Composition Analyzer. A total of 640 cherries, representing a wide ripeness range were investigated. Regression (calibration) equations were constructed between the optical data measured at different wavelengths in the visible region of spectra and the assessed ripeness values with correlation coefficients between 0.927 and 0.949 giving a standard error of calibration between 4.77 and 5.48 in percentage of ripeness of fruit groups differing in ripeness and harvesting date. Within the groups assessed with the same ripeness values, the predicted ripeness values of individuals showed considerable standard deviations. This led to the conclusion, that visual assessment does not give sufficiently homogeneous sample groups. The following summer the ripeness of assessed samples were also predicted on the basis of one of the calibration equations constructed in 1980.

In spite of the fact, that in 1981 the assessed and predicted fruits differed in variety (in 1981 Germersdorf variety cherries were investigated), smaller residual standard deviation between the assessed and predicted values of ripeness were observed, the predicted homogeneity of groups were better, but a bias of about 10 percent occurred.

At the same time, a given amount of unassessed cherries was sorted on the basis of a calibration equation and to conserve the visual impression about sorting, a photo in color was taken.

The photo shows, that the prediction is not bad, but statistical analysis indicates that further investigations are necessary.

The fact, that calibration equations with essentially the same correlation coefficient were found using optical data at different wavelengths implies that the wavelengths, which give the best correlation coefficient, are not absolutely characteristic ones, at least in this case.

Keywords: Non-destructive optical methods, cherry-ripeness, measuring of ripeness

Hungary is traditionally a fruit producing country. Stone fruits play a significant role and this role tends to be increasing. Parallel to production there is an ever increasing demand from the growers and also from the users (canning factories, deep freezing industry, direct consumers) for a rapid, instrumental, non-destructive method for determining the quality of these fruits. The Central Food Research Institute started studies on stone fruits (cherry, sour-cherry, apricot, plum) in order to solve these problems.

KAFFKA and CZABAFFY (1981) published a report on preliminary studies performed in 1979. This report reviewed a list of literature dealing with methodology and instruments used in the quality determination of fruits in general. This present study aims at dealing with the instrumental determination of the quality of cherry using non-destructive optical technique. The study reported now was carried out in 1980 based on results obtained in 1979 and extended by some experiments on the samples picked in 1981.

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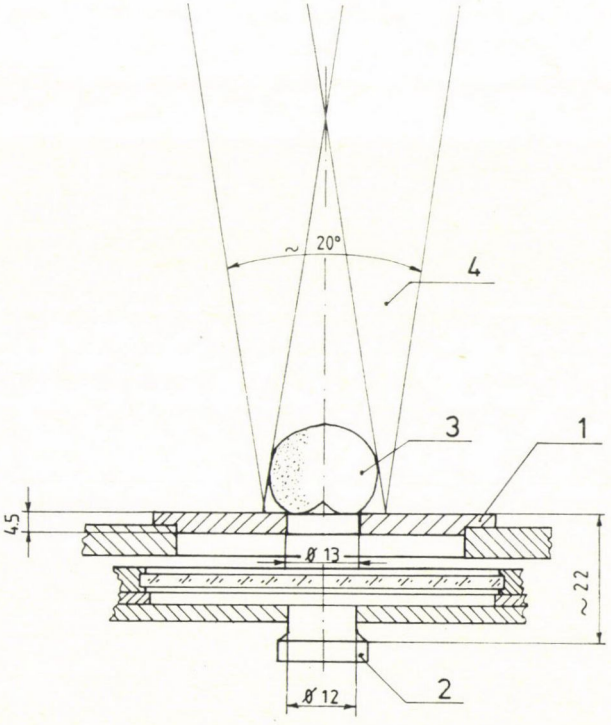

Fig. 1. Geometry used for the measurements. 1. diaphragm, 2. Si detector, 3. sample, 4. illuminating beam

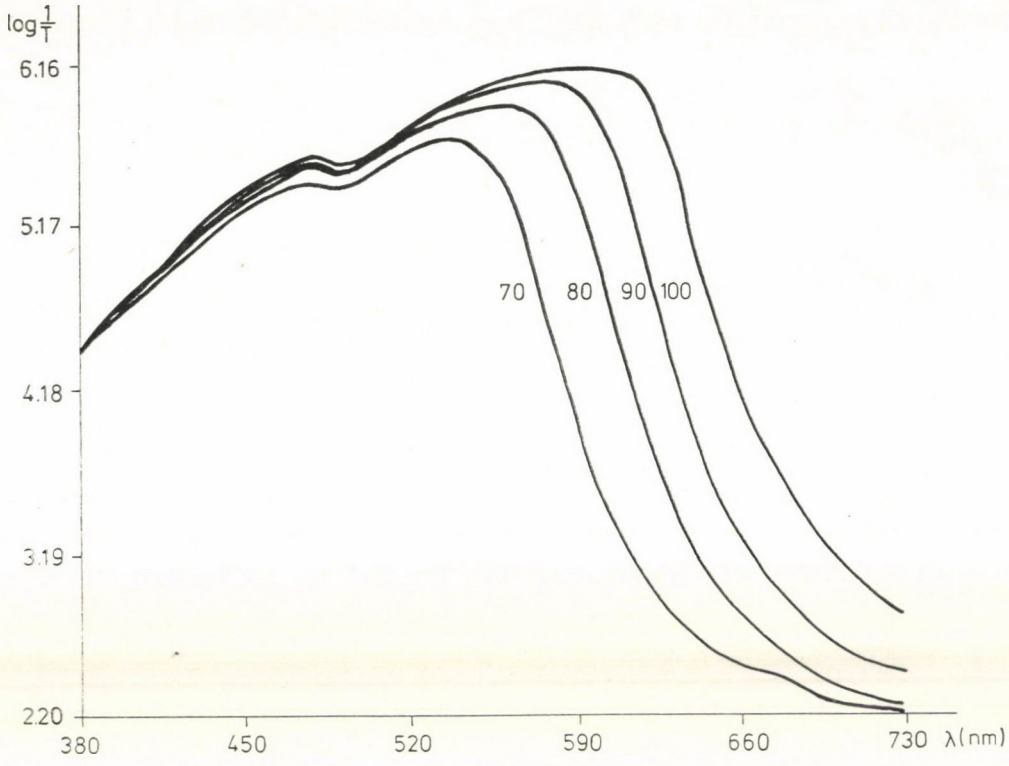

Fig. 2. Typical spectra of cherries representing different ripeness values

the analogue calculations tends to be infinite and the ratio approaches a limit value well determined by the energy reaching the detector through the diaphragm without any sample. This is illustrated in Fig. 2 where in the 380–520 nm range the $\log \frac{1}{T}$ spectra of differently ripened fruits are overlapped. Therefore our investigations were restricted to the 520–730 nm wavelength range.

The spectral data of fruits have been processed by a Nova III type computer built in the Research Composition Analyzer.

The aim of our work in 1980 was to find wavelengths, at which the measured optical data and the assessed ripeness values give the best correlation. We searched the best correlation in the following two forms of equations:

$$R_1 = k_0 + k_1 V(\lambda_1) + k_2 V(\lambda_2) \tag{1}$$

$$R_2 = k_3 + k_4 \frac{V(\lambda_3)}{V(\lambda_4)} \tag{2}$$

where

R_1 and R_2 are the estimated ripeness values,

k_0, k_1, k_2, k_3, and k_4 are constants,

$V(\lambda_1)$, $V(\lambda_2)$, $V(\lambda_3)$ and $V(\lambda_4)$ are the measured optical properties $\left(\log \frac{1}{T}\right)$ at the wavelength given in brackets.

The computation possibilities did not let us run the regression computation with all the 640 spectra. (One floppy disc can store only the data of 200 samples.) Therefore before the regression computation, we averaged the spectra of the samples harvested on the same day with the same assessed ripeness values.

Thus we got 16 averaged spectra in 1980 that we used for computation. To choose the best correlating wavelengths we used the following procedure.

Step 1. The computer calculated the correlation coefficients in one nanometer steps over the 520 nm to 730 nm range fitting the regression equations of the following form:

$$R_1' = k_0' + k_1' V(\lambda_1) \tag{3}$$

From these we chose the λ_1 wavelength giving the best correlation.

Step 2. Considering λ_1 to be fixed, the multiple correlation coefficients also in one nanometer steps over the same spectral range were calculated fitting the regression equations

$$R_1'' = k_0'' + k_1'' V(\lambda_1) + k_2'' V(\lambda_2) \tag{4}$$

From these we chose the best correlating λ_2 wavelength and we got a first wavelength pair λ_1 and λ_2.

Step 3. Considering λ_2 to be fixed, the multiple correlation coefficients were calculated once again as a function of λ_1, and we chose the best correlating λ_1. We found that correlation was better than in step 2.

Step 4. Considering this newly chosen λ_1 to be constant, the correlation coefficients were calculated once again as a function of λ_2 and the best correlating λ_2 was chosen again.

Step 5. From step 3 this procedure was repeated several times and each repetition gave a slightly better multiple correlation coefficient at a slightly different wavelength pair. We continued this iteration until neither the multiple correlation coefficient nor the wavelengths changed any more. We called these wavelengths the optimal pair and the regression equation containing the measured $\log \frac{1}{T}$ values at these wavelengths was called the optimized regression equation. So we got an optimized regression equation in the form of equation No. 1.

Similar procedure was performed to get another optimized regression equation in the form of equation No. 2.

We found, that the regression surface over λ_1 and λ_2 plane was flat in quite a considerable region, implying that the regression equations containing different wavelength pairs within this region do not differ essentially. Therefore we chose two other wavelength pairs, one for a regression equation in the form of equation No. 1. and one in the form of equation No. 2. so we generated four regression equations altogether. Henceforth we call them calibration equations.

Substituting the $\log \frac{1}{T}$ values of each group into the calibration equations, we calculated the estimated ripeness values of groups, the difference of assessed and estimated ripeness values of groups, the standard error of calibration (SEC):

$$\mathrm{SEC} = \sqrt{\frac{\Sigma(Y - \hat{Y})^2}{n - 1 - p}}$$

where

Y is the assessed ripeness value of a group
$\hat{Y}$ is the estimated ripeness value of a group
n is the number of groups
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the (multiple) correlation (MC)

$$\mathrm{MC} = \sqrt{\frac{\Sigma(Y - \hat{Y})^2}{\Sigma(Y - \bar{Y})^2}}$$

where $\bar{Y}$ is the average of assessed ripeness values of all groups and the figure of merit (FM)

$$\mathrm{FM} = \frac{Y_{\max} - Y_{\min}}{2(\mathrm{SEC})}$$

Substituting the $\log \frac{1}{T}$ values of individual fruits into the calibration equations we could "predict" the ripeness of individuals by the calibration equations. As this prediction is not perfectly independent from the calibration, to differentiate this prediction from a perfect one we call this prediction "a prediction of individuals by regression on means", shortly PIM.

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Having studied the results, we chose one of the four calibration equations for predicting ripeness of unassessed fruit samples harvested in 1981. Unfor-

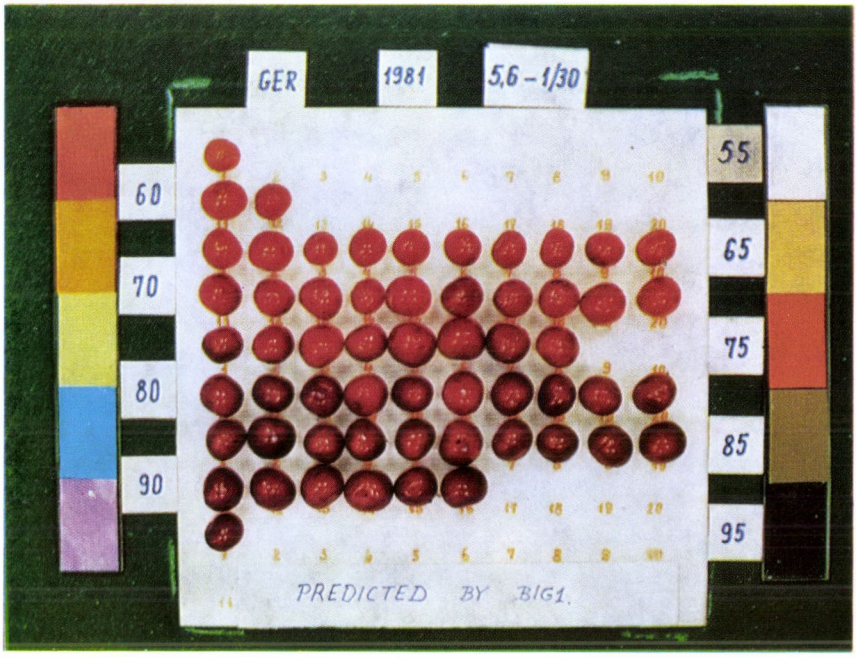

Fig. 3. Germersdorf variety cherries harvested in 1981, predicted their ripeness by calibration equation BIG1 constructed in 1980

tunately in 1981 we could not get samples of the same cherry variety as we got in 1980. In spite of this we did try to use our equations to predict the ripeness of samples.

For that the $\log \frac{1}{T}$ values of unassessed samples were measured at the wavelengths being in the chosen calibration equation and the predicted values were calculated. The samples were arranged on a desk according to the predicted values and a photo in color was taken to illustrate how the regression equation works. Also the assessed samples harvested in 1981 were predicted by each calibration equation. The assessed values and predicted values of groups were then compared by regression analysis. Standard error of predictions, correlation coefficients and biases were calculated. The standard error of predicted ripeness within groups were also calculated by one of the prediction equations.

The spectra of assessed samples were taken and stored on disks for further investigations.

2. Results

The four calibration equations generated on Bigarreau-Burlat cherry samples in 1980 are the following:

$$R_{BIG1} = 41.74 + 22.85\ V(610) - 19.70\ V(660)$$
$$R_{BIG2} = 54.01 + 52.25\ V(624)/V(668)$$
$$R_{BIG3} = 39.56 + 19.19\ V(606) - 15.47\ V(673)$$
$$R_{BIG4} = 168.66 - 71.98\ V(729)/V(616)$$

Equations indexed BIG3 and BIG4 are the optimized ones. Statistical characteristics of the calibrations are summarized in Table 1. It can be seen, that there are no essential differences among the four equations.

In Table 2 the most interesting data of the samples are summarized. Each row refers to a group of samples harvested on the same day and representing the same assessed ripeness by 40 individuals. Each such group has a label for computing reasons. We give the harvesting dates, the labels, the assessed ripeness values, the differences of assessed and estimated values (DAE) gained from calibration equations and the STDPIM values. The STDPIM values characterize the homogeneity of groups and the uncertainty of optical measurements arising partly from the positioning uncertainty of samples and partly from electronic noise.

For prediction the ripeness of samples harvested in 1981, we have tried to use the calibration equation indexed BIG1. Fig. 3 shows the photo taken from Germersdorf variety cherries, the ripeness of which was predicted by the

BIG1 indexed equation. The samples are arranged in 9 rows. Each row has a nominal ripeness value (indicated alternatively at its left or right side with a number) in 5 percent steps from 55 to 95. After the predicted value of a samples had been determined, we placed it in the row with the nearest nominal value of ripeness to the predicted one. This means, that each row contains those samples, the ripenesses of which are predicted within ± 2.5 percent of

Table 1

Statistical characteristics of calibration

Label of the calibration equation	Standard error of calibration	Multiple correlation coefficient	Figure of merit	Wavelengths (nm)
BIG1	4.91	0.946	4.1	610; 660
BIG2	5.48	0.927	3.7	624; 668
BIG3	4.77	0.949	4.2	606; 673
BIG4	5.10	0.937	3.9	616; 729

Table 2

Data of the Bigarreau–Burlat variety samples (1980)

Harvesting date	Label of the group	Assessed ripeness values (%)	Difference of assessed and estimated ripeness values of groups computed by				Standard deviation of predicted ripeness of individuals within groups by regression on means computed by			
			BIG1	BIG2	BIG3	BIG4	BIG1	BIG2	BIG3	BIG4
16–06–1980	BIG10	60	2.2	−0.6	1.7	0.9	4.4	4.7	4.6	4.3
	BIG11	70	5.2	2.5	5.4	3.9	4.5	4.1	4.7	4.0
	BIG12	80	3.8	1.5	4.2	2.8	6.4	4.8	6.4	4.4
	BIG13	90	1.2	−1.2	1.6	0.3	7.5	5.4	7.3	4.2
	BIG14	100	−3.9	−7.9	−3.7	−6.1	5.9	5.6	5.1	4.0
18–06–1980	BIG20	70	0.2	2.1	0.1	2.7	7.2	6.5	7.4	6.8
	BIG21	80	0.4	2.0	0.8	3.2	7.4	5.4	7.5	5.1
	BIG22	100	−5.2	−6.2	−4.8	−5.4	7.6	5.5	7.1	4.0
20–06–1980	BIG30	60	1.2	1.3	0.9	−0.8	4.0	4.2	4.1	4.8
	BIG31	70	−1.4	0.9	−1.4	−0.4	6.1	5.0	6.2	5.8
	BIG32	85	−7.8	−6.9	−7.7	−7.5	9.2	7.5	9.3	7.6
	BIG33	100	−6.4	−8.1	−6.1	−8.1	8.1	6.4	7.7	5.1
23–06–1980	BIG40	70	−3.4	−0.2	−3.5	−2.4	6.3	6.1	6.4	6.8
	BIG41	80	4.4	8.6	4.5	7.5	10.1	7.6	10.0	6.9
	BIG42	90	9.4	10.4	8.7	8.4	9.7	8.4	8.8	6.3
	BIG43	100	0.1	1.7	−0.6	1.0	4.3	5.5	3.1	2.8

the nominal value of the row. It can be seen, that in the adjacent rows there seem to be a few samples, that ought to be placed into the other, and there are one or two that fits a row with 10 percent differing label, but none exists, which would fit a row with 15 percent differing label. This shows, that the resolution of such a prediction is about 10 percent for samples predicted above 80 percent. For the less ripe samples we can see, that every sample fits into the row it has been placed into, so the resolution is better for less ripe samples than for riper ones.

Table 3 summarizes the most interesting data obtained by predicting the ripeness of Germersdorf variety cherries in 1981 using BIG1 indexed calibration equation. Besides the harvesting data and labels of groups consisting of 20 individuals of the same assessed ripeness, it gives the assessed ripeness values, the differences of assessed and predicted ripeness, the STD and the extent within each group.

Table 4 shows the statistical characteristics of predictions obtained by the different calibration equations.

The standard errors of predictions were computed from the squares of differences of assessed ripeness values and the averaged predicted ripeness values of the individuals within a group.

Fig. 4 shows the relation between the assessed and predicted ripeness values of groups obtained by the BIG1 indexed calibration equation.

Regression calculation on the assessed and predicted values gives the result, that the regression line has a slope of about unity (0.981) but it has about 10 percent bias. This can be caused by the different variety of cherries at generating the calibration equation, or by the insufficient color remembrance ability from one year to the other of the assessing experts. The experiments show, that even a short time color remembrance ability is insufficient. In Fig. 4 at 90 percent assessed value there is a group, labelled GER33, which deviates very significantly from the regression line and there is another, namely GER23, which is almost exactly on the line, both with the same assessed values and differing by 10 percent in predicted values. Also the photos in color show, that the two groups of fruit, with the same 90 percent assessed ripeness values differ very much in reality in better accordance with the predicted ripeness values than with the assessed ones. However, the assessing expert did not see both groups at the same time, because two days elapsed between the harvesting of the two groups. These two photos are shown in Fig. 5. The samples are arranged in the photos so, that each two rows have the same assessed value of ripeness indicated on the right side of the rows; on the left side of the rows their label numbers can be found.

Looking at the photos it becomes very clear that if the group labelled GER23 was assessed to be 90 percent, the group labelled GER33 would have had to be assessed about 100 percent ripeness. Also GER32 seems to be

riper than group GER22, their predicted values do differ by 8 percent. But group GER21 seems to be only slightly riper than group GER30, their predicted values are 62 and 60 percent, respectively, in good accordance with the visual impression.

Table 3

Data of the Germersdorf variety samples (1981), predicted on the basis of BIG1

Harvesting date	Label of the group	Assessed ripeness values (%)	Difference of the predicted and assessed ripeness of groups (%)	Standard deviation of predicted ripeness within a group (%)	Extent in predicted ripeness within a group (%)
12–06–1981	GER 10	60	— 7.4	1.4	5.9
	GER 11	70	—13.0	3.1	10.7
	GER 12	80	—13.4	3.1	10.5
	GER 13	90	—15.2	5.4	22.7
15–06–1981	GER 20	65	— 9.3	1.7	5.6
	GER 21	70	— 8.5	2.6	9.2
	GER 22	85	—14.0	3.2	8.7
	GER 23	90	—11.7	4.4	13.8
17–06–1981	GER 30	70	—10.5	2.8	8.5
	GER 31	80	—12.9	3.5	13.2
	GER 32	85	— 6.0	4.1	14.5
	GER 33	90	— 0.7	8.5	26.8
19–06–1981	GER 40	70	—12.3	2.5	12.2
	GER 41	80	—13.5	2.9	11.3
	GER 42	85	— 9.6	3.3	11.4
	GER 43	95	—11.4	4.3	18.5
	GER 44	100	—10.4	4.7	15.7
22–06–1981	GER 50	80	—12.3	3.0	12.1
	GER 51	90	—15.9	4.5	17.7
	GER 52	100	— 9.8	6.4	21.7

Table 4

Statistical characteristics of different predictions of Germersdorf variety cherries in 1981

Label of the equation	Standard error of prediction	Multiple correlation coefficient	Bias
BIG1	3.70	0.955	—10.88
BIG2	3.06	0.968	—15.89
BIG3	3.81	0.957	—11.19
BIG4	3.32	0.962	—13.45

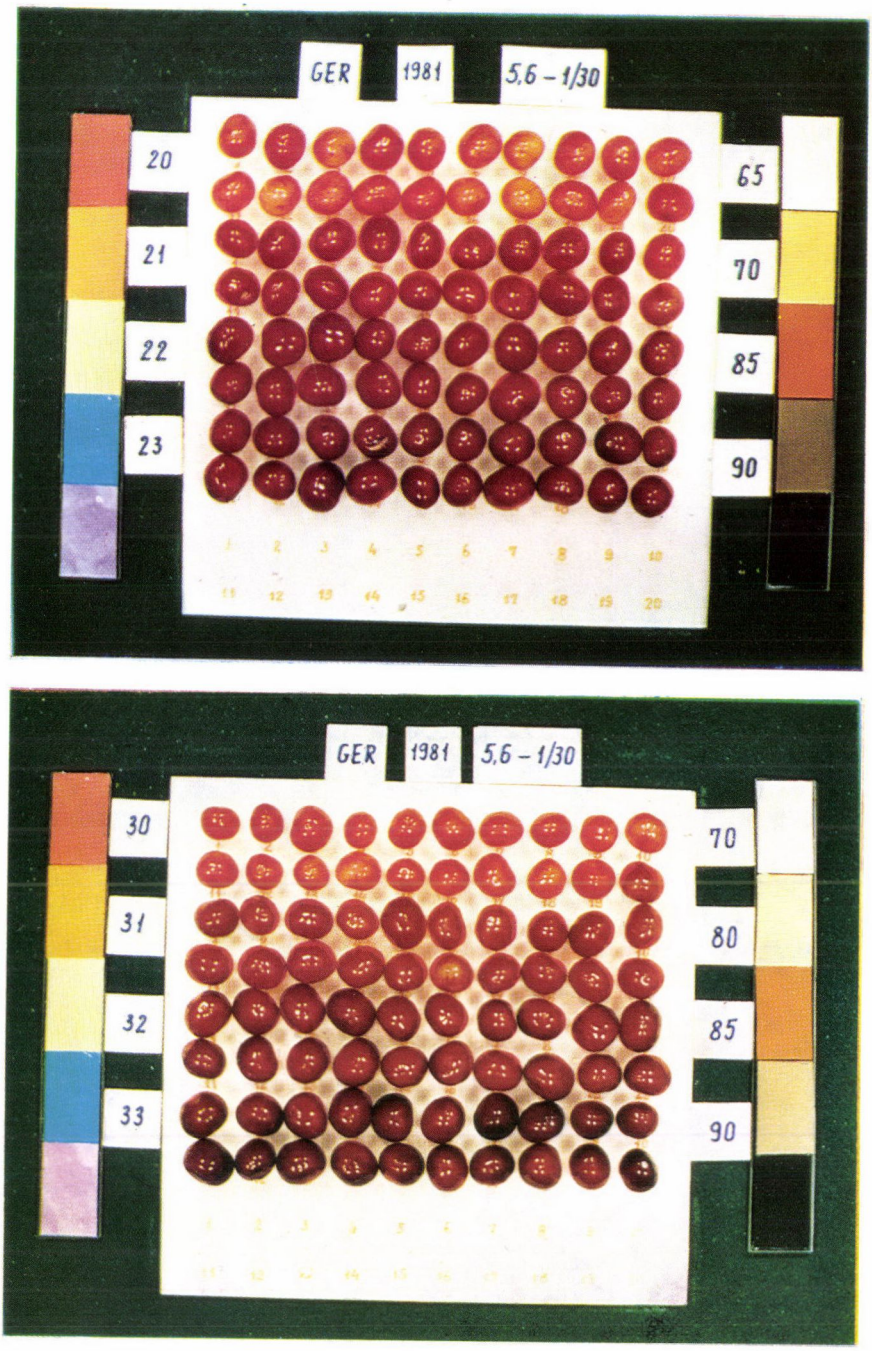

Fig. 5. Assessed Germersdorf variety cherries

Comparing the STDPIM of Table 2 and the standard deviations within the group in Table 3, we can see, that the latter are smaller. This indicates, that the groups were more homogeneous in 1981 than in 1980. It appears also, that the standard deviation of the groups in Table 3 depends on the ripeness value. In Fig. 6 this appears better, where the standard deviation values within

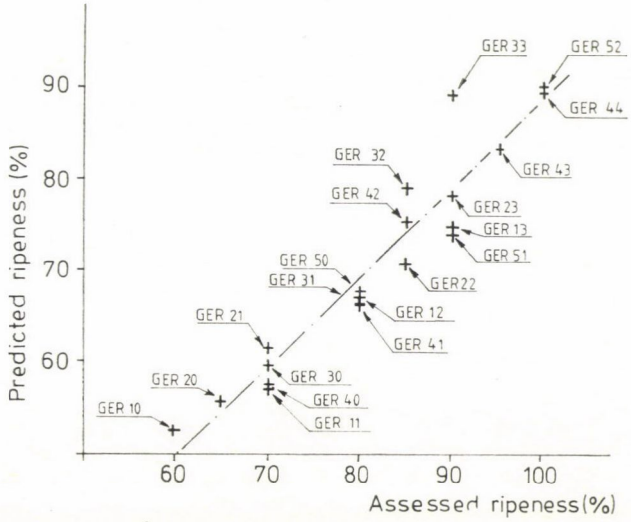

Fig. 4. Relationship of the assessed and predicted ripeness values of cherry groups. Slope = 0.981; intercept = −9.352

the groups are plotted as function of the predicted ripenesses of groups. This seems to justify what we said on the basis of Fig. 3, namely, that the prediction process is better for less ripe samples than it is for riper ones. The standard deviation within groups are affected not only by the prediction errors but also by inhomogeneity of the groups. Taking into account the well-known fact that the ability of the human eye to differentiate colors is lower for darker colors we may say, that the standard error of prediction is somewhat smaller, than it seems to be from Fig. 6. The photo of assessed samples sorted according to their predicted ripeness also shows that the inhomogeneity of the groups increases, as the ripeness value of groups gets higher.

It had been supposed, that the prediction would be influenced by the size of fruit. Therefore each single cherry had been weighed and their diameters in the direction of light propagation had been measured. We expected, that a good correlation could be found between the $\log \frac{1}{T}$ values measured at the selected wavelengths and the weights of samples or the diameters of samples with the same assessed ripeness value. Regressions were computed

for several such groups of cherries, but no good correlation has been found. Also in Fig. 3 it can be seen, e.g. in the third row, that the predicted ripeness of the second big, and the third small fruit are the same showing that the size of visually similarly colored fruits do not influence the predicted ripeness value perceptibly. At present we cannot give any logical interpretation of

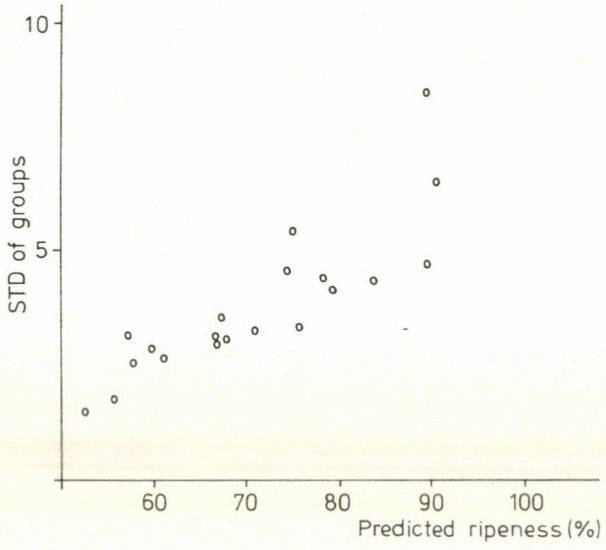

Fig. 6. Standard deviations within groups versus predicted ripeness

this phenomenon. We think that the difference of pigment contents in fruits of different ripeness values causes much more significant absorption differencies than the difference in size within a group can.

3. Conclusions

The experiments show, that ripeness prediction of cherries is possible on the basis of measuring $\log \frac{1}{T}$ values in the visible spectral region at two wavelengths.

To choose the "best" wavelength-pair is problematical, because the correlation surface above the wavelength-pair plane is rather flat in a considerable region and therefore the best wavelength-pair — chosen on the basis of the highest multiple correlation coefficient — is strongly influenced by the random assessing errors. It is also impossible to eliminate its influence theoretically because there is no general theory suitable for the interpretation of

the interaction of light and such materials as fruits. At present only experiments can prove or disprove the results, therefore there is a need to try the prediction procedure at least once again for both varieties of cherries, using calibration equations generated on the basis of the same variety of samples as the samples to be predicted are or will be.

If such repeated experiments prove the practical usefulness of prediction equations, we will be able to conclude, that such an instrumental prediction procedure can help experts assessing ripeness ridding them of monotonous assessing work and can assure a better uniformity in assessing at different places and at different times. But beside this we must not forget that such a prediction procedure is limited by an uncertainty arising partly from technical reasons and partly from the fact that the pigment content is not the single factor of ripeness. An instrument constructed for this purpose will have an arbitrary scale. To avoid it the best solution would be a scale predicting not the ripeness but the pigment content. To achieve that is not a real aim at present and we shall have to put up with an arbitrary ripeness scale causing heavy technical problems.

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ENZYMATIC BROWNING SUBSTRATES IN APPLE CULTIVARS

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The o-dihydroxy phenol composition of the mesocarp of three apple cultivars was compared by two-dimensional thin-layer chromatography on MN 300 cellulose chromatoplates. Methanol extracts of apple flesh were purified by extraction with acetonitrile. The solvent systems used for chromatography were: a) 5% acetic acid, *b)* n-butanol : acetic acid : water (12 : 3 : 5). The o-dihydroxy phenols were detected by spraying with the Hoepfner-reagent. Six identical compounds were found in the chromatograms of all three cultivars: (−)-epicatechin, (+)-catechin, chlorogenic acid and one of its isomers as well as two unidentified compounds (A and B) belonging — according to their colouration — to the catechins. In agreement with data in the literature (−)-epicatechin was the principal o-dihydroxy phenol of all three cultivars. Differences in characteristics of the cultivar were observed in the relative amounts of the other compounds as judged by the colour intensity of the corresponding spots. Beside (−)-epicatechin, the dominating o-dihydroxy phenols were chlorogenic acid and compound A in Golden Delicious, (+)- catechin and compound A in Starking, while in Jonathan these were present only in small amounts (Table 1 and Figs. 3, 4 and 5). A part of these findings is borne out by quantitative determinations of chlorogenic acid and o-dihydroxy phenol contents, those concerning the cultivar Starking have not been assumed earlier. The results are helpful in interpreting the browning behaviour of the apple cultivars investigated.

Keywords: Enzymatic browning, apple cultivars, o-dihydroxy phenol composition, thin-layer chromatography

Enzymatic browning occurs in plant tissues as a result of the action of the enzyme polyphenol oxidase on its endogenous substrates. The number of these substrates has been reported to be restricted in apples to (+)-catechin and (−)-epicatechin as well as chlorogenic acid and its isomers (HULME, 1958; MONTIES, 1966; TÄUFEL & VOIGT, 1963). Out of these substrates, (−)--epicatechin is said to be the most abundant in apples (HERRMANN, 1974; PRABHA, 1982; SIEGELMAN, 1955) and chlorogenic acid to yield the least brown pigments upon enzyme action (SIEGELMAN, 1955; TÄUFEL & VOIGT, 1963). In a study carried on in our laboratory for several years with apples from two locations and of different picking maturity it was found that, on the average, the ratio of chlorogenic acid to o-dihydroxy phenols was significantly lower in the samples of Jonathan than in those of Golden Delicious, while the rate of enzymatic browning polyphenol oxidase activity and o-dihydroxy phenol content itself were very similar in these two cultivars. On the other hand, for the cultivar Starking which showed a markedly higher rate of enzymatic browning and polyphenol oxidase activity, the value of the

above ratio either ranged with that of the cultivar Golden Delicious (in the samples from one location) or was between that of the other two cultivars (in the samples from the other location) (VÁMOS-VIGYÁZÓ et al., 1976; 1980). As no data were found on characteristic differences in the polyphenol composition of apple cultivars, it has been decided to investigate this question more thoroughly. Thin-layer chromatography (TLC) as used for similar purposes by many authors (BERGER & HERMANN, 1971a, b; FORREST & BENDALL, 1969; GOLAN et al., 1977; HEIMANN et al., 1971; MOSEL & HERRMANN, 1974; PAULSON et al., 1980) was selected as method.

1. Materials and methods

1.1. The apples

Apples of the cultivars Jonathan, Golden Delicious and Starking were purchased from the Újfehértó Research Station of the University of Horticulture, Budapest. All the apples were picked in 1979.

1.2. Extraction of the polyphenols

The extraction of the polyphenols from the other components of the apples is the most difficult and delicate step in the procedure. The extraction method selected as relatively best (which made it possible to obtain chromatograms of satisfactory quality) is illustrated in Fig. 1.

Peeled and cored apples were first diced by hand, with a stainless steel knife, then immediately transferred to a blender (Atomix or Biomix, Labor-MIM, Budapest) and homogenized for 3 min in methanol. After standing overnight at 5 °C, solid particles were filtered off and the filtrate was concentrated in vacuo to a solid content corresponding to 1.5–2 g cm^{-3} (concentrate I). Upon standing overnight at 5 °C, a precipitate was formed in the extract which was again filtered off. Vacuum concentration, standing overnight at 5 °C and filtration were repeated twice and finally yielded concentrate III which had a solid content corresponding to 8–10 g cm^{-3}. This was then mixed in a separation funnel with acetonitrile and shaken for 20 min. Then the viscous phase was discarded and the acetonitrile phase used for chromatographic separation in amounts of $(8–22) \cdot 10^{-3}$ mm^3.

1.3. Thin-layer chromatography of o-dihydroxy phenols

Two-dimensional separation was carried out on Macherey and Nagel MN 300 cellulose chromatoplates (Dueren, FRG). The solvents used were 5% acetic acid in distilled water in the first and n-butanol – acetic acid – water (12 : 3 : 5) in the second direction (CRAFT, 1961; PAULSON et al., 1980).

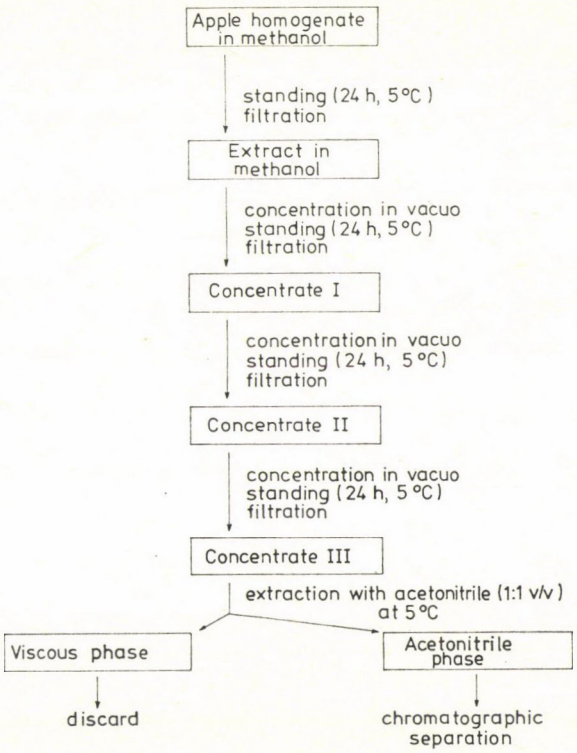

Fig. 1. Method of extracting the o-dihydroxy phenols from apple mesocarp for thin-layer chromatography. The concentration of the initial homogenate and the concentrates I, II and III corresponded to the following concentrations of apple flesh (fresh-weight basis): 0.2 g cm^{-3}, 1.5–2 g cm^{-3}, 4–5 g cm^{-3} and 8–10 g cm^{-3}, resp.

1.4. Detection of the o-dihydroxy phenols

The reagent of HOEPFNER (1932) specific for o-dihydroxy phenols was used as chromogenic spray. The reagent is composed of the following solutions which are applied one after the other to the plate, with drying in between: *1.* 0.5 mol dm^{-3} HCl; *2.* 10% (w/v) $NaNO_2$-10% (w/v) $Na_2MoO_4 \cdot 2H_2O$, *3.* saturated NaOH in ethanol.

After the 3rd spray chlorogenic acids appear as violet and catechins as reddish-brown spots.

1.5. Identification of the separated compounds

The identification of the separated o-dihydroxy phenols was carried out by co-chromatography of authentic chlorogenic acid, (+)-catechin and (−)-epicatechin as well as caffeic acid (all from Fluka, Basel, Switzerland).

1.6. Quantitative assay of the o-dihydroxy phenols

The concentration of o-dihydroxy phenols in the apple samples used for chromatography was determined by a spectrophotometric assay method used earlier for similar purposes in this as well as in a number of other laboratories (SHARPLES, 1964; VÁMOS-VIGYÁZÓ et al., 1976; ZUCKER & AHRENS, 1958). The method is based on a modification of the Hoepfner-reagent (ALMÁSI & MOLNÁR, 1961; ARNOW, 1937). The reaction mixture contained 1 cm^3 of fruit extract in methanol, 1 cm^3 0.5 mol dm^{-3} HCl, 1 cm^3 $NaNO_2$—$Na_2MoO_4 \cdot 2H_2O$ solution (10% w/v each), 1 cm^3 1 mol dm^{-3} NaOH and 1 cm^3 distilled water. Absorbance was measured at 520 nm, o-dihydroxy phenol content calculated on the basis of a Hoepfner-reacted chlorogenic acid calibration curve and expressed as mg chlorogenic acid per 1000 g fruit flesh (fresh-weight basis).

Chlorogenic acid content was determined by direct UV absorption measurements of the above extracts at 328 nm, the specific absorption maximum of this compound (CÔME, 1971). Results were evaluated on the basis of a chlorogenic acid calibration curve and expressed as mg chlorogenic acid per 1000 g fruit flesh (fresh-weight basis).

2. Results

2.1. The o-dihydroxy phenols found in apples

The greatest number of spots (6) reacting with the Hoepfner-reagent could be obtained on applying (20–22) $\cdot 10^{-3}$ mm^3 extract to the chromoplate. A chromatogram showing the distribution of the six spots is schematically represented in Fig. 2.

As can be seen from the chromatogram, no free caffeic acid could be detected in the apple extracts. The two unidentified spots (Nos. 5 and 6) showed a colouration with the Hoepfner-reagent similar to that of the catechins. The "isomer of chlorogenic acid" (No. 1) was present also in the authentic chlorogenic acid standard. It cannot be said which of the isomers it represents, however, its colour reaction identifies it as a compound closely related to chlorogenic acid.

The main o-dihydroxy phenol compound present in the chromatograms of all three apple cultivars was (—)-epicatechin. The differences between the cultivars could be better demonstrated if the amounts chromatographed were adjusted so as to give (—)-epicatechin spots of similar size and colour intensity. This was achieved by applying to the plate (8–10) $\cdot 10^{-3}$ mm^3 of the Starking extract and (12–15) $\cdot 10^{-3}$ mm^3 of the extracts of the other two cultivars. These amounts corresponded to 256–545 mg apple mesocarp (fresh-weight basis).

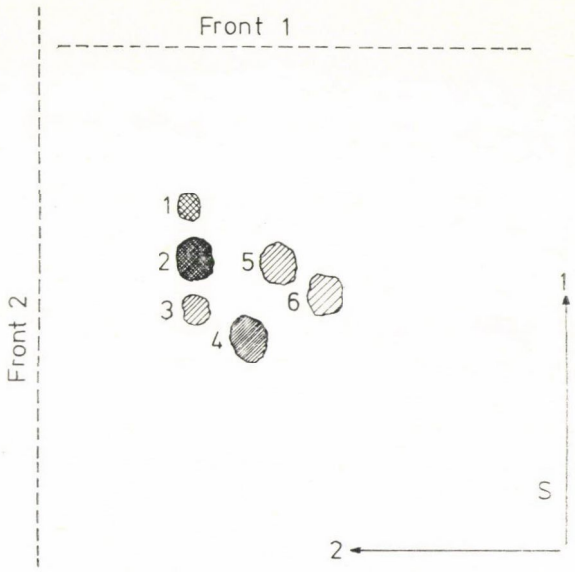

Fig. 2. Schematic representation of the chromatographically separated o-dihydroxy phenols of apples. *Experimental conditions:* (20–22) · 10^{-3} mm³ apple extract prepared according to Fig. 1, MN 300 cellulose chromatoplate. Solvents: 1. 5% acetic acid, 2. n-BuOH : CH_3COOH : H_2O (12 : 3 : 5). Spray: Hoepfner-reagent (*a:* 0.5 mol dm^{-3} HCl; *b:* 10% $NaNO_2$ + 10% $Na_2MoO_4 \cdot 2H_2O$; *c:* saturated NaOH in ethanol). Compounds: 1. isomer of chlorogenic acid, 2. chlorogenic acid, 3. (+)-catechin, 4. (−)-epicatechin, 5. and 6. unidentified o-dihydroxy phenols. Checked spots: violet colouration, striped spots: reddish-brown colouration. S = start

Table 1

Estimation of relative amounts of chromatographically separated o-dihydroxy phenols in apple mesocarp

Experimental conditions: extraction of o-dihydroxy phenols with methanol, purification with acetonitrile, two-dimensional chromatography on MN 300 chromatoplates with 1.) 5% acetic acid and 2) n-BuOH : CH_3COOH : H_2O (12 : 3 : 5). Detection with the Hoepfner spray reagent. The amounts of extract have been adjusted to give spots of identical colour intensity for (−)-epicatechin [(12–15) · 10^{-3} mm³ of Jonathan and Golden Delicious, (8–10) · 10^{-3} mm³ of Starking extract]

Cultivar	Colour intensity					
	Chlorogenic acid	Chlorogenic acid isomer	(+)-Catechin	(−)-Epicatechin	A	B
Jonathan	++	traces	traces	+++++	++	traces
Golden Delicious	+++++	+	traces	+++++	++++	traces
Starking	+++	traces	++++	+++++	++++	traces

A and B: unidentified compounds.

Colouration of the spots: Chlorogenic acid and its isomer — violet; catechins, A and B — reddish brown. +, ++, +++, ++++ and +++++: colour intensities of the spots in increasing order.

The chromatograms obtained for the three cultivars are shown in Figs. 3, 4 and 5 and an evaluation of the relative amounts of the o-dihydroxy phenols is given in Table 1.

Beside the main component (−)-epicatechin, the cultivar Jonathan showed faint spots of chlorogenic acid and one of the unidentified compounds (A). The chlorogenic acid isomer (+)-catechin and the other unidentified compound (B) were present only in traces. In the cultivar Golden Delicious, three components were dominating, i.e. (−)-epicatechin, chlorogenic acid and compound A. The chlorogenic acid isomer gave a somewhat more distinct spot than in Jonathan, while (+)-catechin and compound B were present only in traces. In the cultivar Starking the dominating components were (−)-epicatechin, compound A and (+)-catechin. The chlorogenic acid spot was considerably fainter than in the cultivar Golden Delicious, while the chlorogenic acid isomer and compound B were present only in traces.

Table 2

Ortho-dihydroxy phenol and chlorogenic acid contents in the mesocarp of apple cultivars

Experimental conditions: extraction of o-dihydroxy phenols (ODP) with methanol, determination of o-dihydroxy phenol content with a modified Hoepfner-reagent (ALMÁSI & MOLNÁR, 1961) and reading of the optical density at 520 nm; determination of chlorogenic acid (ChA) content by direct absorbance measurement of the methanol extract at 328 nm. Evaluation on the basis of chlorogenic acid calibration curves (for o-dihydroxy phenol content after treatment with the reagent). Results expressed in mg chlorogenic acid per kg

Cultivar	o-dihydroxy phenols (mg kg^{-1})		Chlorogenic acid (mg kg^{-1})	
	$\bar{x}$	±s	$\bar{x}$	±s
Jonathan (J)	657	73.5	49.9	5.5
Golden Delicious (G)	486	39.9	92.4	6.4
Starking (S)	844	87.5	137	5.4

Results of statistical investigation of distribution homogeneity:
$\chi^2_{ODP} = 1.49 < \chi^2_{crit} = 9.21$, df = 2; $\chi^2_{ChA} = 0.10$, $< \chi^2_{crit} = 9.21$, df = 2; not significant at P = 1% probability level.

Multiple comparison of means (t-test):

ODP:

Cultivar	J	G
G	*	
S	*	**

ChA:

Cultivar	J	G
G	***	
S	***	***

*, ** and ***: differences significant at the levels of probability of 95%, 99% and 99.9%, respectively.

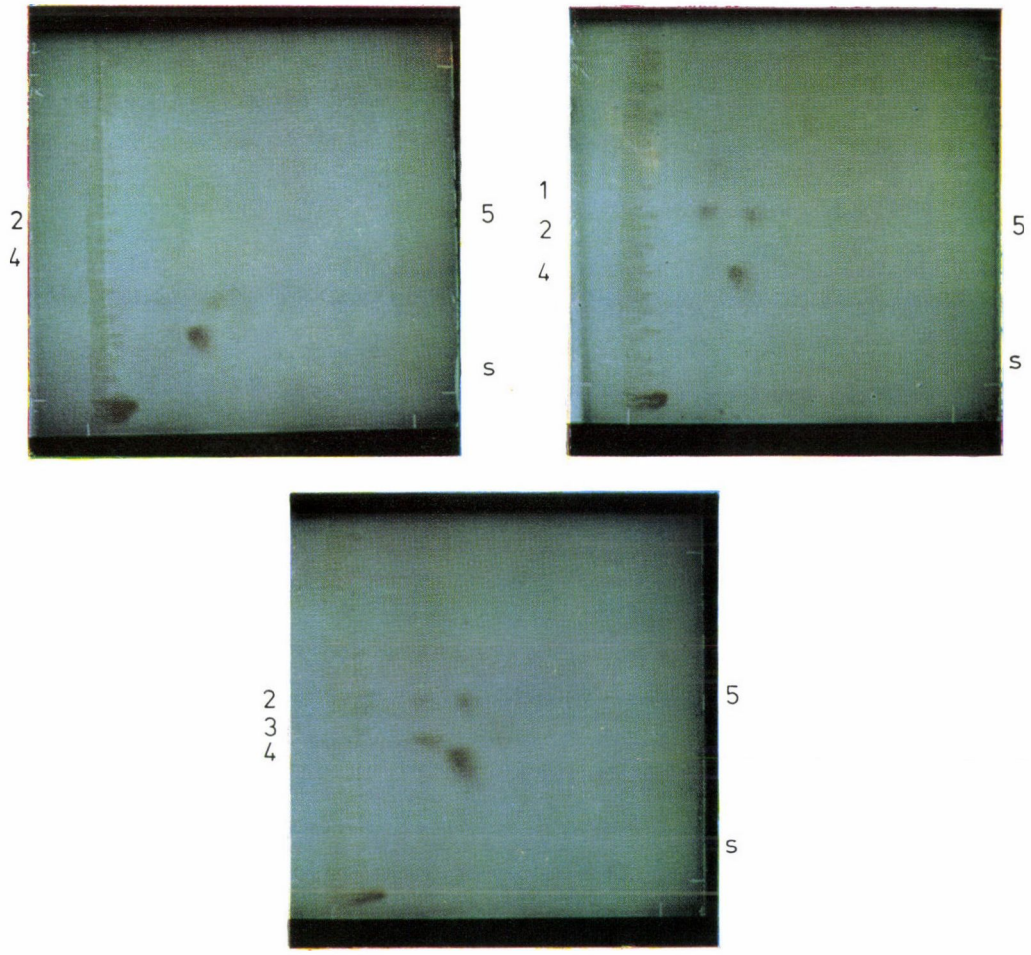

Fig. 3. Chromatogram of the o-dihydroxy phenols of the cultivar Jonathan. Applied amount of extract: $12 \cdot 10^{-3}$ mm³. Experimental conditions, numeration of compounds and symbols as in Fig. 2

Fig. 4. Chromatogram of the o-dihydroxy phenols of the cultivar Golden Delicious. Applied amount of extract: $15 \cdot 10^{-3}$ mm³. Experimental conditions, numeration of compounds and symbols as in Fig. 2

Fig. 5. Chromatogram of the o-dihydroxy phenols of the cultivar Starking. Applied amount of extract: $8 \cdot 10^{-3}$ mm³. Experimental conditions, numeration of compounds and symbols as in Fig. 2

2.2. O-dihydroxy phenol and chlorogenic acid contents of apples

The results of the quantitative determination of the o-dihydroxy phenols and chlorogenic acid are shown in Table 2.

O-dihydroxy phenol content was the highest in the sample of the cultivar Starking and the lowest in that of Golden Delicious, while chlorogenic acid content was the highest in Starking and the lowest in Jonathan. The differences between the cultivars proved to be significant at least at the level of probability of 95%.

The percentage ratio of the chlorogenic acid : o-dihydroxy phenol content was 7.6, 16 and 19 for the samples of Jonathan, Starking and Golden Delicious, respectively. The "non-chlorogenic acid" constituents of the o-dihydroxy phenol content were, in the above order, 607, 707 and 394 mg kg^{-1}.

3. Conclusions

3.1. Thin-layer chromatographic separation of o-dihydroxy phenols

In selecting the conditions of TLC the main point was to obtain well shaped and clearly separated o-dihydroxy phenol spots from the apple extracts and to detect hydroxycinnamic acid derivatives and catechins separated on the same plate.

Methanol extraction of the o-dihydroxy phenols as used for the quantitative assay proved unsatisfactory in itself for chromatographic purposes. This was found to be consistent with most of the findings published by others (BERGER & HERRMANN, 1971a; FORREST & BENDALL, 1969; MOSEL & HERRMANN, 1974; SCHALLER & ELBE, 1970; SCHULZ & HERRMANN, 1980; SHARPLES, 1964; WALTER et al., 1979). However, on subsequent extraction with ethyl acetate as applied by most of the authors (BURAEU et al., 1977; FLEURIET & MACHEIX, 1972; RANADIVE & HAARD, 1971; SCHALLER & ELBE, 1970) the quality of the chromatograms did not meet the requirements outlined above. Acetonitrile which has found application as solvent in high performance liquid chromatography of plant phenolics (BARANOWSKI & NAGEL, 1981; WULF & NAGEL 1980) made it possible to obtain chromatograms of a satisfactory quality.

The Hoepfner-reagent was used by a great number of authors for the detection of o-dihydroxy phenols and, in the first place hydroxycinnamic acid derivatives in chromatograms (CRAFT, 1961; SHARPLES, 1964; WALTER et al., 1979; ZUCKER & AHRENS, 1958). It was found to be best suited for the present study as it permits of a direct comparison with the results of quantitative measurements obtained with the same reagent. A further advantage of this reagent is that it gives different colour reactions with hydroxycinnamic acid derivatives and catechins. Thus, unidentified compounds might be assigned to one of these groups by colouration.

3.2. The composition of o-dihydroxy phenols of the apple cultivars

The o-dihydroxy phenol composition of the apple cultivars Jonathan, Golden Delicious and Starking was essentially identical (see Fig. 2). This composition, as mentioned in the introduction, has been observed by others and also with other cultivars, and seems to be characteristic of the genus. However, the comparison of the chromatograms obtained with the three cultivars (Figs. 3, 4 and 5) revealed minor differences which are in agreement with the results of the quantitative assays (Table 2) and apparently are characteristic of the cultivar. This applies, in the first place, to the higher proportion of chlorogenic acid in the o-dihydroxy phenol composition of the cultivar Golden Delicious. Another difference characteristic of the cultivar is the higher proportion of (+)-catechin in the cultivar Starking. This could not be assumed on the basis of the quantitative assays performed. The higher proportion of catechins (or the higher concentration of "non-chlorogenic acid" o-dihydroxy phenols) might contribute to the higher browning rate of this cultivar. It shall be noted that the results of the quantitative assays are in excellent agreement with those obtained earlier with a total of 37 samples belonging to these three cultivars (VÁMOS-VIGYÁZÓ et al., 1976; 1977; 1980).

The two unidentified spots gave the reddish-brown colouration of the catechins with the Hoepfner-reagent. They might be (+)-gallocatechin and (−)-epigallocatechin, both reported to be present in small amounts in the cultivar Jonathan, while only the former was found in the cultivar Golden Delicious (MOSEL & HERRMANN, 1974). The presence of these compounds in these cultivars seems to be dependent on the location. No data were found on the o-dihydroxy phenol composition of the cultivar Starking.

According to data from the literature compiled by HERRMANN (1974) three chlorogenic acid isomers were found in McIntosh apples, the one present in the largest amount (about 20% of the chlorogenic acid content) being cryptochlorogenic acid. Neochlorogenic acid and isochlorogenic acid were detected only in traces. In apples of the cultivars Victoria and Ontario only the presence of two isomers (neo- and isochlorogenic acids) was reported. Thus, no assumption can be made as to the chlorogenic acid isomer found in the three cultivars investigated. Since this was but a minor component in all three cultivars, its role as enzymatic browning substrate might be considered as of slight importance.

Summarizing the results of the work presented it might be said that chromatographic analysis confirmed the assumed cultivar-dependent difference in the o-dihydroxy phenol composition of apples and revealed additional differences that might be helpful in interpreting the enzymatic browning behaviour of the cultivars investigated.

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CONTENTS

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EVALUATION OF ELDERBERRY (*SAMBUCUS NIGRA* L.) CLONES BASED ON THE QUALITY OF THE FRUIT

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The use of synthetic colouring compounds for the dying of foodstuffs becomes more and more restricted all over the world. In consequence the interest of the food industry in fruits of colouring capacity is steadily increasing. A fruit suitable for the colouring of foods is elderberry belonging to the family of *Caprifoliaceae* (*Sambucus nigra L.*) which is indigenous in Europe, North-West Africa and Western Asia. It can be found over almost the whole of Hungary in the shrub stratum of deciduous forests. It occurs in a large variety in the forest belt in the Hanság (Western Hungary). The fruit contains apart from the pigments a substantial amount of vitamin C, sugars and mineral salts (Andross, 1941).

Elderberry was first cultivated in the United States, in Ohio about 1890. Beside the *S. nigra*, *S. canadensis Hesse* and *S. cerulea Raf.* species or their hybrids were also grown. The first cultivars were produced by Adam and after 1921 Burbank and others improved elderberry cultivars. At present 24 elderberry cultivars are grown in the United States (Ritter & McKee, 1964; Janick & Moore, 1975).

It is grown in Europe, too: in Denmark, Netherland, Belgium and Austria, on a smaller scale.

Keywords: elderberry, analysis of fruit components, anthocyanins, phytometry

We set ourself the task to seek for valuable elderberry plants in the Hanság and propagate them for the purpose of a gene bank on the basis of the determination of their main phytometric and biochemical characteristics. The commencement of this work as early as possible was indicated on one hand by the fact that the formation of large fields required the elimination of several forest belts and on the other the extensively applied spraying of herbicides from helicopters causes occasionally the devastation of the shrub stratum of forest belts.

Based on the above objectives we started the selection of elderberry types suitable for use in the canning industry in the forest belts of the section of the Hanság between Fertőújlak and Kistölgyfa in 1979.

1. Materials and methods

In the course of selection in four different locations a total of 750 parent trees were marked out and fixed topographically. The marked trees were, on the basis of their fruits, visually graded. Detailed analysis was carried out

on 64 stock trees judged to be the best and on their fruit in 1980 throughout 1981.

The phenophases of the stock trees were recorded, the intensity of inflorescence was observed and so was its relation to fruit formation, the composition of the fruit was analysed and the best plants of different ripening time were propagated for the gene bank.

1.1. Analysis of the fruit

Thirty corymbs were picked from each marked out tree. The fruit was considered ripe when it was fully coloured, when it was soft to the touch and became easily detached from the pedicle. The average corymb weight was determined. The number of berries per corymb, the average weight of a berry and of a peduncle was expressed on the basis of the average of the total of berries picked from the 30 corymbs. The difference between the number of original pedicles per corymb and the number of actually developed berries permitted of drawing conclusions as to the extent of fertilization, since elderberry is partly self-fertilizing.

1.2. Analysis of the fruit components

Invert sugar was determined according to AOAC methods. Vitamin C was determined by 2,6 dichlorophenolindophenol (ANDROSS, 1941). For the determination of the titratable acid content a Radelkis type OH–408 apparatus was used. The procedure was as follows: 20 g of pulped fruit were heated with 100 cm^3 distilled water on a water bath for 30 min. When cooled it was filtered and an aliquot of the filtrate of 0.8–1.0 cm^3 was applied to the apparatus.

Elements K and Ca were determined on a AAS–1 type atomic absorption spectrophotometer (ISAAC & JOHNSON, 1975). Phosphorus was determined by a spectrophotometric method (HUMPHRIES, 1956) and the anthocyanins according to GOMBKÖTŐ (1963).

1.2.1. Separation of anthocyanins. The elderberries were pulped in a Biomix apparatus. Six g of fruit pulp were transferred into 50 cm^3 methanol containing 1% HCl. This was kept in the refrigerator for a night and then it was filtered on a glass filter. From the filtrate 0.6 cm^3 was applied to a Whatman 3 chromatographic paper (23 × 55 cm). The start line was marked 5 cm from the lower edge of the paper. The chromatogram was developed by ascending technique in the upper phase of butanol–glacial acetic acid–water (4:1:5) mixture in the dark at 15 °C for 48 h. The chromatograms were dried in an air stream at room temperature.

1.2.2. Photometry of the anthocyanins. According to the method of INGALSBE and co-workers (1965) the homogenate of 10 g elderberry was

mixed with 150 cm^3 70% methanol containing 0.5% HCl for 3 min. Then it was filtered and 2.5 cm^3 filtrate was diluted to 100 cm^3. The anthocyanins were determined at 527 nm on Spectromom type 360 spectrophotometer (MOM, Hungarian Optical Works, Budapest).

2. Results

2.1. Evaluation of the phytometric indices

A difference of 30 days was observed in the full maturity of the different types of elderberry. The earliest date of maturity was 15 August (No. 479 parent tree), the latest 15 September (No. 269 parent tree). The majority of the different types observed was fully ripe between 1 and 10 September.

Great differences were found in the fruit characteristics, too. The weight of a fully ripe corymb varied between 32.1 g (No. 13) and 141.3 g (No. 480). The number of berries per corymb varied between 207 (No. 13) and 925 (No. 480). The average weight of a berry varied between 0.09 g (No. 41) and 0.45 g (No. 500), while that of the peduncle between 1.2 g (No. 42) and 6.6 g (No. 481) (Table 1).

Table 1

Maturity time and components of the yield of marked out elderberry parent plants

Number of clone	Date of maturity	Weight of corymb (g)	Weight of peduncle (g)	Number of berries per corymb	Average weight of a berry (g)
13	20 August	32.1	2.2	207	0.15
26	20 August	39.2	3.2	289	0.35
33	1 September	100.6	3.8	319	0.31
41	5 September	45.7	3.1	487	0.09
42	25 August	40.4	1.2	318	0.12
49	5 September	43.1	3.6	448	0.16
269	15 September	36.5	2.5	265	0.17
472	25 August	46.2	2.6	375	0.12
479	15 August	72.8	4.1	573	0.12
480	2 September	141.3	6.4	925	0.15
481	5 September	80.2	6.6	496	0.16
484	5 September	111.2	3.4	680	0.16
500	1 September	186.2	5.5	410	0.45

The parent plants had a yield of 8–22 kg. (This is only an informatory data, because it is obtained from bushes at different locations and of different ages.) The stock tree No. 33, an outstanding clone, is shown in Fig. 1 and its ripe corymb in Fig. 2.

2.2. Anthocyanins and other components of the fruit

The pH value, dry matter, acid, vitamin C, anthocyanin, sugar and mineral salt content of 13 clones was determined (Table 2). Elderberries have a high dry matter and anthocyanin content.

The components of the different clones vary within a wide range. Clone No. 479 stands out with the highest vitamin C content and one of the highest anthocyanin and dry matter contents.

Clone No. 269 is also worth mentioning with its highest anthocyanin content and high dry matter and vitamin C contents.

The main anthocyanin components were identified first by HARBORNE (1967) and later by REICHEL and REICHWALD (1977) as cyanidin-3-glucoside, cyanidin-3-sambubiozide, cyanidin-3-sambubiozide-5-glucoside. Because of the unavailability of standards we could not undertake to identify components, we separated them only. The spots of seven components were separated

Table 2

Chemical analysis of the fruit components in elderberry
(Trace elements are related to dry weight, the other chemical components to fresh weight)

Number of clone	Dry matter (%)	pH	Titratable acid (in citric acid) (%)	Vitamin C (mg%)	Anthocyanin (E×250 at 527 nm)	Sugar (%)	P (%)	K (%)	Ca (%)
13	28.01	5.07	0.40	18.81	180.0	5.76	0.16	1.22	0.49
26	24.94	4.96	0.32	18.50	145.0	4.94	0.23	1.48	0.50
33	23.95	4.47	0.25	19.20	225.0	4.84	0.19	0.81	0.38
41	23.86	4.54	0.29	25.25	215.0	3.55	0.25	0.98	0.38
42	24.52	4.34	0.26	18.50	157.5	3.80	0.26	1.38	0.47
49	27.67	4.95	0.64	13.70	179.75	3.80	0.21	0.96	0.48
269	25.34	4.38	0.59	23.15	362.5	3.32	0.15	0.94	0.41
472	20.75	4.72	0.44	16.30	205.5	3.55	0.24	1.10	0.57
479	26.91	4.78	0.35	31.25	270.0	5.34	0.22	1.12	0.37
480	25.68	4.12	0.32	12.55	209.5	4.26	0.23	1.08	0.49
481	24.92	4.48	0.60	21.75	210.5	2.60	0.33	1.32	0.57
484	23.55	4.32	0.38	10.95	201.25	4.44	0.28	1.22	0.45
500	23.30	4.72	0.48	7.15	84.75	5.34	0.24	1.25	0.54

(E = extinction)

Fig. 1. Productive stock tree of No. F. 33 elderberry clone

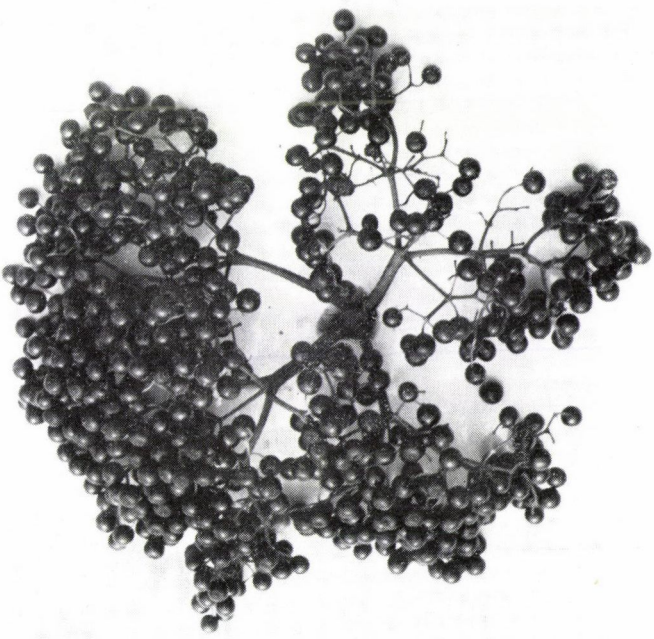

Fig. 2. Mature corymb of No. F. 33 elderberry clone

(Fig. 3). The R_f values were determined (Table 3). The most intense spots were obtained at R_f values 0.31, 0.26, 0.17. The spots appearing at $R_f = 0.05$ and 0.37 were very faint, therefore they are not seen on the photograph.

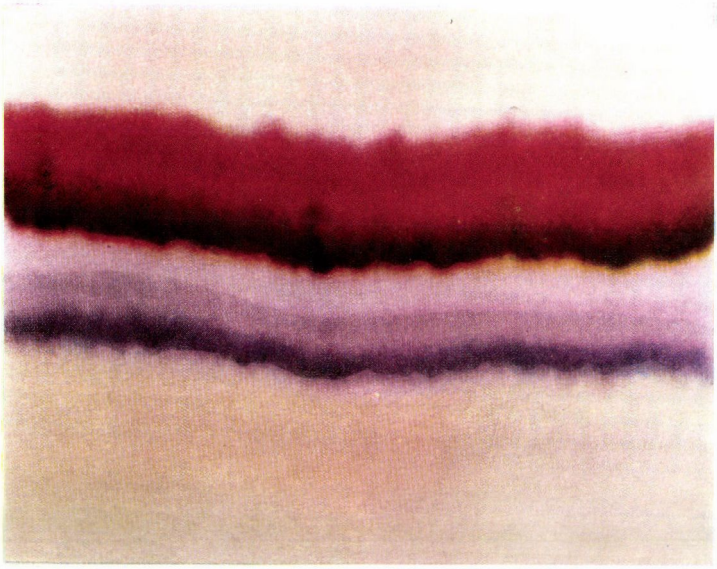

Fig. 3. Chromatogram of the pigments of elderberry clone No. 269

Table 3

R_f values belonging to the spots in the chromatogram of the anthocyanin pigments of No. 269 elderberry clone

	Separated spot						
	1	2	3	4	5	6	7
R_f value	0.05	0.11	0.17	0.21	0.26	0.31	0.37

3. Conclusions

The selection of elderberry clones suitable for food colouring was carried out. At four different locations 750 parent trees were marked out. The analysis of the fruit composition and the components of the yield was carried out on 64 stock trees.

Between the full maturity of the various clones a difference of 30 days was observed. Clone No. 479 is the earliest, it was fully ripe on the 15 August, while clone No. 269 was the latest with becoming fully ripe on 15 September. The majority of the other clones became ripe between the 1st and 10th of

September. The number of berries per corymb was found to be between 207 and 925. The average weight of the berries varied between 0.09 and 0.45 g, while the weight of the peduncles between 1.2 and 6.6 g. The total yield per parent plant amounted to 8–22 kg.

The pH value, dry matter, acid, vitamin C, anthocyanin, sugar and mineral salt content of 13 elderberry clones were determined. Elderberries were found to have a high dry matter and anthocyanin content. Great differences were observed between the fruit components of the different clones.

The highest vitamin C content (31.25 mg %) was found in clone No. 479 and this clone was very rich in anthocyanin (270.0) and dry matter (26.9%), too. Clone No. 269 had the highest anthocyanin content ($E \times 250 = 362.5$).

Paper chromatography of the anthocyanins revealed the presence of 7 components. The spots most intense were found at R_f values 0.31 and 0.17.

The results show that the elderberry types suitable for the colouring of fruit products appear in Hungary in a large variety. The ban on the use of synthetic dyes draws attention to the collection and cultivation of elderberries, suitable to replace the dyes because of their valuable components. Our aim was to provide the raw material by seeking out the best individual plants.

Of the different elderberry types several clones have economic characteristics directly utilizable and these may form the basic material of cultivation. Therefore the collection and placement in the gene bank of elderberry clones was reasonable and necessary.

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DATA ON PLANT ENERGY RELATIONS MODIFYING THE MASS OF ORGANIC COMPOUNDS PRODUCED IN PLANTS

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Based on the biosynthesis paths of organic vegetable compounds formerly unavoidable losses are expressed in the mass (or energy) equivalents of unit simple carbohydrate (minimum equivalent [ME], productivity value [1/ME]).

Taking into account the energy content of the organic compounds biochemical energy efficiencies were calculated to these measuring numbers.

The calculated biochemical energy efficiency value for starch instead of the earlier 0.90 was found to be 1.00, that of general plant carbohydrate instead of 0.87, 0.98, that of fibre instead of 0.80, 0.95, that of oil instead of 0.34, 0.86, that of protein (from oxidized nitrogen and sulfur forms) instead of 0.40, 0.90 and of protein (from reduced nitrogen and sulfur patterns) instead of 0.57, 1.22.

The energy efficiency as calculated for the sterol and fatty acid composition of vegetable oils showed slight differences.

The values calculated for the amino acid sum of cereal grains, normal and endosperm mutants (opaque, floury) of corn and high oil and high protein variants, differed only slightly. The energy efficiency for the sum of the acid-soluble fraction of the opaque form was reduced.

It was established that the simultaneous increase of the product mass and the proportion of oil and protein is limited not only by the stoichiometric determinateness of organic compounds and the unavoidable losses of biosynthesis, but by plant energy factors, too.

Keywords: plant energy, bioeconomic minimum, organic compounds in plants

It is natural and therefore comprehensible effort of plant cultivation to achieve greater and greater product masses on a given unit of field. Mostly, the selection of the place of cultivation, the plant variety and the technique of cultivation is directed towards this aim.

From the aspect of utilization of the product the proportion in which the organic components are present (starch, cellulose, lignin, oil, protein, etc.) is not indifferent. This applies equally to both animal and plant products.

Many efforts are made to increase the oil or protein content of plants and thereby improve their nutritional value.

In Hungary a tendency of diminishing protein level is observed in various crops, particularly in cereals. However, for economic reasons the primary task is to increase the quantity of products (GONDA, 1979).

The production of energy, moreover in a predetermined form is considered the main task of agriculture (BALOGH, 1978).

GYŐRFFY (1976) studied, in relation to corn cultivation, how far the technical energy investment can be increased with the simultaneous increase of the energy of product mass. Intensive plant cultivation involves the progressive increase of investment and pertinent expenses.

Profitable cultivation is based on sensible energy economy. This is valid for the energy produced by a living organism, too (KURNIK, 1977).

Naturally, the production of organic plant components is a question of energy. The energy of the sun is stored in the chemical bonds and conformations of organic matter.

The contemplation of the thermodynamics of transformations leads to the conclusion that the mass and energy output of a process is always lower than the input. This applies to processes in living organisms, too. By increasing the intensity of a process beyond the limit its efficiency decreases (NETTER, 1969). Efficiency can be raised by highly developed technique or in living organisms by satisfying their biological requirements.

Photosynthesis is considered the central process of plant energy exchange and metabolism. The primary stable products are the reduction and energy equivalents, such as $NADPH_2$ (reduced nicotinamide adenine dinucleotide phosphate) and ATP (adenosine triphosphate), etc. In addition to maintaining the viable state they ensure growth and propagation. To consider the CO_2 and O_2 metabolism as a basis may be misleading (HOFFMANN, 1976).

The formation of carbon compounds provides a possibility for the increasing, continued and reversible storage of hydrogen of high energy value. Carbohydrates, oils and proteins play the role of intermediary hydrogen vehicles (HOFFMANN, 1977).

The energy stored in the course of photosynthesis covers the total requirements of all vital processes. The formation of protein is also in close relationship with photosynthesis (PÁL & BENEDEK–LÁZÁR, 1973).

In the processes of biosynthesis reactions are in stoichiometric correlation with one another (BUVET, 1977).

The energy content of the organic compounds formed in plants can be measured or approximately calculated. However, the volume of chemical energy stored accounted for e.g. in joule is not a satisfactory measure of the organic product, although there exists a correlation between the proportion of their organic substance content and the mass and energy content of the product.

Efforts to correlate great organic masses with their oil and/or protein content have not been successful. For example the mass of seeds obtained in plants serving as oil and protein sources is substantially lower than that of cereal crops.

The aim of this study was to reduce this contradiction by means of a specific evaluation method in showing the energy efficiency of the formation of organic plant materials. Thus it may be possible to reduce the specific utilization of plant products for nutrition and other purposes.

1. Materials and methods

Earlier on the basis of the biochemical synthesis of organic components of plants, a measuring number was introduced for maximum masses (production value, 1/ME). Such a value is for starch 0.90, for vegetable carbohydrate in general 0.87, for fibre (with 10% lignin content) 0.80, for vegetable oil 0.34 and for protein 0.40 (NEHÉZ, 1971a). The unavoidable loss of mass is represented by the relation 1–1/ME. The values are, naturally, only approximate.

It was shown that the mass of various organic plant components is limited for stoichiometric and biochemical reasons.

Values were given for nearly thousand organic compounds. To offset the inevitable losses calculated for particularly frequent biosynthetic paths it was suggested the use of the measuring number of minimum equivalent (ME). The stochiometric determination of the chemical elements of organic compounds was marked as an elementary equivalent (EE) in accordance with unit simple carbohydrate. The bioeconomic minimum equivalent (ME) contains also the elementary equivalent (EE) (NEHÉZ, 1971a, 1971b, 1974a, 1974b, 1975a). Plant production and energy problems related to the measuring numbers were also investigated (NEHÉZ, 1974b, 1975a, 1975b).

The above mentioned measuring numbers were later confirmed numerically (PENNING DE VRIES et al., 1974) based on a somewhat differing basic conception. The investigations of Penning de Vries supported the acknowledgement in Hungary and abroad of the results achieved by the author. The same subject was treated by the following authors: BHATIA and RABSON (1976); MITRA and co-workers (1979); MITRA and BHATIA (1979).

As the cause of low vegetal yields frequently non-biological difficulties, inadequacies of cultivation technique and management are considered. MÁNDY (1972) found that in influencing productivity it has to be taken into account that the productivity of viable organisms is in question.

In the cultivation of plants varieties are used. It would be highly desirable if a variety would combine all the advantageous properties. Demands on a plant variety are the toleration of disadvantageous environmental conditions, high productivity, good quality of the product and even the toleration of cultivation inadequacies.

Since the end of the 60's an effort was made in this Institute to increase the protein content, to modify the amino acid composition of corn and wheat

by selection in the laboratory and screening. The results were very modest (NEHÉZ, 1977d). It became apparent already at the beginning of this work that the simultaneous achievement of a great mass of crop and high protein content is very difficult.

Although the biological and within this the biochemical processes of the building up of organic compounds in plants are fairly well known, research work on the possibility of utilizing this knowledge is scanty (PENNING DE VRIES et al., 1974).

In evaluation of productivity beside the amount of the product the energy relations of the production of organic substances have to be taken into account, too (NEHÉZ, 1970, 1975b). In this work some new concepts were introduced.

The elementary equivalent (EE) is the mass, relative to a simple carbohydrate (unit: $-(CH_2O)-$), determined by the proportion of the chemical components in the organic compounds of plants. For instance in the case of oleic acid ($C_{18}H_{34}O_2$) 1/EE = 0.523, a simple carbohydrate ($C_{18}H_{36}O_{18}$) EE = = 1.000. The mass relations of the chemical elements of compound determine the EE value.

The bioeconomic minimum equivalent, in short the minimum equivalent (ME) means the maximal efficiency of biosynthetic paths related to a simple carbohydrate unit. For instance, oleic acid, 1/ME = 0.348, simple carbohydrate, ($C_{27}H_{54}O_{27}$) ME = 1.000. At the same time correlation 1–1/ME gives the minimum loss for the most frequent biosynthetic paths. Value 1/ME is called also biochemical mass efficiency.

The biochemical energy efficiency is the 1/ME value relative to the energy of the simple carbohydrate unit.

Taking into account the minimum equivalents reduces the negative correlation between the mass and the oil and protein content of the crop (NEHÉZ, 1971a). The advantages of the production of wheat varieties was proven which, while of a slightly lower productivity, have a high protein content (NEHÉZ, 1971b). Even the elementary equivalents show that plants of high oil and protein level cannot be expected to give great masses of product (NEHÉZ, 1974a). The utility of minimum equivalents was shown for the amino acid composition of plant proteins as obtained by analyzer in comparison with the energy content of amino acids (NEHÉZ, 1974b). The measuring numbers were applied to resolve partially the problems of corn cultivation and improvement. The aspirations relative to quality were aided by providing the theoretical basis. The sum of amino acids in corn proteins was evaluated with the measuring numbers (NEHÉZ, 1975a). To resolve certain problems of barley cultivation and improvement the introduction of minimum equivalents was proposed, this being a help in selecting barley varieties of high protein content (NEHÉZ, 1975b). The cultivation of corn varieties of higher oil and

protein content than earlier was suggested (NEHÉZ, 1976). To achieve better fodder for animals the amino acid content and the sum of amino acids was evaluated. The energy content of the amino acids was accounted for, too (NEHÉZ, 1977a). It was suggested the National Institute for Variety Selection to use the bioeconomic evaluation system in accepting new varieties and to achieve better quality (NEHÉZ, 1977b). The high productivity value of vegetable oils in relation to practical experiences, was shown (NEHÉZ, 1977c). On the basis of energy relations of the measuring numbers the cultivation of corn varieties of high oil and protein content was proposed (NEHÉZ, 1978a). The capacity of different nitrogen fertilizers to modify plant production was proven (NEHÉZ, 1978b). It was shown the disadvantage of the onesided selection for higher mass production. Minimum equivalents were calculated for the sum of fatty acid and sterol content in the oils and the sum of amino acids of various plants (NEHÉZ, 1979a). As regards productivity of a location it was established that productivity is better expressed by biochemical efficiency than the mass of product (NEHÉZ, 1979b). Enzyme energies were used to evaluate the energy relations of environmental factors and transportation processes at a preliminary study level (NEHÉZ, 1980a). With the aid of the measuring numbers suggested it is possible to achieve higher yields at lower energy input by producing nutrients of better quality. Enzyme energy relations were established for carbohydrates, fatty acids and amino acids (NEHÉZ, 1980c). In giving enzyme energies ($NADH_2$ and ATP) the simplifications established by PENNING DE VRIES and co-workers (1974) were accepted. Energy relations of transformations of nitrogen and sulfur and the concomitant changes of valency were given with enzyme energies (NEHÉZ, 1980c). Plant breeders are forced by economic reasons to apply onesided selection for mass only. To avoid unsuccessful aspirations it was suggested to use in improvement the measuring numbers (NEHÉZ, 1981a). Only slight results were achieved in corn improvement. The reduced biochemical energy efficiency in endosperm mutant corn varieties reflects their deficiency as mutants and this is apparent also in the reduced mass of their product. Laboratory selection has to be carried out in the knowledge of energy efficiencies (NEHÉZ, 1981b). Since there is not great difference in the biochemical energy efficiency of starch, carbohydrate in general, oil and protein the best way to achieve energy efficiency is the increase of product mass (NEHÉZ, 1981c). Energy efficiency indicates sufficiently why is not possible to achieve highest yield simultaneously with highest oil and protein content (calculated in mass). In quantitative attributes (e.g. oil or protein level) it is possible to modify the judgement on inheritence in the evaluation of dominancy or recessivity (NEHÉZ, 1981d).

At present the efficiency of the measuring numbers is given. Efficiency in mass was converted into energy taking into account the energy content of chemical compounds, groups of materials. The differences in state of energy

were considered between carbon and carbon dioxide, hydrogen and water, nitrogen and nitrate and ammonia (^{+V}N and ^{-III}N), sulfate and hydrogen sulfide (^{+VI}N and ^{-II}S).

Earlier values were complemented with biochemical energy efficiency values.

2. Results and discussion

Mass and energy efficiency of the biosynthesis of plant organic substances may differ.

Biochemical efficiencies in mass and energy of some carbohydrates, lignin models, fatty acids (Table 1) and some amino acids, showing their nitrogen and sulfur valencies ("oxidation number"), are tabulated (Table 2).

Table 1

Biochemical efficiency in mass and energy of the formation of some vegetable carbohydrates, lignin and fatty acids

Compounds	Mass, 1/ME	Energy, 1/ME
Glucose	1.000	1.000
Fructose	1.000	0.998
Saccharose	0.950	0.997
Starch	0.900	1.000
Cellulose	0.900	1.000
Lignin (Erdtmann poly-acid monomer)	0.543	1.000
Lauric acid	0.371	0.879
Myristic acid	0.362	0.885
Palmitic acid	0.356	0.892
Stearic acid	0.351	0.896
Arachidic acid	0.347	0.899
Behenic acid	0.344	0.903
Lignoceric acid	0.341	0.905
Lauroleic acid	0.367	0.853
Myristoleic acid	0.359	0.864
Palmitoleic acid	0.353	0.872
Oleic acid	0.348	0.877
Gadoleic acid	0.345	0.884
Erucic acid	0.342	0.889
Selacholeic acid	0.339	0.891
Linolic acid	0.346	0.861
Linolenic acid	0.343	0.843

Efficiencies expressed in mass may show great differences while those expressed in energy much smaller ones. The inevitable loss of lignin models and fatty acids in mass is great while in energy is small.

Efficiencies as calculated for amino acids differ substantially. In the case of oxidized nitrogen and sulfur patterns the unavoidable loss in mass is greater, than that in energy. Because of the high energy content of reduced nitrogen and sulfur patterns the energy efficiency of amino acids can be even positive in comparison to that of simple carbohydrates.

The mass and energy efficiencies as calculated for several important groups of organic compounds as averages of a large number of data are tabulated in Table 3. In the exact knowledge of the chemical compounds of organic plant substances efficiences can be calculated in detail.

Table 2

Biochemical efficiency of the formation of vegetable amino acids in mass and in energy from nitrate, sulfate (^{+V}N, ^{+VI}S), *ammonia and hydrogen sulfide* (^{-III}N, ^{-II}S)

Amino acids	Mass, 1/ME ^{+V}N, ^{+VI}S	Energy, 1/ME ^{+V}N, ^{+VI}S	Mass, 1/ME ^{-III}N, ^{-II}S	Energy, 1/ME ^{-III}N, ^{-II}S
Glycine	0.452	0.579	0.708	0.907
Cysteine	0.467	0.880	0.870	1.253
Cystine	0.465	0.831	0.870	1.201
Alanine	0.536	0.830	0.840	1.301
Serine	0.632	0.977	0.991	1.170
Aspartic acid	0.678	0.703	0.978	1.013
Asparagine	0.485	0.706	0.868	1.263
Threonine	0.607	0.845	0.875	1.218
Proline	0.404	0.729	0.511	0.922
Glutamic acid	0.514	0.628	0.650	0.796
Glutamine	0.403	0.645	0.603	0.966
Valine	0.457	0.865	0.597	1.130
Methionine	0.469	0.800	0.752	1.282
Histidine	0.356	0.694	0.608	1.185
Leucine	0.379	0.762	0.458	0.921
Isoleucine	0.458	0.921	0.580	1.166
Lysine	0.404	0.840	0.603	1.254
Arginine	0.340	0.704	0.640	1.361
Phenylalanine	0.439	0.886	0.522	1.054
Tyrosine	0.481	0.848	0.573	1.011
Tryptophan	0.377	0.793	0.485	1.020

Table 3

Biochemical efficiency of the formation of some organic plant compounds

Compounds	Mass, 1/ME	Energy, 1/ME
Starch	0.90	1.00
Vegetable carbohydrate	0.87	0.98
Vegetable fibre	0.80	0.95
Vegetable oil	0.34	0.86
Vegetable protein (from energy level: ^{+V}N, ^{+VI}S)	0.40	0.90
Vegetable protein (from energy level: ^{-III}N, ^{-II}S)	0.57	1.22

As it can be seen in the Table efficiencies given in energy are much more favourable than those given in mass while there is not much difference in the efficiency of different organic compounds.

The main reason for not being able to produce varieties of high oil and/or protein content and at the same time of great yield is to be found in this fact. Maximum yield in mass is incompatible with maximum oil or protein content. A limit is imposed by the law of indestructibility of matter.

Considered, however, from another aspect it can be said that plant productivity in mass can be increased jointly with a more advantageous proportion of the inherent organic substances (particularly with a higher oil and protein level).

The improvement of plants with respect of their oil and protein content has been successful. KURNIK (1977) succeeded in increasing the oil content of sunflower seed from 28–30% to 48–50%. He has shown that the product may be increased not only by the mass of seeds but by the oil content, too. He considers a further increase possible up to the biological limit. It was established already in 1968 that to increase the oil content by 3–4% an increase of the product mass by 10% is necessary. Fortunately the increase in oil content was accompanied by the reduction in fibre content and growing thin of the seed-coat.

Efficiencies were calculated for the fatty acid and sterol composition in the oil of different seeds. Data were taken from the manual of ALTMAN and DITTMER (1972). In addition to the minimum equivalents, calculated earlier (NEHÉZ, 1980c) biochemical efficiencies were also calculated and tabulated in Table 4. For the sterols the mean of the measuring numbers of sitosterol and cholesterol was used for calculation (1/ME in mass 0.269, in energy 0.737).

As can be seen from the data, there is not much difference in the efficiencies as obtained for different oils. The energy efficiency of rape, peanut and corn is relatively higher, while that of linseed lower.

Table 4

Fatty acid and sterol composition and biochemical efficiency in mass and energy of various seed oils

Fatty acids and sterols	Fatty acids and sterols (g per 100 g fatty acid)					
	Corn	Sunflower seed	Rape seed	Soybean	Linseed	Peanuts
Lauric acid				0.2		
Myristic acid	1.4			0.1		
Palmitic acid	10.2	5.6	1.0	9.8	6.3	8.3
Stearic acid	3.0	2.2		2.4	2.5	3.1
Arachidic acid		0.9		0.9	0.5	2.4
Behenic acid					0.2	3.1
Lignoceric acid						1.1
Lauroleic acid						
Myristoleic acid				0.1		
Palmitoleic acid	1.5			0.4		
Oleic acid	49.6	25.1	32.0	28.9	19.0	56.0
Erucic acid			50.0			
Selacholeic acid						
Linolic acid	34.3	66.2	15.0	50.7	24.1	26.0
Linolenic acid			1.0	6.5	47.4	
Sterols	0.79	0.40	0.43	0.27	0.40	0.22
Total	100.79	100.40	99.43	100.27	100.40	100.22
Sum	1.000	1.000	1.000	1.000	1.000	1.000
1/ME in mass	0.348	0.347	0.344	0.346	0.347	0.348
1/ME in energy	0.873	0.867	0.880	0.866	0.862	0.876

It is important to know the biochemical efficiency of the protein content of various products in general and the formation of their amino acids in particular.

In an earlier paper minimum equivalents were calculated based on FAO data of amino acid sums in wheat, rye, barley and corn (NEHÉZ, 1977a). The biochemical energy efficiencies calculated with the above data are presented in Table 5.

It is evident from the data that the efficiencies as calculated for various plants do not differ significantly. The biochemical energy efficiency is relatively high for corn and low for wheat.

In breeding cereals for higher quality the discovery of the modified protein and amino acid composition of endosperm mutants seemed to mark a turning-point. These mutants are used to improve protein, to increase the

Table 5

Amino acid composition and biochemical efficiency in mass and energy of cereal grains

Amino acids	Amino acid in grain (g per 100 g)			
	Wheat	Rye	Barley	Corn
Glycine	0.483	0.522	0.442	0.322
Cystine	0.336	0.227	0.258	0.181
Alanine	0.525	0.507	0.453	0.669
Serine	0.600	0.525	0.459	0.454
Aspartic acid	0.627	0.845	0.669	0.555
Threonine	0.387	0.399	0.380	0.328
Proline	1.344	1.185	1.307	0.917
Glutamic acid	3.762	2.865	2.760	1.809
Valine	0.577	0.518	0.609	0.447
Methionine	0.228	0.227	0.293	0.188
Histidine	0.301	0.274	0.252	0.293
Leucine	0.857	0.724	0.786	1.207
Isoleucine	0.420	0.418	0.387	0.344
Lysine	0.397	0.408	0.384	0.239
Arginine	0.604	0.499	0.545	0.356
Phenylalanine	0.493	0.520	0.590	0.461
Tyrosine	0.324	0.236	0.310	0.330
Tryptophan	0.194	0.115	0.177	0.067
Total	12.459	11.014	11.061	9.167
Sum $+^{V}_{N}, +^{VI}_{S}$	1.000	1.000	1.000	1.000
1/ME in mass	0.480	0.485	0.471	0.475
1/ME in energy $-^{III}_{N}, -^{II}_{S}$	0.725	0.731	0.736	0.745
1/ME in mass	0.665	0.676	0.660	0.662
1/ME in energy	1.001	1.013	1.013	1.028

mass of basic amino acids, mainly of lysine and thereby the nutritive value of corn and also of barley and sorghum (BÁLINT, 1977).

The corn mutants opaque and floury, and others are used for hybridization. Strains of high oil and protein content are used in combinations. Most of the mutants are, however, biochemically deficiency mutants. They inhibit the accumulation in the endosperm of the alcohol-soluble proteins (prolamins), highly characteristic of cereals, to the relative advantage of other proteins.

It was established that in the presence of opaque-2 gene the lysine, tryptophan, arginine contents substantially increased while the glutamic

acid, alanine, leucine, tyrosine and phenylalanine contents were reduced (TAMIR, 1980). ZUBER (1975) found that it is possible to select for high lysine content even in the absence of genes opaque-2 and floury-2.

When the productivity and protein content of corn hybrids were compared only a few hybrids were found to have slightly higher protein content with hardly reduced yield (NÉMETH & SZÉL, 1977). A negative relationship was found between the oil and protein content of opaque strains (KOVÁCS–SCHNEIDER et al., 1976).

Table 6

Amino acid composition and biochemical efficiency in mass and energy in the germ and endosperm of corn grain

	Germ		Endosperm	
	normal	opaque	normal	opaque
	Amino acid as percentage of total amino acid (%)			
Glycine	5.4	5.5	3.24	4.02
Cystine	1.0	0.9	1.79	2.35
Alanine	6.0	5.8	8.13	6.99
Serine	5.5	5.0	5.17	4.99
Aspartic acid	8.2	9.2	6.17	8.45
Threonine	3.9	3.7	3.48	3.91
Proline	4.8	5.3	9.67	9.36
Glutamic acid	13.1	13.9	21.30	19.13
Valine	5.3	4.4	4.68	4.98
Methionine	1.7	1.5	2.83	2.00
Histidine	2.9	2.9	2.82	3.55
Leucine	6.5	5.6	14.29	11.63
Isoleucine	3.1	2.5	3.82	3.91
Lysine	6.1	5.9	2.00	3.39
Arginine	9.1	9.2	3.76	5.10
Phenylalanine	4.1	3.6	5.29	4.96
Tyrosine	2.9	2.2	5.26	4.71
Ammonia	2.2	2.1	3.28	3.41
Total	91.8	89.2	106.98	106.84
Sum $+^{V}_{N}, +^{VI}_{S}$	1.000	1.000	1.000	1.000
1/ME in mass	0.477	0.479	0.474	0.478
1/ME in energy $-^{III}_{N}, -^{II}_{S}$	0.756	0.749	0.759	0.756
1/ME in mass	0.690	0.707	0.673	0.685
1/ME in energy	1.147	1.139	1.114	1.129

Table 7

Amino acid composition and biochemical efficiency in mass and energy in corn proteins

	Protein soluble in					
	acid		alcohol		alkali	
	normal	opaque	normal	opaque	normal	opaque
	Amino acid as percentage of total amino acid (%)					
Glycine	2.6	3.1	2.1	2.6	3.8	3.3
Alanine	5.4	4.8	10.2	8.8	4.7	4.5
Serine	3.9	3.7	6.5	5.6	3.7	3.7
Aspartic acid	4.8	7.4	6.0	5.8	6.9	6.8
Threonine	2.4	2.3	3.4	3.4	3.4	3.3
Proline	10.9	9.9	10.5	10.5	10.1	8.7
Glutamic acid	15.7	15.9	26.0	23.5	14.6	12.9
Valine	2.4	2.4	4.5	4.2	5.6	5.4
Methionine	1.4	0.8	2.5	1.6	1.1	1.4
Histidine	1.2	6.5	1.6	2.1	4.2	3.8
Leucine	10.1	7.4	20.3	18.8	8.6	8.1
Isoleucine	3.0	2.2	4.2	4.2	3.4	3.4
Lysine	1.8	5.9	0.3	1.0	3.6	3.7
Arginine	2.8	10.0	2.5	3.1	5.3	5.8
Phenylalanine	3.2	3.2	7.1	7.1	3.8	3.8
Tyrosine	3.4	2.8	5.4	4.8	3.1	2.9
Ammonia	3.1	2.8	2.5	4.5	2.2	1.9
Total	78.1	91.1	115.6	111.6	88.1	83.4
Sum $+^{V}_{N}, +^{VI}_{S}$	1.000	1.000	1.000	1.000	1.000	1.000
1/ME in mass	0.469	0.458	0.468	0.468	0.469	0.469
1/ME in energy $-^{III}_{N}, -^{II}_{S}$	0.757	0.747	0.757	0.791	0.755	0.758
1/ME in mass	0.668	0.675	0.659	0.662	0.671	0.673
1/ME in energy	1.125	1.011	1.069	1.124	1.112	1.115

Minimum equivalents were calculated for the amino acids of fractions of the germ and endosperm of normal and opaque variants soluble in acid, alcohol and alkali (NEHÉZ, 1975a; 1977a). Data on the composition of the amino acids were taken from the work of NELSON (1969). With the sum of these amino acids the biochemical efficiencies as well as the energy efficiencies are presented in Tables 6 and 7.

Only slight differences were found between the values obtained. Merely the reduction of the energy efficiency calculated for the sum of amino acids

of the acid-soluble fraction (albumins and globulins) of the opaque mutant was striking.

The minimum equivalent was calculated for the sum of amino acids of corn variety W 64 A, its opaque variants, its floury endosperm mutant, the high protein and high oil variants (NEHÉZ, 1975a; 1978c). Calculations were based on the data of NELSON (1969) and HARVEY (1970). The biochemical and energy efficiencies are tabulated in Table 8.

Table 8

Amino acid composition and biochemical efficiency in mass and energy in corn variety W 64 A and other variants

	normal	opaque	floury	high protein	high oil
	Amino acid as percentage of total amino acid (%)				
Glycine	3.0	4.7	3.5	2.8	3.4
Cystine	1.8	0.9	1.8	1.4	1.5
Alanine	10.1	7.2	8.0	9.9	6.4
Serine	5.6	4.8	4.8	5.5	4.8
Aspartic acid	7.0	10.8	8.1	6.0	6.1
Threonine	3.5	3.7	3.5	3.3	3.2
Proline	8.6	8.6	8.3	6.8	9.3
Glutamic acid	16.0	19.8	19.1	12.8	13.9
Valine	5.4	5.3	5.2	4.6	2.1
Methionine	2.0	1.8	3.2	1.1	1.2
Histidine	2.9	3.2	2.2	2.2	2.3
Leucine	18.8	11.6	13.3	7.8	15.2
Isoleucine	4.5	3.9	4.0	3.5	4.0
Lysine	1.6	3.7	3.3	2.0	3.2
Arginine	3.4	5.2	4.5	3.9	4.6
Phenylalanine	6.5	4.9	5.1	5.7	2.9
Tyrosine	5.3	3.9	4.8	4.1	3.5
Tryptophan				0.4	0.6
Ammonia	0.3	0.7	0.8		
Total	106.3	104.7	103.7	83.8	88.2
Sum $+^{V}_{N}, +^{VI}_{S}$	1.000	1.000	1.000	1.000	1.000
1/ME in mass	0.437	0.480	0.479	0.487	0.472
1/ME in energy $-^{III}_{N}, -^{II}_{S}$	0.757	0.746	0.752	0.759	0.745
1/ME in mass	0.661	0.677	0.674	0.686	0.660
1/ME in energy	1.044	1.054	1.061	1.060	1.034

According to the data the energy efficiency as calculated for ^{+V}N and ^{+VI}S is lower in the opaque variant than in the normal variant and in the high protein variant higher than in the high oil variant.

The summary values as obtained for carbohydrates, fatty acids and amino acids do not differ considerably. This shows that their formation is not solely dependent on plant energy. It is an individual characteristic of plants in which organic compounds and to what extent they accumulate the energy of the sun.

Table 9

Chemical composition, biochemical efficiency in mass and energy and the achievable medium yield of seeds

Seeds	Carbohydrate (%)	Oil (%)	Protein (%)	Ash (%)	Moisture (%)	Mass, 1/ME $+^{V}N$, $+^{VI}S$	Energy, 1/ME $+^{V}N$, $+^{VI}S$	Mass, 1/ME $-^{III}N$, $-^{II}S$	Energy, 1/ME $-^{III}N$, $-^{II}S$	Mass yield (ton ha^{-1})
Wheat	71.7	1.8	12.3	1.7	12.5	0.696	0.829	0.717	0.868	3.9
Rye	73.4	1.7	12.1	1.8	11.0	0.711	0.833	0.731	0.882	1.5
Barley	78.0	1.0	8.2	1.8	11.1	0.739	0.855	0.654	0.881	3.1
Corn	73.0	4.0	8.8	1.2	13.0	0.696	0.829	0.711	0.857	5.0
Sunflower seed	29.2	44.0	18.5	3.3	5.0	0.511	0.831	0.542	0.890	1.6
Rape seed	22.3	43.6	20.4	4.2	9.5	0.465	0.777	0.501	0.842	1.3
Soybean	34.8	18.1	34.9	4.7	7.5	0.551	0.811	0.610	0.922	1.5
Linseed	30.3	35.9	24.0	3.6	6.2	0.518	0.822	0.558	0.898	1.2
Peanut	24.3	42.8	26.2	2.7	4.0	0.489	0.842	0.533	0.926	1.5

On the other hand this proves that energy relationships in plants are of general validity. This is not surprising since there is a great likeness in their life processes and biochemical processes.

Mass and energy efficiences were calculated for the chemical composition of plant seeds (SPECTOR, 1956). The oil content of sunflower seed was modified from 27.8% to 44%. Table 9 includes data on the medium mass yields achievable in Hungary per hectare.

As it can be seen in the Table efficiency expressed in mass decreased with increasing protein and oil level while the efficiencies measured in energy do not differ significantly. The energy efficiency of oil plants and of soybeans is similar to that of cereals.

Plants rich in oil and protein are more efficient in the feeding of animals, that is plant energy can be used more economically.

BIRÓ (1966) attributes great importance to energy transformation in the production of animal body. In the animal organism the efficiency of trans-

formable plant and other energy differentiates according to energy and protein level. By the transformation indices the possibility of the production of higher grade meat, milk, egg, wool and fat is given.

The useful parts of product, the mass yield and chemical components are differentiated according to area and place of cultivation, plants and their varieties. Taking into account the mass yield and its chemical composition it is easier to select the variety giving the best crop and the most suitable cultivation method.

It seems that the energy equivalent of the maximum mass of organic compounds formed in biosynthesis is not a poor index of the production of organic matter. In the chemical bonds of oil and protein per mass units. the accumulation of plant energy is higher than e.g. in starch.

In plant cultivation we deal with varieties. The recognition of improved plant varieties depends on their maximal achievable product while in cultivation practice the actually achieved product mass is of decisive importance. Of the wheat varieties generally cultivated variety Tiszatáj has had the highest protein level for several years (PARÁDI et al., 1977). It is not favoured for cultivation, however, because of its reduced yield.

The energy bound in organic compounds is required in a definite form in agriculture (BALOGH, 1978).

It has been calculated that the protein requirement of the world-population could be provided by growing alfalfa on an area of 780 000 quadrat kilometres. After extracting the half of the protein content the about equal amount of residual protein fed to cows would produce further protein in the form of milk and beef (STAHMANN, 1968).

Alfalfa yields a high green mass of advantageous chemical composition. The minimum equivalent measuring numbers were first described in the proposition for the improvement of alfalfa cultivation (NEHÉZ, 1965).

It seems that preference is given the cultivation of cereals.

Because of the reduction of mass yield in several parts of the world the efforts to increase the oil, protein, certain fatty acids or amino acid content are treated with scepticism. It would be wrong to do this. In judging this problem the biochemical efficiency differences as measured in mass and energy have to be taken into account, too.

If plants are improved with the aim of giving maximum yield and varieties doing this are cultivated then the proportion of oil and/or protein shows a diminishing tendency. However, this can be helped by satisfying the requirements of the plants (selection of place of cultivation, agrotechnique adapted to variety, use of nitrogen fertilizer, etc.).

Efficiency can and should be improved not only by the increase of yield, but by the enhancement of certain chemical components (mainly oil and protein). By increasing the mass of products the amount of these components

increases, too. It is easier to achieve a higher protein yield by e.g. the cultivation of corn instead of peas or soybeans.

The most important is, however, the yield. Percentage does not show effectively the produced yield, in itself not even productivity. Since the energy efficiency of carbohydrates, oils, proteins is not very different the biochemical energy efficiency of their formation is most simply achieved by increasing the mass of yield.

*

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STUDIES ON THE EVALUATION OF THE IN VITRO BIOLOGICAL VALUE OF FOOD PROTEINS

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The authors present a novel chemical index which describes the correlation between the amino acid composition of the sample and the amino acid requirement of the consumer by a normal distribution function. The usefulness of this index is superior to that of previous ones and shows a closer correlation between the predicted biological value and the in vivo results and it can be used in optimization more effectively.

Considering the variance in chemical indices, originating from amino acid analysis data, it can be concluded that the error sensitivity of this new index is of the same order as that of the Mørup–Olesen index and is lower than that of the traditional chemical indices.

Among the applications of the new index here two computer-aided formula optimizations are presented: the utilization of a brewer's yeast based protein concentrate as fortification agent to increase the nutritive value of some bakery products; and the optimization of the formulae of breads containing plant protein of various sources.

Keywords: food proteins, biological value, chemical index, Mørup—Olesen index

Nowadays, there is a growing interest in the protein quality of foods. The causes are complex, here we are taking into consideration only some of them. The fact that the same protein shows different nutritional value for different consumers and the biological value varies also with the age of the consumer offers a new possibility of effective and economical management of proteins: sources of protein can be utilized in the form in which they represent the highest biological efficacy.

Another cause determining the significance of the problem lies in laws and regulations specifying the necessity of ample information. In some countries the biological value of marketed foods must be declared and the registration of new products is bound to declaration of protein quality.

Exact methods enabling the assessment of the biological value of proteins are essential in order to be able to propagate modern standards of nutrition, to increase the assortment of foods, to enhance the protein content of staple foods of low biological value or to satisfy the special requirements of certain strata of consumers (babies, sportsmen, etc.).

Analogous problems arise when the task is to maintain the biological value of food where the protein of animal origin is substituted by protein of plant origin.

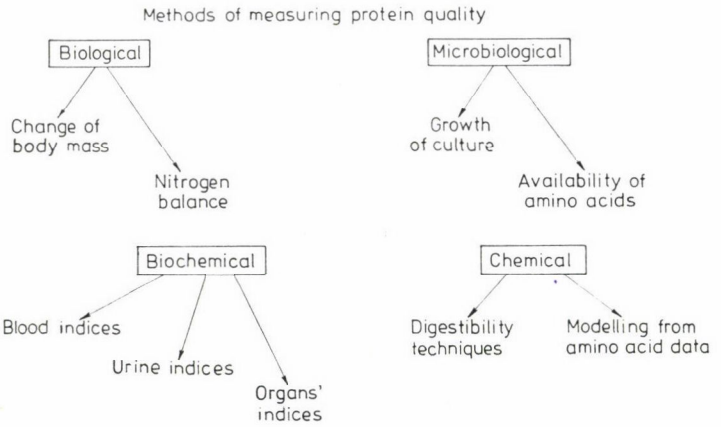

Fig. 1. Grouping of methods used for evaluating protein quality

Thus, the biological value of proteins depends on the relationship between essential amino acids present in the utilizable proportion of the proteins and the essential amino acid requirement of the consumer (KOFRÁNYI & JEKAT, 1967; KOFRÁNYI, 1972). This definition proves that the quality of a protein is determined in addition to its chemical composition by the biological and biochemical parameters (digestibility, utility, requirement). There are, therefore, two approaches to the determination of the nutritive value of proteins (Fig. 1).

At present, for several reasons, the in vitro methods are coming into prominence. It has been mentioned above that the same protein has different value for different living organisms. Thus, experiments carried out on animals have to be adapted to human requirements (e.g. PER value) and therefore must be considered as indirect methods. The correlation of these data to human requirement is, in most cases, not at all better than that of some in vitro methods (KOFRÁNYI, 1973; KÁRPÁTI et al., 1979). Finally, the cause of in vitro methods coming to the foreground is of economic nature. Determination of the quality of protein by biological methods requires much time and it is very expensive. In Table 1 the expenses of the two, most frequently used in vivo methods (BV, PER) are compared to the expenses of chemical methods. The data in Table 1 become really convincing when it is made clear that some of the chemical methods show a correlation of 0.85–0.95 to results of biological experiments (BODWELL, 1977).

In this study we cannot discuss in detail the different chemical methods. We give here the chemical indices obtained by calculation from data of amino acid composition and possibilities of their utilization.

The chemical index comparable more or less with the biological value (BV) is a function of the sample and the amino acid composition of a reference

protein. Thus, the chemical indices differ from one another in the number and quality of the essential amino acids to be considered in the reference protein and mainly in the type of function used (BÉKÉS & ZSIGMOND, 1980).

Here four index formulae, typical of the development of index formulation, are shown.

The MEAI index (modified amino acid index) is the hundredfold geometrical average of the quotients of the 8 amino acids to be considered and that of the egg selected for reference (OSER, 1951; MITCHELL, 1954).

$$\text{MEAI} = \sqrt[n]{\prod_{i=1}^{n} (a_i/a_{i,R'})} \tag{1}$$

The Korpáczy index corrects the weighted value of the MEAI index by the quantity of the non-essential amino acids, accounted for by an adequate function (KORPÁCZY et al., 1961).

$$\text{KORPI} = 75 \sqrt[n]{\prod_{i=1}^{n} (a_i/a_{i,R})} + 25 \left(1 - \frac{\sum_{j=1}^{10} b_j - \sum_{j=1}^{10} b_{j,R}}{\sum_{j=1}^{10} b_j} \right) \tag{2}$$

The FAO-WHO 72 index is understood to mean the numerical value of the sum of essential amino acids divided by the individual essential amino acids or the lowest quotient obtained by dividing the essential amino acids of the reference protein by the individual ones (FAO-WHO, 1972).

$$\text{FWI} = \left[\left(a_i \Big/ \sum_{i=1}^{8} a_i \right) \Big/ \left(a_{i,R} \Big/ \sum_{i=1}^{8} a_{i,R} \right) \right]_{\min} \tag{3}$$

It is valid for all three indices that they are suitable only for ranking proteins of the same sort and cannot be used as measuring numbers. They are

Table 1

Time and cost requirement of bioassays after BODWELL (1977)

Bioassay	Time requirement (days)	Cost per sample (US $)
Nitrogen-balance method (Biological Value, BV)	35—45	12 000—18 000
Protein Efficiency Ratio (PER)	28	100—300
Chemical indices	1.5— 2	50

not at all utilizable in relation to protein mixtures (KOFRÁNYI, 1973; BÉKÉS & ZSIGMOND, 1980). They do not describe the maximum type function of mixing proportion and biological value as observed in biological experiments.

Function formation of the Mørup–Olesen index differs theoretically from the previous ones (MØRUP & OLESEN, 1976; OLESEN & MØRUP, 1975). Here the compound fractions, analogous to the FAO-WHO index but using another reference pattern, as data of the amino acid composition, are compared with parameters obtained in biological experiments. The result is the weighting factors of individual essential amino acids. Thus, the Mørup–Olesen index, although a chemical index, an in vitro method, contains parameters originating from biological experiments. Due to this basic difference and the exact data obtained by Kofrányi in human biological assays (KOFRÁNYI, 1973; KOFRÁNYI & JEKAT, 1965, 1969; KOFRÁNYI et al., 1969, 1970) the Mørup–Olesen index is suitable to measure the biological value on a 0 to 141 scale.

$$q_i = (a/\sum_{i=1}^{8} a_i)/(a_{i,R}/\sum_{i=1}^{8} a_{i,R}) \tag{4a}$$

where

$$\mathrm{MOI} = 10^{\alpha_0} \prod_{i=1}^{8} q_i^{\alpha_i} \tag{4b}$$

It can be used for protein mixtures because it describes the anomaly, namely that the biological value of a protein mixture is not the sum of the biological values of the components weighted by weight fractions.

The correlation coefficient of the Mørup–Olesen index and the biological assays is above 0.8.

At the Department of Biochemistry and Food Technology, Technical University, Budapest, the in vitro determination of the biological value of food and feed proteins has been the subject of investigations for five years. In this paper the results obtained in developing and utilizing chemical indices based on amino acid data are summarized and a new index, usable more efficiently than the earlier ones, is presented. We present further the variances of chemical indices as functions of the standard errors of amino acid analyses as well as the computer program package by which the BV data obtained by in vitro methods can be calculated and used as objective functions in food optimization and complementation calculations.

1. Methods

1.1. Calculation of chemical indices

In calculating the four chemical indices by eqs (1)–(4) data contained in Table 2 were used.

1.2. Gaussian index and determination of its weighting factors

In calculating both the FAO-WHO and the Mørup–Olesen indices the following algorithmic constraint must be satisfied: if the vaue of a compound fraction representing the relative ratio of individual essential amino acids, is above 1, its reciprocal is used.

As it can be seen in Fig. 2a, the amount of a given amino acid is most efficient in increasing the BV if its ratio within the total amount of essential amino acids corresponds to its ratio in the reference protein or, in other words, to the consumer's requirement (Fig. 2a).

The new function was obtained by replacing this sophisticated maximum function by a function quite general in natural processes, by the Gaussian function. The maximum of the biological value as determined from the ratio of the essential amino acids in the sample and required by the consumer, equals to 1, that is, the requirement is just satisfied and the standard deviation from this is taken into account as a normal distribution. If the sample does not contain the desired amino acid at all the BV is equal to zero. This shows that the half-width (3σ) of the bell curve is equal to the amino acid requirement (Fig. 2b).

Table 2

Parameters accounted for in chemical indices

Amino acids	Chemical indices			
	MEAI and Korpáczy	FAO-WHO 72	Mørup-Olesen	α_i
THR	47	40	99	2.40
MET + CYS	57	35	89	0.77
VAL	66	50	140	0.0
ILE	54	40	110	0.0
LEU	86	70	179	0.0
PHE + TYR	93	60	212	0.60
LYS	70	55	141	0.41
TRP	17	10	30	0.21
Total essential	490	360	1000	
Total non-essential	—	510	—	—

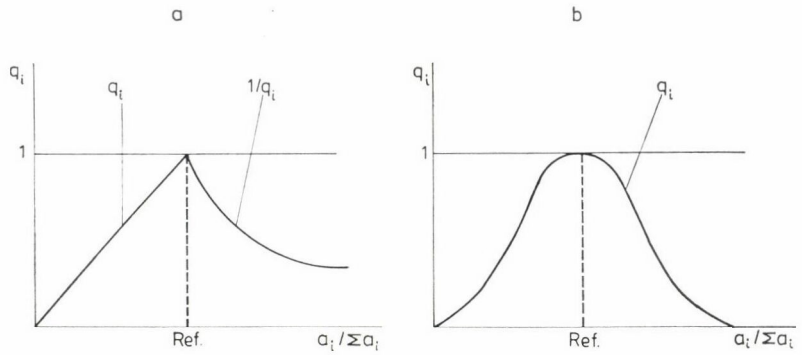

Fig. 2. Representation of the Mørup–Olesen (a) versus Gaussian (b) type of q_i functions in modelling protein quality

Thus, the q_i function of the i-th amino acid is as follows:

$$q_i = \exp\left[(-0.5)\left[\frac{(a_i/\sum_{i=1}^{n} a_i - a_{i,R}/\sum_{i=1}^{n} a_{i,R})}{\frac{a_{i,R}/\sum_{i=1}^{n} a_{i,R}}{3}}\right]^2\right] \tag{5}$$

1.3. Mathematical considerations

Using Kofrányi's experimental biological data and equation (5) for transforming the amino acid composition and look for β_i weighting factors where the sum of $\sum_{j=1}^{27} (BV_j - PV_j)^2$ squared differences is minimal:

$$BV \approx PV = \sum_{i=1}^{n} q_i^{\beta_i} \tag{6}$$

The problem as formulated above — in contrast with the text describing the Mørup–Olesen index (MØRUP & OLESEN, 1976) — cannot be considered as a regression on the logarithm of (6) because it has to be fulfilled for every β_i

$$\beta_i \geq 0 \tag{7}$$

as it is laid down in the paper cited.

Thus, the mathematical problem, satisfying constraint (7) is the minimization of the above sum of quadratic differences in accordance with (6) non-linear correlation where, due to (7) the vector β_i is looked for as follows:

$$\beta_i = a + b \sin^2 \gamma_i \tag{8}$$

where

$$a = 0 \text{ and } b = 1$$

The non-linear optimization was carried out by the OPT program of the computer program package, applying the Hooke–Jeeves algorithm (BOJARINOV & KAFAROV, 1973).

1.4. Combining of amino acids

Using the mathematics presented in para. 1.3. weighting factors β_i were determined for different combinations of essential amino acids. Arginine was taken into account as an essential amino acid and the sulfur containing and aromatic amino acids were considered individually as well as summarized. The eight patterns thus conceived are summarized in Table 3.

Table 3

Essential amino acid combinations

Amino acid combination	ILE	VAL	LEU	LYS	TRP	THR	MET + CYS	TYR + PHE	CYS	MET	TYR	PHE	ARG
1	+	+	+	+	+	+	+	+					
2	+	+	+	+	+	+	+	+					+
3	+	+	+	+	+	+		+	+	+			
4	+	+	+	+	+	+		+	+	+			+
5	+	+	+	+	+	+	+				+	+	
6	+	+	+	+	+	+	+				+	+	+
7	+	+	+	+	+	+			+	+	+	+	
8	+	+	+	+	+	+			+	+	+	+	+

These eight different amino acid compositions were calculated (hereafter marked [a]) as well as by the bell curve technique of the authors (hereafter [b]), using the set of 27 BV-amino acid composition data as utilized in the development of the Mørup–Olesen index.

1.5. Derivation of the amino acid-variance dependent variances of the chemical indices

The five chemical indices investigated (MEAI index, Korpáczy index, FAO-WHO index, Mørup–Olesen index and the author's Gaussian-type index) naturally carry the errors inherent in the analytical data used in the calculations. The variances of the different types of indices as functions of amino acid data variances were derived by using the law of error propagation.

$$\text{var}\,[y] = \sum_{i=1}^{k} \left(\frac{\partial y}{\partial x_i}\right)^2 \text{var}\,[x_i] \tag{9}$$

where y stands for the different chemical indices,

x_i stands for the amino acid data used in the calculations.

1.6. Application of the chemical indices as objective functions in optimizations and complementation

The composition of food and feed mixture formulae is a typical optimization task: the optimum proportions of mixing are sought for by the use of an economic or nutritional objective function. The so-called "least cost" optimization has been used in the mixed feed industry for many years and there is a large number of publications pertinent to this subject (BÉKÉS et al., 1979; HIDVÉGI et al., 1983; ÖRSI et al., 1980; PÓDER et al., 1980).

To maximize 3 nutritional characteristics a suitable biological value function used as objective function, is needed. The traditional chemical indices are not suitable for this purpose or their use is difficult and the results obtained are only approximate, although several essays were made to apply them (CAVINS et al., 1972; WADSWORTH & HAYES, 1979; INGLETT et al., 1969). The main difficulty of these attempts is that the FAO-WHO index does not describe adequately the quality of the protein in case of mixtures and, therefore, it can be applied only by establishing a separate inequality for each of the essential amino acids. However, the proportion of the amino acids is not defined even then. A similar formalization is used by GELENCSÉR and coworkers (1981a, b, c) in linear optimization as the objective function of the Mørup–Olesen index.

Indices of the Mørup–Olesen type, thus the Gaussian index as well, are suitable to describe anomalous biological values of protein mixtures: BV-weight fraction correlations are maximum functions. Thus, substituting a_i in equations (4) or (5) for the following term

$$a_i = \sum_{j=1}^{m} a_j A_{i,j} \tag{10}$$

the resulting $BV = f(x_j)$ function can be used to maximize biological value by a non-linear optimization.

A sharp distinction is drawn between *complementing* and *formula optimization*. While in the case of complementing the proportion of a product specified by a formula and a complementing material is sought for, in formula optimization the weight fractions of all the components of the mixture change.

The Mørup–Olesen type indices (indices containing weighting factors and established in biological experiments) were used to solve a variety of fortification and formula optimization problems. Here we present a fortification and an optimization problem.

In the work carried out jointly with the Central Food Research Institute (HALÁSZ et al., 1981) the fortification characteristics of a brewer's yeast based protein concentrate were studied in bakery products. Considering the

norms of the baking industry we looked for the amount of brewer's yeast which would raise the biological value of the product to a maximum.

The most typical formula optimization task is the optimization of a mixed feed formula.

To resolve this problem the evaluation parameters of chemical indices specific to species, age and breeding conditions were stored in the data bank of the program package (BÉKÉS et al., 1981; BÉKÉS et al., 1982, 1984; HIDVÉGI et al., 1984; KEMÉNY et al., 1984; LÁSZTITY et al., 1984).

Here we present a food industrial application. Based on the average yearly consumption data of two different strata of the Egyptian population (undernourished and nourished on an average level) as given by HUSSEIN (1978), bread formulae containing in addition to wheat some other grain (corn, millet, rice, soybeans, fenugreek) were optimized. The formulae were optimal in relation to the total food consumption during a year.

In these optimization the x_i weight fractions of the bread formulae were sought for and the PV values, maximized from the amino acid composition of the yearly food consumption is as follows:

$$C_j = g\ B_j + \sum_{i=1}^{6} (1-g)\ x_i\ D_{i,j} \tag{11}$$

where

C_j the amino acid level of the total food,
g ratio of the non-bread-like food consumed per year,
B_j brutto amino acid content of the non-bread foods,
x_i weight fractions in the bread formula to be determined,
$D_{i,j}$ amino acid composition of the basic materials of bread,
j considered coordinate of the essential amino acids,
i coordinate of the basic materials considered in bread production.

The annual consumption structure of the two strata of Egyptian population: undernourished (UN) and nourished at an average level (AV) is shown in Table 4.

The essential amino acid composition in g, based on data in Table 4, is given in Table 5.

The bread formula was optimized by way of maximizing the following four objective functions:

A. Gaussian index,

B. Gaussian index per cost,

C. Gaussian index corrected by the specific factor (F) of the average yearly produce in Egypt,

D. Combination of B and C, i.e. GAI index per cost $\times$ F.

Table 4

The annual food consumption of two strata of the Egyptian population: nourished at an average level (AV) and undernourished (UN) after HUSSEIN (1978)[a]

Food	Consumption (kg per year per capita)	
	AV	UN
Beef	6	1
Lamb	7	3
Fish	2	2
Milk	46	15
Cabbage	27	20
Lettuce	30	24
Beets	10	10
Snap beans	18	10
Green peas	5	4
Beans	10	10
Cereals (as bread)	240	260

[a] Fruits are not indicated

Table 5

Amino acid composition in the food of the two strata of Egyptian population (Annual consumptions, cereals not included)

Amino acid	Consumption (g amino acid per kg food)	
	AV	UN
THR	1.50	0.76
CYS	0.56	0.51
VAL	1.38	0.93
MET	0.62	0.49
ILE	1.70	1.75
LEU	2.90	2.55
TYR	1.60	1.19
PHE	1.61	1.54
LYS	2.61	2.35
HIS	0.94	0.85
TRP	0.39	0.34
ARG	1.51	1.57

1.7. Biolert computer program package

The so-called Biolert computer program package was written in ALGOL-1204 programming language for the Odra 1204 computer, made in Poland. The task of the package is to perform the calculation functions related to the nutritional value of food and feed proteins:

— off-line evaluation of the signs of an automated amino acid analyzer,
— calculation of chemical indices on the basis of amino acid data,
— formula complementing and optimization,
— management of the data bank of food and feed science,
— statistical and biometrical calculations.

The input and output system of the program package is extremely flexible, it is suitable for the processing of the most differing data series and the preparation of tables and figures. A detailed description of the original version of the program was given by WÖLLER and co-workers in 1977. The new version was given by BÉKÉS and co-workers in 1982.

2. Results

2.1. Determination of the beta-weighting factors of new chemical indices

The beta-weighting factors defined on the basis of equations (4) and (5) in case of essential amino acid selection as described under para. 1.4., are summarized in Table 6. The last column of the Table contains the correlation coefficients of the biological values of the 27 points as measured and calculated.

2.2. Deduction of the variance correlations of chemical indices

The variance of the chemical indices studied as functions of the variances of the amino acid data are as follows:

$$\text{var } [\text{MEAI}] = \left(\frac{\text{MEAI}}{9}\right)^2 \sum_{i=1}^{9} \left(\frac{\text{var}\,[a_i]}{a_i}\right) \tag{12}$$

$$\text{var } [\text{KORPI}] = \left(\frac{75}{8}\sqrt[8]{\prod_{i=1}^{8} (a_i/a_{i,R})}\right)^2 \sum_{i=1}^{8} \left(\frac{\text{var}\,[a_i]}{a_i}\right) + \left(\frac{25}{\sum_{j=1}^{10} b_{j,R}}\right)^2 \sum_{j=1}^{10} \text{var } (b_j) \tag{13}$$

Table 6

Weighting factors and correlations

Type of index	β_0	βa_i					
		ILE	VAL	LEU	LYS	TRP	THR
1/a	2.14	0	0	0	0.41	0.21	2.40
1/b	2.16	0	0	0	0.28	0.19	3.32
2/a	2.16	0	0	0	0.61	0.23	1.70
2/b	2.16	0	0.10	0.12	0.21	0.71	6.14
3/a	2.16	0	0	0	0.15	0.29	2.81
3/b	2.06	0	0	0	0.10	0.20	4.29
4/a	2.16	0	0	0	0.63	0.26	1.20
4/b	2.11	0	0	0	0.43	0.06	2.71
5/a	2.16	0	0.66	0.72	0.34	0.10	2.04
5/b	2.01	0	0	0	0.27	0.09	2.75
6/a	2.14	0	1.29	0.62	0.39	0.03	2.25
6/b	2.06	0	1.07	0.59	0.18	0.08	4.14
7/a	2.16	0	0.31	0.21	0.21	0.15	2.63
7/b	2.06	0	0	0	0.09	0.09	5.09
8/a	2.16	0	0.24	0.45	0.12	0.12	2.82
8/b	2.16	0	0	0	0.48	0.07	4.23

a: calculated amino acid compositions
b: amino acid composition determined by bell curve technique of the authors

$$\operatorname{var}[\mathrm{FWI}] = \left[\left(\frac{\sum_{i=1}^{n} a_i - a_i^*}{\sum_{i=1}^{n} a_i}\right) \Big/ \left(\frac{a_{i,R}^*}{\sum_{i=1}^{n} a_{i,R}}\right)\right]^2 \operatorname{var} a_i^* + \left(\frac{\sum_{i=1}^{n} a_{i,R}}{\left(\sum_{i=1}^{n} a_{i,R}\right)^2}\right)^2 \sum_{\substack{i=1 \\ i \neq *}}^{n-1} \left(\frac{a_i}{a_{i,R}}\right)^2 \operatorname{var} a_i \qquad (14)$$

$$\operatorname{var}[\mathrm{MOI}] = (\mathrm{MOI})^2 \left[\sum_{i=1}^{9} \left(\frac{\alpha_i}{a_i} \operatorname{var}[a_i]\right) + \sum_{i=1}^{9} \alpha_i \left(\frac{\sum_{i=1}^{9} a_i}{\operatorname{var} \sum_{i=1}^{n} a_i}\right)\right] \qquad (15)$$

$$\operatorname{var}[\mathrm{GAI}] = \left(\frac{9}{\sum_{i=1}^{n} a}\right)^2 \sum_{i=1}^{n} \left[\alpha_i \left(a_i - \frac{a_{i,R} \sum_{i=1}^{n} a_i}{\sum_{i=1}^{n} a_{i,R}}\right)\left(a_i - \sum_{i=1}^{n} a_i\right)\right].$$

of the different chemical indices

MET + CYS	TYR + PHE	CYS	MET	TYR	PHE	ARG	r
0.60	0.77	—	—	—	—	—	0.920[c]
0.67	0.72	—	—	—	—	—	0.986
0.46	0.81	—	—	—	—	0	0.946
0.61	0.72	—	—	—	—	0	0.953
—	0.73	0.07	0.38	—	—	—	0.943
—	0.36	0	0.14	—	—	—	0.959
—	0.81	0	0.34	—	—	0	0.931
—	0.57	0	0.21	—	—	0	0.922
1.19	—	—	—	0	1.06	—	0.892
0.11	—	—	—	0.14	0.48	—	0.803
0.09	—	—	—	0	1.08	0	0.882
0.07	—	—	—	0	0.87	0	0.825
—	—	0	0.42	0	0.69	—	0.939
—	—	0	0.17	0.18	0.71	—	0.913
—	—	0	0.38	0.14	0.64	0	0.904
—	—	0	0.39	0.11	0.51	0	0.910

[c] The original Mørup–Olesen index.

$$\cdot \left(\frac{a_{i,R} \sum_{i=1}^{n} a_i}{\sum_{i=1}^{n} a_{i,R}} \right)^{-2} + \sum_{\substack{j=1 \\ j \neq i}}^{n} \alpha_j a_j \left(\frac{a_{J,R}}{\sum_{j=1}^{n} a_{j,R}} \right)^{-2} \Bigg\} \operatorname{var}[a_i] \qquad (16)$$

After incorporating the calculating algorithms of equations (12) to (16) into the Biolert program model series of numbers (amino acid data of systematically changing standard error) and data from the literature (FAO NUTRITIONAL STUDIES, 1970; SOUCI et al., 1974; DE VUYST, 1968) were run. Results are shown in Fig. 3 and Table 7.

2.3. Applications of the Mørup–Olesen-type indices

2.3.1. Determination of the biological value of mixed proteins. Hereafter the original Mørup–Olesen index as marked 1a and 1b in Table 4 and the Gaussian index, based on the same selection of amino acids has been used. The biological value of mixed proteins as affected by the two different calculations of q_i are shown in Fig. 4.

In the Figure the biological value and two kinds of protein efficiencies are shown as functions of the three mixtures of different proportions.

Table 7

The mean value and standard deviation of in vitro protein quality of some foods and feedstuffs

Item	Number of analyses	MEAI index	Korpáczy index	FAO-WHO 72 index	Mørup-Olesen index	Gaussian index	Limiting amino acid
Perennial ryegrass	4	71.9±27.7	72.6±85.8	81.9± 5.4	97.9±25.5	103.0±18.7	MET + CYS
Barley	4	59.9±16.0	56.9±40.8	65.9± 1.1	78.4± 9.9	100.9±22.7	LYS
Barley germs	11	63.9±12.8	63.2±34.4	89.8± 1.1	105.5±10.0	128.0±32.7	LEU
Broken barley grains	5	57.7±28.2	52.9±71.9	45.9± 3.5	65.7±21.4	90.4±17.9	TRP
Straw from barley	4	75.8±24.5	76.5±72.0	81.8± 1.0	96.3±16.5	78.7±30.3	LYS
Field peas	4	59.2±13.2	57.4±39.9	40.2± 3.4	70.7± 6.8	85.7±14.2	TRP
Potato	8	57.9±28.9	56.0±80.5	68.0± 3.5	79.6±16.9	74.1±40.2	ILE
Wheat	4	56.1±22.2	55.2±56.4	37.7± 2.2	55.8± 7.8	15.7± 9.5	LYS
Wheat germs	4	61.5±18.1	60.3±38.1	46.8± 2.7	83.2± 6.8	106.5±23.9	TRP
Flour middlings	7	62.1±20.0	57.9±53.7	77.9± 2.8	117.7±23.4	133.0±77.1	LYS
Wheat flour	3	52.9±30.2	49.7±59.3	60.4± 8.2	54.2± 9.8	64.9±22.7	LYS
Chaff from wheat	4	78.4±43.9	73.8±131.2	86.5± 6.9	68.8±20.5	49.1±18.9	TYR+PHE
Straw from wheat	6	70.1±37.8	68.2±112.4	75.2± 8.4	38.1±10.7	25.1±10.8	TYR+PHE
Fodder beet top with leaves	5	67.9±21.1	62.9±60.9	73.1± 4.1	54.7± 7.9	33.6±19.7	ILE
Beet pulp	8	74.3±28.4	68.8±79.4	71.8±12.8	92.1±11.5	118.2±33.9	TRP
Silage of beet pulp	8	75.2±28.6	72.3±85.2	74.4±15.9	92.4±14.5	117.1±40.7	TRP
Dried beet pulp	5	67.9±34.2	61.5±90.1	81.5 ± 4.5	92.8±29.6	115.1±11.9	LEU
Lupine	4	62.9±26.5	63.3±89.7	42.4± 3.9	40.8± 6.6	28.9± 7.8	MET+CYS
Skim milk	10	66.0±22.8	66.7±71.9	38.9±12.3	79.1±13.4	109.3±28.6	TRP
Herring meal	15	72.9±16.5	71.5±46.2	81.8± 5.2	97.4±11.0	118.5±29.3	MET+CYS
Meat meal	28	49.8±10.1	50.8±29.9	56.2± 4.2	103.0± 9.5	126.8±32.5	TRP
Marrowstem kale	4	62.8±27.6	66.6±89.6	46.9± 5.3	23.1± 6.9	29.1± 4.0	MET+CYS
Coconut meal	13	57.0±16.1	55.5±46.4	64.2± 2.2	84.2± 9.4	99.4±30.2	LYS
Maize	4	59.1±20.1	54.9±40.9	41.4± 2.7	87.0± 9.2	92.9±23.0	LYS
Maize germs	3	58.9±25.9	52.9±69.2	33.1± 1.8	75.2±12.4	99.2±33.9	TRP
Maize gluten meal	7	57.4±25.9	54.6±77.1	31.8± 3.0	28.5± 5.5	27.8±10.7	LYS
Horse been	4	63.4±18.5	59.8±37.9	57.1± 2.4	66.8± 8.9	77.1±21.3	MET+CYS
Corn spurrey	4	68.6±28.8	71.9±81.0	67.5± 4.9	45.8± 5.4	33.1± 8.9	MET+CYS
Manioc	5	65.1±35.9	59.7±93.4	83.8± 3.7	66.9±22.9	53.3±54.9	LEU
Siletta	3	68.0±39.3	62.1±101.9	66.1± 6.1	60.6±14.2	63.9±39.3	MET+CYS
Brewer's yeast (pressed)	3	68.0±37.1	61.5±83.9	44.4± 0.0	58.9±22.2	50.0±13.1	TRP
Meadow grass	7	75.9±26.8	74.4±74.7	85.5± 6.8	81.7±12.7	93.9±35.9	LYS

Rye	4	60.7±12.3	57.5±36.3	72.8± 2.2	85.5± 5.3	109.7±16.1	LYS
Serradella	4	68.1±27.5	68.4±84.4	54.4± 3.7	48.9± 8.7	40.1±21.1	MET+CYS
Sesame cake	5	62.4±28.5	58.4±68.9	50.9± 2.2	45.8± 8.1	42.5± 5.5	LYS
Soybean meal	10	68.5±19.1	64.2±52.1	80.1± 3.4	94.0±13.3	121.9±32.6	THR
Vetch grass	3	77.9±30.3	73.9±94.4	70.4± 3.2	93.1±18.4	113.2±48.6	MET+CYS
Fodder beet	6	43.9±40.4	40.5±104.3	37.0±10.3	29.9±12.5	41.6±11.8	TRP
Turnip	4	63.4±24.9	68.3±84.7	63.2± 9.9	29.5± 5.3	7.8± 3.0	MET+CYS
Turnips' green leaves	4	68.3±29.8	67.9±88.4	60.8± 4.8	44.1±17.2	23.4± 4.2	MET+CYS
Whole milk powder (roller)	4	58.3±29.7	58.9±87.4	20.7± 2.4	68.9±14.9	101.2±41.1	TRP
Dried skim milk (roller)	5	61.7±29.0	60.9±85.3	39.7± 5.8	45.4±14.2	55.9±18.8	TRP
Dried whey	3	73.5±46.3	67.9±132.3	52.1±12.7	42.3± 9.3	55.2±17.3	TRP
Sorghum	7	60.7±34.9	56.9±109.9	37.5± 3.3	48.3±11.1	52.2±18.6	LYS
Scraps of pork fat	3	52.7±52.9	53.7±155.0	71.5± 6.8	59.1±23.8	54.8±64.6	THR
Hen eggs	8	74.9±23.5	73.4±68.1	68.1±13.1	89.9± 9.8	89.1± 8.1	TRP
Large spelt wheats	4	60.3±24.8	57.4±72.8	45.5± 2.9	53.9±13.9	32.9±11.5	LYS
Blood meal	12	65.3±17.3	65.5±56.9	91.4± 2.3	110.3±14.1	132.9±41.5	VAL
Oats	4	67.9±29.8	64.8±86.4	55.8± 1.2	65.9±10.9	54.4± 1.8	LYS
Chaff from oats	4	73.9±27.9	73.9±93.8	73.1± 1.7	89.9±11.1	98.3±19.4	LYS
Straw from oats	4	68.4±30.3	67.2±80.9	73.9± 2.7	74.1±12.7	92.9±39.8	LYS

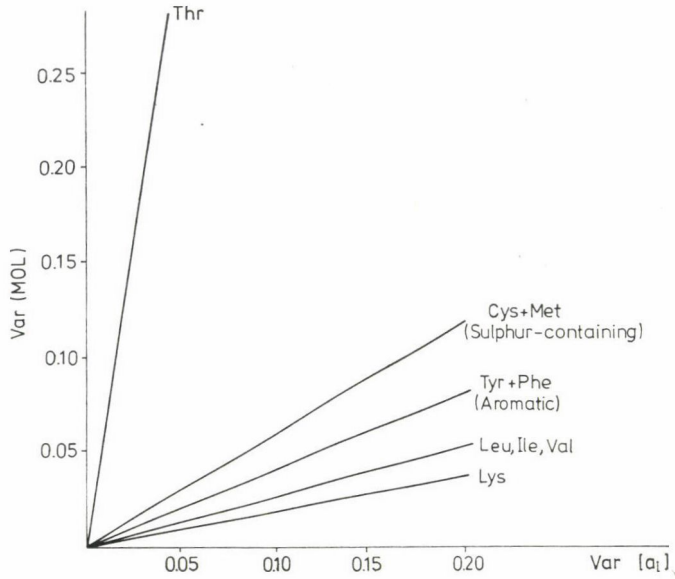

Fig. 3. Dependence of the variance of the Mørup–Olesen index on the variances of the individual essential amino acids

Table 8

Fortification of bakery products with brewer's-yeast-based protein concentrate

Products	PV-value	PV-value of fortified products	Complementing agent content (%)
Fine white bread	49.7	121.2	9.6
White bread	75.3	111.4	6.4
Buffet bread	80.6	107.8	6.3
Wheat loaf	72.7	114.7	7.1
Fine rye bread	100.9	123.9	1.7
Soroksári bread	93.2	128.9	2.3
Watery dough	61.8	118.1	9.7
Milky dough	90.9	122.3	8.4
Dough with butter	92.4	121.8	8.3
Egg-fortified dough	95.9	119.9	8.0
Short pastry	92.1	123.1	8.1
Dough with butter	96.5	121.2	8.2
Potato containing dough	59.1	126.4	8.1

Compositions of the products are prescribed by the Official Norms of Bakery Products in 1968.

The amino acid data are results of HALÁSZ and co-workers (1981)

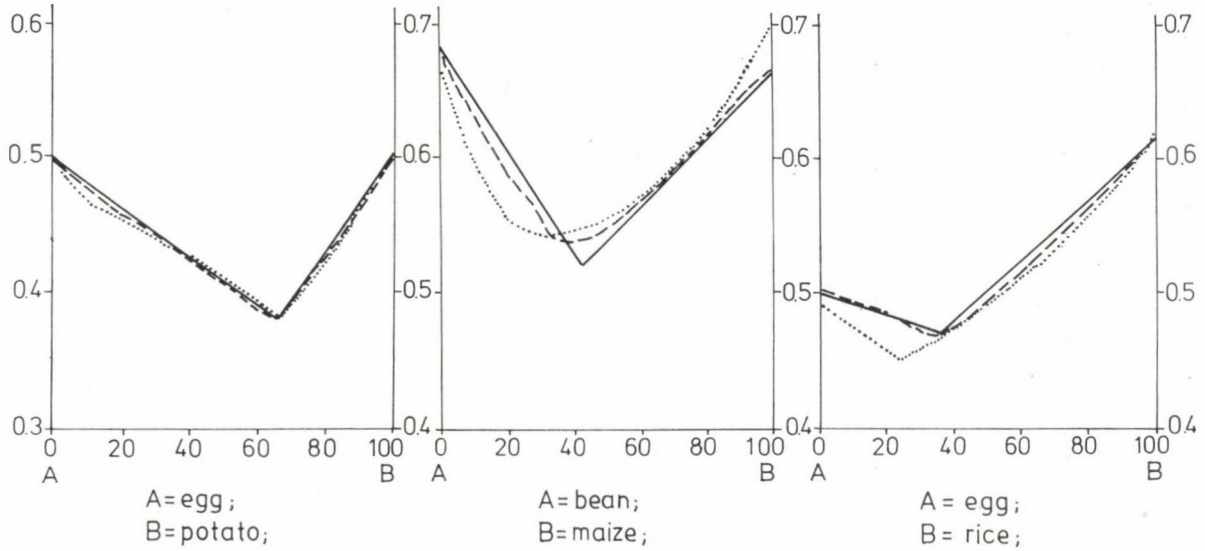

Fig. 4. The changing protein quality of blends. BV of protein mixtures as a functionof the ratio of components
——— BV: after KOFRÁNYI and JEKAT (1967, 1969)
. . . . . PV_{MOI}: after MØRUP and OLESEN (1976)
— — — PV_{GAI}: calculated by equations (5), (6) and (10), respectively, based on data in Table 6
X-axis: protein (%)
Y-axis: protein (N×6.25) requirement per kg of body mass

2.3.2. Complementing bakery products with protein concentrates gained from baker's yeast. The protein efficiency values (PV) of various bakery products as calculated from the formula norms, the brewer's yeast requirement as established by complementing, and the PV-s of the complemented products are listed in Table 8.

2.3.3. Bread formulae optimized on the basis of the Egyptian structure of nutrition. Table 9 shows the bread formulae optimized on the basis of essential amino acid data calculated by equation (11).

Table 9

Optimized bread formulae for the Egyptian consumption structure

Type of the objective function	Recipes						PV of the total food	Relative cost of the bread
	wheat (%)	maize (%)	millets (%)	rice (%)	fenugreek (%)	soy (%)		
AV population								
A	81	2	0	6	0	11	131.45	1.000
B	0	95.4	3.3	1.3	0	0	121.36	0.400
C	62.2	37.8	0	0	0	0	118.68	0.773
D	70	13.5	8	8	0.5	0	99.22	0.846
UN population								
A	3	3	21	0	0	74	77.43	2.606
B	0	97	0	3	0	0	48.64	0.393
C	0	100	0	0	0	0	62.01	0.400
D	84.5	14.4	0	0	1.1	0	75.48	0.947

3. Conclusions

3.1. Development of a new type of index

Protein quality, its determination and the role of the index thus obtained in the research and development activities of the food industry came into the foreground of the scientific interest. The importance of the problem is not challenged, only the solution is argued.

On the basis of financial and scientific considerations the spreading of in vitro methods comparable with in vivo nutritional experiences containing actual information seems inevitable in the praxis of the food industry.

These methods are far superior to the information content of traditional chemical indices, they actually measure and not only rank the quality of the protein in the samples studied and in the case of mixtures, too.

The Mørup–Olesen index was the first, by correlating in vivo data to amino acid compositions, to bridge the distance between in vitro and in vivo methods, thereby promoting the development of more advanced indices.

As it was proven in successful feeding experiments, the Mørup–Olesen-type indices may be extended over the measurement of biological values in different living organisms e.g. domestic animals (BÉKÉS et al., 1984; SOLTÉSZ & BÉKÉS, 1979).

The mathematical apparatus, as described under para. 1.2. is used for the processing of the BV data obtained in feeding experiments of various animals and of the amino acid composition of feeding rations, chemical indices specific of species, age and breeding conditions can be derived. These indices invalidate the remarque of KÁLLAI and KRALOVÁNSZKY (1978) according to which traditional chemical indices are two-armed balances with one arm missing (the amino acid requirement of the living organism).

The aim of this work is the highest possible exploitation of possible applications and to explore the theoretical and practical consequences. The Mørup–Olesen index, due to difficult function formation and accounting for excess amino acid intake, is not a good model of the description of physiological facts by in vitro methods.

The function of Gaussian character, as used by the authors eliminates this defect, in addition the correlation coefficient of the PV calculated by this index to the BV obtained by in vivo measurements is higher than the one obtained by the formation of reciprocal. Another advantage of the Gaussian function is that, being a continuous function, it can be differentiated along the whole of the x axis and therefore when the PV thus calculated is applied as an objective function the most efficient end value searching methods can be used.

The combination of the essential amino acids taken into account in calculating the indices, gave very interesting results:

— as it can be seen from data in Table 6, the correlation BV–PV is affected in every case when ARG is taken into consideration (in case of indices 1–2, 3–4, 5–6 and 7–8),

— the joint application of sulfur containing and aromatic amino acids is indicated because the highest correlation coefficient is thus achieved (index 1b).

Although there is no theoretical connection between the numerical value of weighting factors and the importance of individual amino acids, it is interesting to note, that in the case of the sulfur containing amino acids not only MET but β_{CIS} is also equal to zero. Similar is the situation for the relation PHE—TYR, but when β_{TYR} is not zero $\beta_{PHE} \gg \beta_{TYR}$.

The formation of the β of hydrophobic amino acids is also surprising. In the 16 cases investigated β_{ILE} did not differ significantly from zero and the value of β_{VAL} was above zero where that of β_{LEU} was also.

This behaviour of the weighting factor of hydrophobic amino acids and their analogous reaction pathways in metabolism are not in unambiguous correlation, particularly if there is no physiological explanation for the exceptionally high value of β_{THR}. It is possible, however, that this higher value is the crosseffect with the data series of another amino acid. Drawing of the final consequences requires further investigation of the problem.

3.2. Possible uses of the chemical indices

Indices of the Mørup–Olesen type can be used as measuring numbers of protein quality and thus, the program package developed for their calculation is suitable for the solution of many practical problems (WÖLLER et al., 1977; HUSSEIN et al., 1976a, b). The authors wish to illustrate by the calculation of two recipes (complementing and optimization) that these chemical indices are suitable for the solution of more complex tasks in addition to the characterization of the protein quality of the product.

However, the above considerations do not mean that the chemical indices used as in vitro methods can completely replace in vivo biological methods. As it is described in various publications (BODWELL, 1977; PELLETT, 1978) informations obtained by chemical indices are limited. Six limiting factors were discussed in detail by Pellett:

1. While the amino acid data used for calculation represent the composition of the hydrolysate containing the total amino acid set of the protein in question, in reality only a fraction of the protein is digestible and of the digested part only a fraction is accessible.

2. The non-specific nitrogen introduced by way of the essential amino acids is not accounted for in the indices.

3. The metabolism of the essential amino acid is not known in sufficient depth to account for every crosseffect in the calculations.

4. It is evident that the chemical indices cannot indicate the effect on the biologica value of the antinutritive and toxic materials consumed with the protein.

5. Chemical indices in protein below BV=40 are not valid.

6. Reproducibility of the chemical indices is dependent on the systematic error of the analytical methods.

Of the 6 limiting factors as stated by PELLETT (1978) four can be eliminated. A possible way of accounting for non-specific nitrogen was shown already in 1961 by KORPÁCZY and co-workers. For the BV low-quality proteins the Mørup–Olesen-type indices give much better correlation than the formulae used by Pellett.

Successful efforts were made in this institute to account for the digestible amino acids in the chemical indices, by adapting the multi-enzyme rapid digestibility test, developed by HSU and co-workers (1977). SALGÓ and

JÉCSAI (1981) and SALGÓ and co-workers (1983) found that the data thus obtained can be incorporated in the PV value describing the biological value and the correlation of data calculated in this way is thereby highly improved.

The sixth factor given by PELLETT (1978) requires more detailed discussion. The relations as deduced in para. 2.2. and the calculations based on them prove that the different chemical indices carry the analytical error to a different extent, depending on the formula used in calculation.

In the case of the FAO-WHO index and the Mørup–Olesen-type indices propagation of the error pertinent to individual essential amino acids differs within the index. In the FAO-WHO index the variance of the limiting amino acid becomes dominant. As regards the Mørup–Olesen-type indices, variations are caused by the numerical differences in the weighting factors, as shown in Fig. 3. It can be seen from data in Table 7 that, if the amino acid analysis is carried out with sufficient care and precision and the average relative error does not exceed 5–10%, the variation of chemical indices is substantially lower than the scatter of the in vivo experiments. It is a deplorable fact that most of the food analysts, in order to save expenses and time, do not carry out the analyses in parallel and thus have no idea of the extent of error. In the experience of the authors, if preparation of the sample is sufficiently exact, separation is sharp and evaluation is carried out, possibly not manually, precisely the error can be kept within the above mentioned limits (ZSIGMOND et al., 1982, 1984).

Of the factors listed by PELLETT (1978) Nos. 3 and 4 are considered really serious, these, however are sufficient to lead to the conclusion that by using in vitro experiments only the number of in vivo experiments can be reduced and the parameters of high information content can be prognosticated. These advantages permit considerable savings of expenses and time and enable accounting for the quality of protein in fields where in vivo methods give not sufficient information.

*

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Symbols

a_i concentration of the essential amino acids accounted for in calculating the chemical indices;

b_j concentration of non-essential amino acids accounted for in calculating chemical indices;

$\cup_R$ reference pattern;
$\cup^*$ limiting essential amino acid;
n number of essential amino acids accounted for;
q_i increment of the i-th amino acid in the index;
$\alpha_i\beta_i$ weighting factors of the i-th amino acid;
BV biological value, determined in biological experiments;
PV predetermined value, determined by calculation, analogous to BV;
MEAI Modified Essential Amino Acid Index;
KORPI Korpáczy index;
FWI FAO-WHO index;
MOI Mørup–Olesen index;
GAI Gaussian index;
x_j mass fraction of the j-th component to be mixed;
$A_{i,j}$ the i-th amino acid of the j-th component accounted for dimension: g amino acid per 100 g sample;
m number of the components to be mixed.

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SIMULTANEOUS DETERMINATION OF CALCIUM, MAGNESIUM, PHOSPHORUS AND CHLORIDE CONTENT IN BASIC FEED MATERIALS AND FEEDSTUFFS

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In the mineralization of the weighed-in sample sodium acetate was used as additive. This promoted the even charring of the material and at the same time it served as an anion binding reagent. Subsequent to charring a dilute nitric acid stock solution was prepared. In this the chloride ions were determined by direct potentiometry, the phosphate as phospho-vanado-molybdate by spectrophotometry. To determine the alkali earth metal ions atomic absorption technique was applied and the yellow solution containing $P_2O_5 - V_2O_5 - 22MoO_3 \cdot n\,H_2O$ complex was sprayed into the air-acetylene flame. This was indicated not only from the aspect of reducing the number of dilution operations, because the metavanadate and molybdate ions render the sign independent of the phosphate concentration, and reduce the sensitivity of measurement to an optimum degree. The reliability of the results for each of the four components determined corresponds to practical requirements; the method is suitable for serial tests and its introduction seems economical in laboratories engaged in routine tests of large sample series.

Keywords: atomic absorption technique, Ca, Mg, P, Cl content in feedstuffs, direct potentiometry, spectophotometry

The aim of this work was to develop a rapid method, suitable for serial tests to determine the calcium, magnesium, phosphorus and chloride content of basic feed materials and feedstuffs. Beyond the self-evident requirement that the reliability of the method should meet conventional expectations, as partially formulated in standards, the following points of view were accounted for:

- all the components in question shall be determined from the same stock solution;
- the number of portioning and dilution operations shall be as low as possible;
- the techniques applied shall be capable of providing rapid results;
- the measuring intervals shall be sufficiently wide;
- the constant presence of highly qualified experts shall not be necessitated either by the intricacy of measurements or by safety of labour;
- the specific cost of reagents shall not be extremely high.

Since chlorides were also among the components to be determined it seemed expedient to carry out mineralization by gentle incineration and then

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prepare a dilute nitric acid solution. Alcoholic sodium acetate solution was added as charring aid in view of the following considerations.

In order to prevent losses at ignition and abundant smoke formation, it is advisable to use some suitable additive to promote charring (VARJÚ, 1972); the generally applied method is incineration in the presence of alcohol. Some additives of basic character are also used to promote incineration, because, thus, losses due to evaporation can be eliminated when certain anions or anion forming elements are determined. Such an additive may be magnesium acetate (GROSSFELD, 1920), magnesium oxide (ANALYTICAL METHODS COMMITTEE, 1960), sodium carbonate (VOGT, 1921), lithium carbonate (BADER & BRANDENBERGER, 1968), etc. The advantage of sodium acetate as used in the present study is that it fills the role of both the additive promoting incineration (alcohol) and the reagent binding anion. (The breaking down of the organic salts is accompanied by sodium carbonate formation.) Another advantage is, that it mixes well and evenly with the sample.

The chloride ions in the ash solution were determined by direct potentiometry, the phosphate by spectrophotometry, the alkali earth metal ions by atomic absorption technique.

In this study we refrain from discussing in detail the chloride determination method, we refer only to the basic study of PUNGOR and TÓTH (1970). Further on we shall show some of the experimental results.

The phosphate was determined in the form of phospho-vanado-molybdate which has the advantage over the method based on phosphorus molybden blue formation, as follows:

— to form the phospho-vanado-molybdate complex all the necessary reagents can be contained in the same solution and this can be stored almost infinitely under adequate storage conditions;

— the yellow colour formed subsequent to the mixing of the sample solution and the reagent solution reaches its maximum within a few minutes and remains stable for a long time. (E.g. in a solution of 15 μg cm^{-3} phosphorus content the optical density as measured subsequent to development, at 460 nm, remained unchanged for 10 days.)

It is known that the method originally suggested by MISSON (1908) for the measurement of phosphorus in steel found a wide field of use in agricultural and food analysis (KITSON & MELLON, 1944; RAUTERBERG, 1951; GERICKE & KURMIES, 1952; MUNK & LÖSING, 1959; KÖRMENDY, 1960; PULSS, 1961; THAMM et al., 1968; SARUDI, 1980; SARUDI & PŐCZ, 1980, etc.). In the present case in selecting the conditions of determination we used mainly the observations of KITSON and MELLON (1944) and THAMM and co-workers (1968). However, the light absorption of the solution containing the coloured $P_2O_5 \cdot V_2O_5 \cdot 22MoO_3 \cdot n\ H_2O$ complex (UPOR et al., 1978) was measured

at a wavelength much higher than that suggested by the above mentioned authors. This was indicated mostly by the extension of the upper limit of the measuring range. The observation as described by several authors (RAUTERBERG, 1951; GERICKE & KURMIES, 1952), that the disturbance caused by iron (III) ions may be eliminated by the use of wavelengths of increased length, supported also this concept.

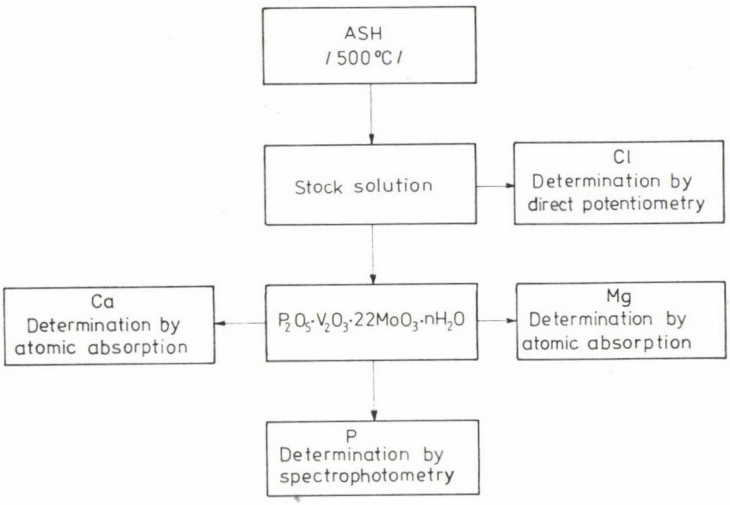

Fig. 1. Recommended scheme for determination of basic feed materials and feedstuffs

The main characteristic of the procedure developed (Fig. 1) is that the same coloured solution is sprayed into the air–acetylene flame for the determination of the alkali earth metals as is used for phosphorus. This is very useful not only because it means the reduction of the number of dilutions but, at the same time, the phosphate reagent proved to act as a very favourable matrix in calcium determinations. As it was shown earlier (SARUDI, 1980):

— it renders the sign independent of phosphate concentration and thereby the application of a specific exempting material becomes unnecessary (e.g. lanthanum chloride);
— reduces, at the same time, the sensitivity of measurement which in the case of a rather high calcium content is definitely useful.

It should be noted that the disturbance of phosphate could have been eliminated by replacing the air component of the gas mixture by dinitrogen oxide (WILLIS, 1965; AMOS & WILLIS, 1966; FODOR et al., 1974). However, this would have meant an undesirable increase of the sign and the specifications of safety in the laboratory would have to be tightened.

1. Materials and methods

1.1. Materials

–Additive applied with incineration: 70% ethyl alcohol saturated with sodium acetate at room temperature;

–5 *M* HNO_3 solution;

–Reagent (R): 5 dm^3 aqueous solution prepared with 200 g $(NH_4)_6Mo_7O_{24} \cdot 4\,H_2O$, 10 g NH_4VO_3 and 1260 cm^3 conc. HNO_3. Standard stock solution (S): 2.4975 g $CaCO_3$ was dissolved in nitric acid diluted 1:1, then it was evaporated to dry over a water bath. The residue was washed with distilled water in a 1 dm^3 volumetric flask. Further 0.5068 g $MgSO_4 \cdot 7\,H_2O$ and 1.7574 g KH_2PO_4 were dissolved in the same flask and this was filled to the mark with distilled water. The solution, thus prepared, contained 1000 μg Ca^{2+} ion, 100 μg Mg^{2+} ion and 400 μg P per 1 cm^3.

The standard solutions are shown in Table 1.

Table 1

Standard solution of phosphor — calcium — magnesium

	In 200 cm^3 final volume		P	Ca	Mg	P	Ca	Mg
	R [cm^3]	S [cm^3]	($\mu g \cdot cm^{-3}$)			(%)[a]		
S_0	20	0	0	0	0	0	0	0
S_1	20	5	10	25	2.5	2	5	0.5
S_2	20	10	20	50	5.0	4	10	1.0
S_3	20	15	30	74	7.5	6	15	1.5
S_4	20	20	40	100	10.0	8	20	2.0
S_5	20	25	50	125	12.5	10	25	2.5

[a] For 1.00 g sample
R : reagent solution
S : standard stock solution

The reagents used were of analytical grade and the distilled water was free of alkali as established by flame photometry.

1.2. Measuring instruments

Radelkis type OP-261 instrument (Hungary)
Spectrophotometer: Zeiss type VSU-2 P (GDR)
Atom absorption instrument: Pye Unicam type SP 1900 (USA)

1.3. Procedure

1.3.1. Charring and preparation of stock solution. Generally 1.00 g of the ground sample was weighed in a porcelain dish, 20 cm^3 alcoholic sodium acetate were added and after ashing it was incinerated at 500 °C for 8 h. The ash

was dissolved in 10 cm^3 5 *M* HNO_3, then washed through a filter with hot distilled water into a 100 cm^3 Kohlrausch flask. The flask containing about 60 cm^3 solution was kept over a boiling water bath for 25 min in order to hydrolyze the pyrophosphate. After cooling it was made to mark with water. In the case of grain or samples containing calcium below 0.5% 10 g of the sample were weighed in.

1.3.2. Determination of chloride. Determination was carried out according to the description in the manual accompanying apparatus OP-261 (Radelkis, Hungary).

1.3.3. Determination of phosphorus and alkali earth metals. Five cm^3 of the stock solution were pipetted into a 100 cm^3 volumetric flask. Ten cm^3 of the reagent were added with an Oxford pipette and the flask was made to mark with distilled water. The yellow solution, thus prepared, was ready for the determination of phosphorus, calcium and magnesium.

The calibration curves plotted on the basis of the P—Ca—Mg standard solutions, as shown in Table 1, are presented in Figs. 2 to 4. It is worth mentioning, however, that to determine the alkali earth metals the scale was extended sufficiently to permit to read the results, expressed in percentage related to the sample, directly from the display of the atomic absorption apparatus.

Parameters of measurement: are shown in the legends to Figures.

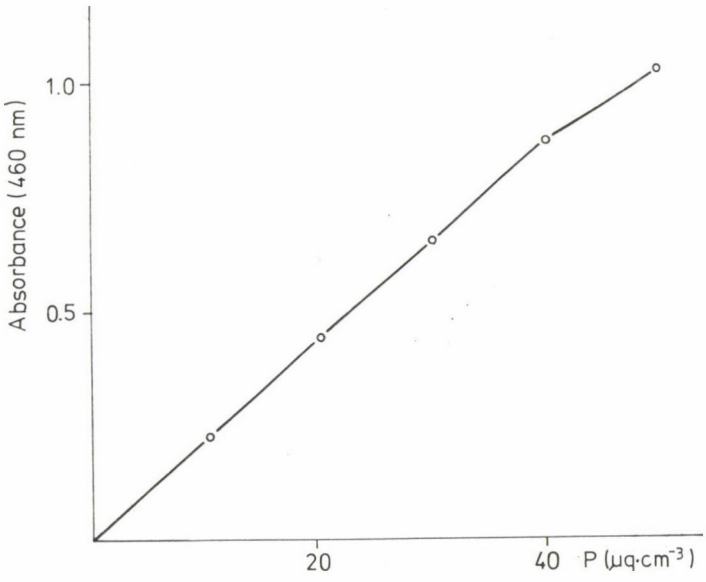

Fig. 2. Calibration curve of phosphorus determination by the phospho-vanado-molybdate method ($\lambda = 460$ nm; width of the glass cuvettes 1.00 cm)

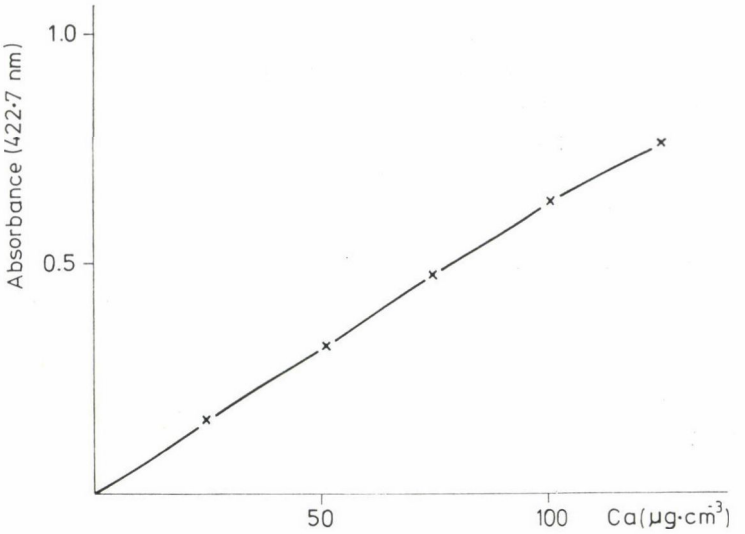

Fig. 3. Calibration curve of calcium determination by the atomic absorption method (Apparatus: Pye Unicam SP 1900; air: flow rate = 4.8 cm^3 min^{-1}; acetylene: flow rate = 0.8 dm^3 min^{-1}; λ = 422.7 nm; slit = 0.1 mm; torsion of the burning head around its axis = 10°; observation height = 8 mm)

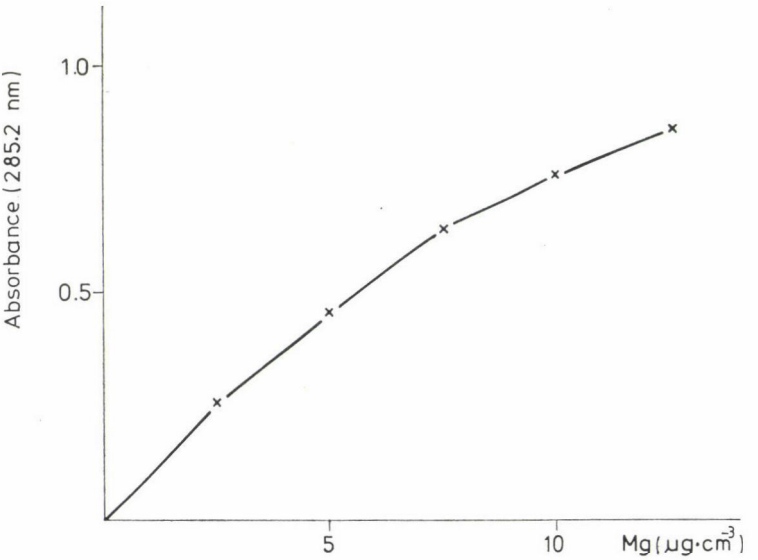

Fig. 4. Calibration curve of magnesium determination by atomic absorption method (λ = 285.2 nm; burning head torsion around its axis: 90°; all other parameters correspond to those used in calcium determination)

1.4. Methods of comparison

The sample prepared for comparative test was impregnated with alcohol prior to ashing, but anion binding reagent was not used. Otherwise mineralization was carried out as described in para. 1.3.1. Since in chloride and phosphorus determination the aim of the comparative test was to find out whether the basic additive used in the procedure developed was necessary (i.e. efficient), the methods applied were as described above. The comparative determination of the alkali earth metals was also carried out by the described method (AAS method, air–acetylene flame) only the solutions were diluted so as to have the calcium ions in the range of 1–10 $\mu g\ cm^{-3}$ and the magnesium ions between 0.1–1.5 $\mu g\ cm^{-3}$. To eliminate the disturbance caused by phosphate 1 g per 100 cm^3 lanthanum chloride was applied (TRENT & SLAVIN, 1964; FODOR et al., 1974).

2. Results and conclusions

A total of 36 different mixed feeds or premixes were subjected to comparative analysis. Generally two parallel determinations were carried out of each sample, the first by the recommended method and the second by a comparative method. The two exemptions were a feed and a premix sample of which 5 parallel determinations were made each, in order to study reproducibility. The deviations were compared by F-test, while the mean values generally by Student's *t*-test. If however, the difference between deviations proved to be significant (Table 3) the mean values were compared by the so called t_f statistics according to Welch (VINCZE, 1975).

Table 2

Comparative chloride (common salt) determinations (I)

	Method	
	Recommended (A)	Comparative (B)
Number of samples	36	
Range (mg NaCl in 100 mg sample)	0.03—12.36	0.20—11.17
Mean value of results (mg NaCl in 100 mg sample)	$y = 2.6220$	$\bar{x} = 2.3583$
t-test	$t = 6.183^{***}$	
Regression equations	$y = 1.0772x - 0.0747$	$x = 0.9272y - 0.0747$

*** = very highly significant at P = 0.001% probability level ($t_{tabl.} = 3.551$)

Table 3

Comparative chloride (common salt) determination (II)

	Duck rations		Premix	
	A	B	A	B
n	5	5	5	5
s	0.026	0.069	0.234	0.598
v	7.56	27.60	2.02	5.71
F-test	$F = 7.04^*$		$F = 6.53^*$	
Mean value of results	$\bar{y} = 0.344$	$\bar{x} = 0.250$	$\bar{y} = 11.61$	$\bar{x} = 10.48$
t_f-test (Welch)	$t_f = 2.85$		$t_f = 3.48$	

A: recommended method
B: comparative method
n: number of determinations
s: standard deviation of individual results (mg NaCl in 100 mg sample)
v: coefficient of variation (%)
$\bar{y}$: mean value of results (mg NaCl in 100 mg sample) obtained with method A
$\bar{x}$: mean value of results (mg NaCl in 100 mg sample) obtained with method B
Tabulated values: $F_{0.01} = 15.98$; $F_{0.05} = 6.39$
$t_{0.01} = 4.032$; $t_{0.05} = 15.98$
* significant at $P = 0.05\%$ probability level

The results in Table 2 and 3 show that the difference between the results obtained by the two methods of chloride (common salt) determination was very highly significant: results when anion binding reagent was used, were much higher. A further observation is, as seen from the results in Table 3,

Table 4

Reliability of chloride (common salt) determinations by the so called addition method

Sample	NaCl content in the original sample (%)		NaCl (mg) added to 1 g sample	Recovered NaCl additive (%)	
	A	B		A	B
Duck rations	0.325	0.262	2.14	108.0	85.0
Piglet rations	0.424	0.356	2.14	97.4	76.1
Cow with calf rations	0.513	0.463	2.14	100.4	85.9
Cow in milk rations	0.952	0.796	4.28	96.2	88.8
Duck breeding premix	6.16	5.94	42.8	100.0	83.3
Breeder rabbit premix	8.04	7.77	42.8	96.1	77.8
Cow in milk premix	11.13	10.81	42.8	101.7	92.5

A: recommended method
B: comparative method

that difference is significantly higher when the additive is not used. Thus, it was presumed that in the latter case a high loss by evaporation occurs, which, however can be — partly or completely — eliminated by the addition of sodium acetate. The correctness of this presumption was proven finally by the so called additive method (Table 4). A known amount of sodium chloride was added in the form of solution to the sample weighed-in, prior to ashing.

In the case of phosphate the results obtained by two tested methods showed a good agreement and this proves that if the 500 °C incineration temperature is strictly controlled there is no need to apply the basic additive (Tables 5 and 6).

Table 5

Comparative phosphorus determinations (I)

	Method	
	Recommended (C)	Comparative (D)
Number of samples	36	
Range (mg P in 100 mg sample)	0.30—9.15	0.28—9.08
Mean value of results (mg P in 100 mg sample)	$\bar{y} = 2.876$	$\bar{x} = 2.900$
t-test	$t = 1.773$	
Regression equations	$y = 0.9960x - 0.0163$	$x = 0.9943y + 0.0192$

Tabulated value: $t_{0.05} = 2.03$

Table 6

Comparative phosphorus determinations (II)

	Duck rations		Premix	
	C	D	C	D
n	5	5	5	5
s	0.019	0.045	0.157	0.198
v	2.95	7.08	2.10	2.64
F-test	$F = 5.40$		$F = 1.585$	
Mean value of results	$\bar{y} = 0.664$	$\bar{x} = 0.636$	$\bar{y} = 7.476$	$\bar{x} = 7.512$
t-test	$t = 0.28$		$t = 0.318$	

C: recommended method
D: comparative method
n: number of determinations
s: standard deviation of individual results (mg P in 100 mg sample)
v: coefficient of variation (%)
$\bar{y}$: mean value of results (mg P in 100 mg sample) obtained with method C
$\bar{x}$: mean value of results (mg P in 100 mg sample) obtained with method D
Tabulated values: $F_{0.05} = 6.39$
$t_{0.05} = 2.31$

As it can be seen in Tables 7 to 10, the difference between mean values of the results of both calcium and magnesium determinations carried out by the recommended and the comparative method, resp., was not si gnificant.

Table 7

Comparative calcium determinations (I)

	Method	
	Recommended (E)	Comparative (F')
Number of samples	36	
Range (mg Ca in 100 mg sample)	0.50—29.18	0.42—29.42
Mean value of results (mg Ca in 100 mg sample)	$\bar{y} = 8.433$	$\bar{x} = 8.461$
t-test	$t = 1.774$	
Regression equations	$y = 0.9958x + 0.0118$	$x = 1.0040y - 0.0103$

Tabulated value: $t_{0.05} = 2.03$

Table 8

Comparative calcium determinations (II)

	Duck rations		Premix	
	Method			
	E	F'	E	F'
n	5	5	5	5
s	0.045	0.0825	0.167	0.135
v	3.34	6.15	0.87	0.70
F-test	$F = 3.358$		$F = 1.53$	
Mean value of results	$\bar{y} = 1.348$	$\bar{x} = 1.342$	$\bar{y} = 19.20$	$\bar{x} = 19.15$
t-test	$t = 1.428$		$t = 0.522$	

E : recommended method
F' : comparative method
n : number of determinations
s : standard deviation of individual results
v : coefficient of variation (%)
$\bar{v}$: mean value of results (mg Ca in 100 mg sample) obtained with method E
$\bar{x}$: mean value of results (mg Ca in 100 mg sample) obtained with method F'
Tabulated values: $F_{0.1} = 4.11$
$t_{0.1} = 1.86$

Table 9

Comparative magnesium determinations (I)

	Method	
	Recommended (G)	Comparative (H)
Number of samples	36	
Range (mg Mg in 100 mg sample)	0.18—2.07	0.14—2.09
Mean value of results (mg Mg in 100 mg sample)	$\bar{y} = 0.7985$	$\bar{x} = 0.7915$
t-test	$t = 0.728$	
Regression equations	$y = 0.9943x + 0.0115$	$x = 1.0016y - 0.0083$

Tabulated value: $t_{0.1} = 1.69$

Table 10

Comparative magnesium determinations (II)

	Duck rations		Premix	
	G	H	G	H
n	5	5	5	5
s	0.009	0.013	0.036	0.024
v	3.21	2.78	1.76	1.18
F-test	$F = 2.20$		$F = 2.19$	
Mean value of results	$\bar{y} = 0.280$	$\bar{x} = 0.278$	$\bar{y} = 2.040$	$\bar{x} = 2.034$
t-test	$t = 2.07$		$t = 1.24$	

G: recommended method
H: comparative method
n: number of determinations
s: standard deviation of individual results (mg Mg in 100 mg sample)
v: coefficient of variation (%)
$\bar{y}$: mean value of results (mg Mg in 100 mg sample) obtained with method G
$\bar{x}$: mean value of results (mg Mg in 100 mg sample) obtained with method H
Tabulated values: $F_{0.1} = 4.11$
$t_{0.05} = 2.31$

To sum up the value of the method developed, the reliability of results for the four components determined, meets practical requirements; the procedure is suitable for serial tests and is economical for laboratories carrying out a large number of routine tests.

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STUDY OF THE MECHANICAL PROPERTIES OF APPLE WITH SPECIAL REGARD TO JUICE EXTRACTION

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Pressure and shear strength and firmness according to Magness-Taylor's method as well as cutting resistance in apple flesh were studied.

The effect of variety, ripeness, storage and heating on the above characteristics, was investigated. Substantial difference in the resistance to pressure of varieties grown in Hungary was not observed.

Ripening and heating cause the reduction of resistance to pressure. The change in the resistance to pressure of the heated texture depends on the rate of loading.

The rate of loading affects also shear strength. A substantial reduction in shear strength occurs upon heating. At 75 °C its value is about half of that measured at 70 °C.

Cutting resistance and firmness decrease as an effect of ripening and storage.

The apple varieties grown in Hungary are ranked according to their cutting resistance as follows: Starking, Jonathan, Golden Delicious.

Measured according to Magness-Taylor the firmness of Golden Delicious is higher than that of Jonathan. Thus ranking as established by measurement of cutting resistance differs from firmness as established according to Magness-Taylor, due to difference in mechanical stress.

Keywords: mechanical properties of apple, juice extraction, firmness, pressure and shear strength

The mechanical strength of apple tissue is very important from the aspect of both techniques of juice extraction: pressing and diffusion.

Of the mechanical stress parameters instrumentally measurable the following were studied:

— elasticity modul,
— tensile strength,
— flexibility of a thin disc,
— pressure and shear strength,
— firmness according to Magness–Taylor's method
— cutting resistance.

The results of measurements related to the first three parameters and their change as the function of variety, ripeness, storage and heating, were summed up in a previous paper (VUKOV et al., 1981).

In the present paper the results of measurement of pressure and shear strength, of firmness according to Magness–Taylor and of resistance to cutting, are presented.

These characteristics are related to the possiblity of slicing an apple and its hydrodynamic behaviour during juice extraction.

These relations are comprehensible in the knowledge of the most important requirements upon the apple slices used for juice extraction:

- the equivalent thickness of the slice should be the lowest permitted by the elasticity of the raw material;
- its ruggedness factor should be the lowest possible (VUKOV, 1977a).

The cutting of thicker slices may be necessary for two reasons: the apple to be sliced is either too brittle or too soft to permit of cutting thin slices free from fragments. Brittle slices are characterized by a high elasticity modulus, while soft slices by low elasticity modulus and low cutting resistance.

If the cutting resistance is unusually high, knives become soon blunt or clogged. However, the presence of pip and core are responsible for this mainly. The capacity of the slicing machine is reduced due to frequent exchange of knives and this loss of capacity can be balanced by cutting of thicker slices only.

The ruggedness factor of the slice is usually high if the edge maintaining capacity of the knife is low or it is not exchanged often enough or is not cleaned in time.

In an extremely disadvantageous case this may become impossible. The ruggedness factor is affected also by the gas content of the slice and the bubbles adhering to its surface. This problem will be discussed in a forthcoming paper.

The pressure and shear strength affect the ruggedness factor indirectly, too. The slice of low strength in consequence of the mechanical stress during extraction can break down further and the ruggedness factor becomes worse. Pressure and shear strength are interesting from this point of view, too (VUKOV & MONSZPART 1977).

This study was aimed at obtaining more detailed knowledge on the pressure and shear strength, cutting resistance and firmness of the apple tissue in view of the new technology of apple juice production by diffusion extraction.

1. Materials and methods

1.1. Materials

The raw material used in this study was the same as described in on earlier paper (VUKOV et al., 1981). Apple varieties Jonathan and Starking (1976, 1977) and recently Golden Delicious (1978, 1979) were used. In 1981 all three varieties obtained from the Model Farm of University of Horticulture harvested on September 22 and 28, were studied.

Pressure and shear strength were tested on apple pieces cut from 12 apples each. Cutting resistance was tested on samples obtained from 15 apples. Firmness was measured on two places of 30 apples.

Generally samples were randomly taken from 50 apples, some time from 30 or 100.

1.2. Methods

1.2.1. Determination of pressure strength. Pressure strength was established by a pressure test, similarly to the determination of the elasticity modulus, using the same test samples and the same instrument (VUKOV et al., 1981).

The cylindrical test samples had a diameter of 1.13 cm and a height of 1.0 cm. The tests were carried out on the Höppler consistometer with a plastometer insert, at room temperature. On this instrument a load equal to 2.5 N force or the multiple of this can be achieved. The load was continuously increased till the test sample collapsed. The pressure required was expressed in Pa units.

The pressure was increased at two different rates:

– slow rate: pressure was increased by 25 kPa per minute,

– high rate: pressure increased by 75 or 100 kPa per minute.

1.2.2. Determination of the shear strength. Cutting resistance is understood to mean the work falling to unit cut surface, needed to cut the test sample with an edge agreed upon.

Applying the method of Tegze, Sipos and Vukov (VUKOV, 1977b) a test sample of 1.60 cm diameter and 3 cm length was cut crosswise with a drop balance, fitted with a steel wire of 0.4 mm diameter as a cutting edge. The work required is calculated from the empty drop of the balance and the deviation after cutting. Fifteen parallel measurements were carried out on each sample.

Shear strength was measured, similarly to the determination of pressure strength, with the Höppler consistometer, using a circular insert and a fitting plunger (HÖPPLER, 1940). Dimensions can be seen in Fig. 1.

The test samples were exposed to both loading rates: gradual slow rate and sudden high rate. Loading was increased to the total shearing of the test sample.

The basic load and the increase was the same as applied to measure pressure strength.

1.2.3. Determination of firmness according to Magness–Taylor. Firmness was measured with a hand-operated penetrometer (Fruit tester, Renato Lusa, Italy, diameter of plunger 11.11 mm). On measuring with the hand-operated

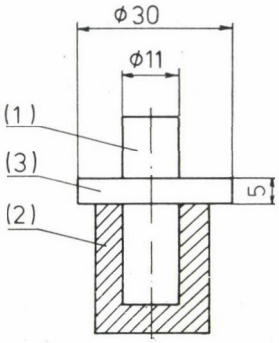

Fig. 1. Dimensions of the fitting used in measuring shear strength and of the test sample. 1. Plunger; 2. sample holder; 3. test sample

penetrometer the resistance of fruit flesh at a given penetration depth, the force required to compress the spring is read in pound force units. The dimensions of penetrometer head can be seen in Fig. 2.

In the knowledge of the surface of the plunger the values were calculated in Pa units.

2. Results

2.1. Pressure strength

Results related to pressure strength are summarized in Table 1.

Results relate to gradual, slow loading. Above 70 °C a rapid softening can be observed.

In raw apple flesh there is no difference in the pressure strength as measured at gradual or sudden loading. The difference is, however, high with heated samples (Table 2).

2.2. Shear strength

Shear strength was measured in fresh apple tissue and in apple tissue heated to 70 and 75 °C. Jonathan and Golden Delicious varieties were tested. Results are shown in Table 3.

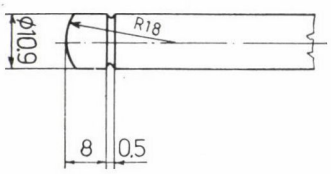

Fig. 2. Penetrometer used in determining the firmness value (Fruit Tester, Renato Lusa penetrator)

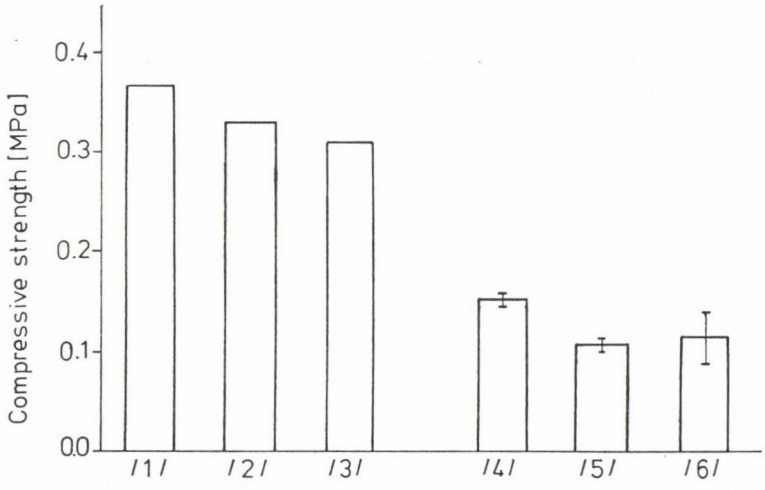

Fig. 3. Pressure strength of raw apple as affected by ripening and storage **1: unripe; 2: ripe; 3:** overripe; 4: stored-in; 5: 60 days of storage at 45% R.H.; 6: 60 days of storage at 84% R. H.

Table 1

Pressure strength (MPa) in freshly harvested and stored apple varieties Jonathan and Golden Delicious and after 20 min treatment at different temperatures

	Jonathan		Golden Delicious	
	upon harvest	stored[a]	upon harvest	stored[a]
Raw	0.249±0.012	0.275±0.012	0.295±0.012	0.288±0.01
Heated				
65 °C		0.084±0.019		0.090±0.01
70 °C		0.092±0.065		0.090±0.02
75 °C		0.035±0.012		0.055±0.01

[a] Storage period: 30–90 days, storage temperature: +3 °C, relative humidity: 90%

Table 2

Pressure strength of apple flesh under gradual and sudden loading (MPa)

	Gradual loading	Sudden loading
Raw	0.286±0.028	0.286±0.028
Heated for 20 min at 65 °C	0.086±0.010	0.050±0.002

Table 3

Shear strength of apple flesh under gradual and sudden loading (MPa)

Treatment	Gradual loading	Sudden loading
Raw	0.180±0.03	0.104±0.004
Heated for 20 min		
at 70 °C	0.075±0.014	0.029±0.001
at 75 °C		0.016±0.001

2.3. Cutting resistance

Cutting resistance tests were carried out on the three apple varieties: Jonathan, Starking and Golden Delicious. The apples were harvested in 1979 and 1981 at 3 different stages of ripeness and were stored. They were tested immediately upon harvest and after 6 months storage in controlled atmosphere. Results were evaluated by analysis of variance and are given in Tables 4, 5 and 6.

Table 4

Cutting resistance (kJ m^{-2}) during ripening according to variety

Variety	Date of harvest in 1979				
	5 September	17 September	25 September	Average	Upon storage (average)
Jonathan	0.22 ±0.06	0.17 ±0.046	0.14 ±0.023	0.173±0.043	0.17±0.037
Golden Delicious	0.21 ±0.024	0.15 ±0.029	0.11±0.022	0.156±0.025	
Starking	0.23 ±0.031	0.16 ±0.037	0.14 ±0.030	0.173±0.033	
Average	0.216±0.041	0.156±0.037	0.13 ±0.025		

Table 5

Cutting resistance (kJ m^{-2}) according to variety of apples after 8 months storage under controlled atmosphere

Variety	Date of storing in 1979				
	5 September	17 September	28 September	Average	On testing (average)
Jonathan	0.105±0.036	0.102±0.045	0.068±0.047	0.092±0.042	0.084±0.033
Golden Delicious	0.091±0.036	0.066±0.023	0.056±0.028	0.071±0.029	
Starking	0.097±0.022	0.100±0.035	0.068±0.024	0.088±0.027	
Average	0.098±0.031	0.088±0.035	0.064±0.034		

Table 6

Cutting resistance (kJ m^{-2}) of apple varieties Jonathan, Golden Delicious and Starking

Variety	Date of harvest in 1981		
	22 September	28 September	Average
Jonathan	0.132±0.017	0.136±0.029	0.134±0.017
Golden Delicious	0.105±0.014	0.126±0.014	0.115±0.018
Starking	0.150±0.008	0.150±0.016	0.150±0.010
Average	0.129±0.022	0.137±0.017	

2.4. Firmness according to Magness–Taylor

Tests were carried out on the three apple varieties Jonathan, Starking and Golden Delicious. The samples were gathered in the autumn 1979 at 3 different stages of ripeness. They were tested immediately upon harvest and after storage. Prior to the test the apples were peeled. Average values of measurements are given in Table 7.

Table 7

Firmness according to Magness–Taylor (MPa) of fresh apples as a function of harvest time
(Measurements carried out on the day after harvest)

Variety	Date of harvest in 1979			
	5 September	15 September	25 September	Average
Jonathan	0.83	0.72	0.65	0.73
Golden Delicious	0.83	0.79	0.73	0.78
Starking	0.81	0.85	0.75	0.80
Average	0.82	0.79	0.71	

3. Discussion

3.1. Pressure strength

As can be seen in Table 1 the pressure strength of the two apple varieties tested (Jonathan, Golden Delicious) raw and after heating (boiling) for 20 min at 65–75 °C, did not differ practically.

NIKOLAEV and co-workers (1973, 1978) found substantial difference between apple varieties when tested in the raw state. The results were as follows:

Melba: 0.243 MPa
Antonovka: 0.481 MPa
difference: 0.238 ± 0.098 MPa

NIKOLAEV and co-workers (1973, 1978) observed similarly to the authors of this paper that the effect of storage on this parameter depends largely on the relative humidity of the atmosphere. The effect of temperature, as seen in Table 1, does not change the pressure strength between 65 and 70 °C after heating for 20 min, but during 20 min treatment at 75 °C it rapidly decreases.

POWERS and BOARD (1973) used a different method to establish the effect of heating. They measured the time needed to cause a change in pressure strength when apples were heated at 100 °C. Thus, they established only trends and their results cannot be compared with those of the authors.

As it can be seen in Table 2, the extent of change of pressure strength depends largely on the loading rate. When loading is slow the cell-sap seeps through, the cell walls amass and thus the pressure strength increases. In case of sudden loading the cell-sap can escape only through the leaks in the cell walls caused by the collapse of the tissue.

Pressure strength as affected by different factors (based on data taken from the literature and the authors observations) is shown in Fig. 3.

3.2. Shear strength

Shear strength both in raw and in heated apples depends largely on the rate of loading (Table 3).

By increasing the heating temperature from 70 °C to 75 °C the shear strength is substantially reduced. Since according to the measurements of the authors neither the pressure nor the shear strength is practically changed by the time of heating between 20 and 60 min, a 20 min treatment causes a stable limit value.

The shear strength shown at 75 °C is about the half of that found at 70 °C.

3.3. Cutting resistance

As can be seen in Tables 4 and 5 cutting resistance changes substantially during storage. As an effect of ripening and storage cutting resistance is significantly reduced.

DOMONKOS (1980) found a 30% reduction in the cutting resistances of apples after 3 months cold storage. The observations of the authors show a 50% reduction after storage in controlled atmosphere for 6 months.

The cutting resistance of the apple varieties studied are ranked on the basis of the authors' experiences, supported by the findings of DOMONKOS (1980) as follows: Starking > Jonathan > Golden Delicious.

3.4. Firmness according to Magness–Taylor

As it can be seen in Table 7, firmness diminishes in every variety with ripening. In Jonathan apples firmness diminished during storage, too (Table 8).

Table 8

Firmness according to Magness–Taylor (MPa) in stored Jonathan apples
(90 day-storage in a commercial store)

Place of test	Firmness	Average	Standard deviation
Green side	0.56	0.57	±0.05
Red side	0.59		

Storage temperature: +3 °C, relative humidity: 90%

Data taken from the literature on the firmness of apple tissue (HÁMORI–SZABÓ, 1974; KOMÁNDY–ERDŐDI & TOMCSÁNYI, 1971; POWERS & BOARD, 1973; STANKINA et al., 1970) as a function of stage of ripeness are given in Table 9, while those according to variety in Table 10.

Table 9

Magness–Taylor firmness values as a function of ripeness, found in the literature

References	Varieties	Stage of ripeness			
		Unripe	Ripe for picking	Ripe for consumption	Overripe
HÁMORI–SZABÓ, 1974	5	—	0.80	0.49	0.38
KOMÁNDI–ERDŐDI, 1971	Jonathan	0.90	0.66	—	—
STANKINA et al., 1970	Jonathan	0.83	0.71	0.67	—
POWERS & BOARD, 1973	4	1.26	0.97	—	—
Extreme values		0.83—1.26	0.66—0.97	0.49—0.67	0.38
Averages		1.0	0.78	0.56	0.38

Collating data in Table 7 and 8 and considering those in Table 9, it is evident that in spite of certain overlapping of boundaries firmness permits of grading apples in practically acceptable ripeness classes. As shown by the measurements of the authors and data in Table 10 of the two apple varieties most widely cultivated in Hungary (Golden Delicious and Jonathan) at the time of testing Golden Delicious was found to be firmer.

Table 10

Magness–Taylor firmness values according to variety

Variety	Source in literature				
	HÁMORI-SZABÓ (1970) Ripe for		POWER & BOARD (1973) Ripe for		CHETVERTAKOV aud co-workers (1977)
	picking	consumption	picking	consumption	
Red delicious	0.82	0.47	1.22	0.84	—
Golden Delicious	0.82	0.54	0.96	0.80	—
Winesap	0.87	0.60	1.46	1.29	—
Jonathan	0.69	0.45	—	—	—
Red Roma	0.80	0.40	—	—	—
Gravenstein	—	—	1.38	1.02	—
Antonovka	—	—	—	—	1.15
Pepin Safranyï	—	—	—	—	1.01

4. Conclusions

Based on all the data it can be concluded that during the process of ripening and storage every strength factor deteriorates and thereby reduces the technological value of the fruit for juice extraction. In contrast the taste and aroma components get enriched and the diffusion constant increases during ripening and storage.

Thus, an optimum technological ripeness exists when the parameters of apple are most advantageous for both pressing and extraction. This optimum condition is not the same for the two different technologies. Because of the decisive roll of the diffusion constant for extraction fully ripe end even the stored apples are most suitable while these are not at all suitable for pressing (LÜTHI & GLUNK, 1979; EMCH, 1979; AMOS et al., 1980; PELEG & CALZADA, 1976; CHETVERTAKOV et al., 1977).

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QUANTITATIVE DETERMINATION OF ZEARALENONE IN RED PEPPER

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The red pepper is an important spice used widely both in Hungary and abroad for flavouring food. In the course of processing it is possible for the small number of mould fungi present on the plant in the field to multiply and pro duce poisonous substances (mycotoxins). The examinations were carried out on samples found by routine official checks to contain large quantities of mould (including Fusarium species). The method of determination consists of the ext-raction of the sample, chemical purification, and of qualitative and quantitative analyses thus justifying spot checkson the zearalenone content. by means of capillary gas chromatography. Twelve randomly selected samples containing a large number of fungi were examined in and no zearalenone could be identified in the samples.

Keywords: zearalenone, red pepper, capillary gas chromatography

Moulds develop on almost all the organic matter to be found in the human environment. In the course of their metabolism, moulds synthesize secondary metabolites which contain antibiotics suitable for use in curing human and animal diseases, and mycotoxins capable of fatally poisoning humans and animals alike.

The climate of Hungary chiefly favours the multiplication of moulds belonging to the genera Fusarium and Penicillium. Some strain of Fusarium occur in greater or smaller numbers on virtually all materials originating from field production.

One group of secondary metabolites arising from the life functions of these fungi (e.g. fusaric acid) is phytotoxic to the host plant (Jakucs et al., 1980). Another group of metabolites (zearalenone and its derivatives, compounds with a trichothecene skeleton) are toxic to both humans and animals (Mirocha et al., 1974a; Szathmáry et al., 1976).

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If the technical specifications are not adhered to during ripening, processing and storage, the pepper may become infected with mould, or rather the fungi may multiply, using the pepper as a substrate (CHRISTENSEN et al., 1967).

Various analytical methods are available for the demonstration of fusariotoxins, including zearalenone. Of these, thin layer chromatography (SARUDI, 1974; BALZER et al., 1978; JEMMALI et al., 1978) can be used for both qualitative and quantitative determinations. The reliability and accuracy of data obtained using the TLC method do not satisfy all the aspect of practical requirements. Consequently, methods involving gas chromatography or a combination of gas chromatography and mass spectrometry are gaining ground (DRUCKER, 1974; BATA et al., 1980; MIROCHA et al., 1974b; RODRICKS & HESSELTIME, 1977).

The aim of the experiment was to determine the quantity of zearalenone which might occur in random samples, and to elaborate an analytical method suitable for the routine determination of any toxins that might be present.

1. Materials and methods

The red pepper samples used in the experiment originated from the Kalocsa region and were very mouldy, because of having been stored under unfavourable conditions.

1.1. Preparation of samples and formation of derivatives

Fifty g sample of finely ground red pepper was extracted with 200 cm^3 ethyl-acetate for two hours at room temperature in a 500 cm^3 Erlenmeyer flask with frequent shaking. The extract was filtered and the filtrate was evaporated almost to dryness over a water bath. The oily residue was dissolved in 25 cm^3 chloroform and purified using the "alkaline" purification procedure, in the course of which the chloroform solution was extracted twice with 10 cm^3 1 *M* sodium hydroxyde in a 100 cm^3 separating funnel. In order to prevent the formation of an emulsion, 10 cm^3 saturated sodium chloride was added to the solution. The aqueous fractions were collected, united and used in the further stages. The aqueous phase was further purified with twice 5 cm^3 chloroform and the chloroform phases were discarded. The negative ion concentration of the aqueous phase was adjusted to 9.5 pH with 2 *M* phosphoric acid and was then extracted three times with 15 cm^3 chloroform. The chloroform solvent was united, freed from water on a column containing sodium sul-

phate and evaporated to dryness over a water bath. The dry residue was dissolved in 5 cm^3 chloroform. A 1 cm^3 sample of the chloroform solution was placed in a screw-cap vial and evaporated to dryness in a stream of N_2. One hundred mm^3 *N,o*-bis-(trimethylsilyl)-trifluoro-acetamide reagent (BSTFA) were added to the dry residue. After closing the vial the sample was kept at 60 °C for 15 min. After cooling, a 1 mm^3 sample of the reaction mixture was injected into the analyzer.

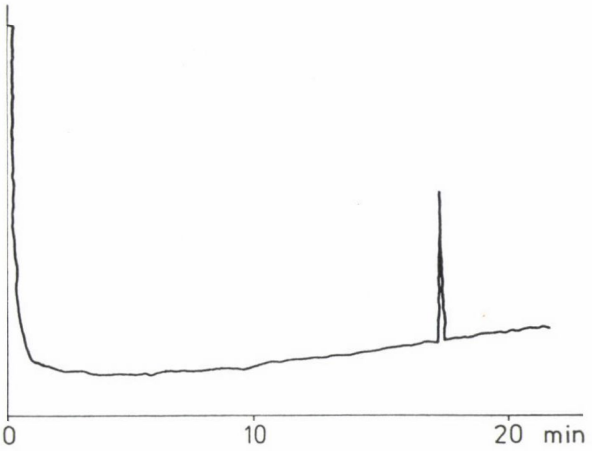

Fig. 1. Chromatogram of standard zearalenone
Column: 14 m long, 0.25 mm I.D. glass capillary wetted with SE 52; thermostat: 200–260 °C at 3 °C/min; injector: 260 °C; detector: 260 °C; carrier gas: H_2; pressure of carrier gas: 40 kPa; amplification: 10^{-11}A

1.2. Gas chromatography

A Packard 427 gas chromatograph was used, connected to an HP 3390 A type integrator. A 14 m long, 0.25 mm i.d. glass capillary prepared by the method of GROB and GROB (1976) and GROB and co-workers (1977) wetted with SE 52 stationary phase and tested by the same method of GROB and co-workers (1978) was used in the equipment.

2. Results

In order to check the method a known quantity of maize containing a known concentration of zearalenone was mixed with good quality red pepper which did not contain zearalenone. In the course of three parallel determinations using the procedure described above, the zearalenone was recovered with an efficiency of 78%. The chromatogram of a pepper sample containing zearalenone is presented in Fig. 2.

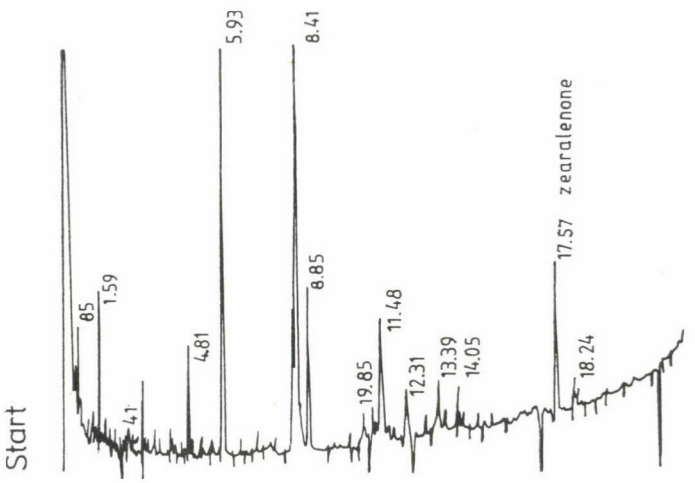

Fig. 2. Chromatogram of a pepper sample containing zearalenone
Column: 14 m long, 0.25 mm i.d. glass capillary wetted with SE 52; thermostat: 200–260 °C at 3 °C/min; injector: 260 °C; detector: 260 °C; carrier gas: H_2; pressure of carrier gas: 40 kPa; amplification: 10^{-11} A

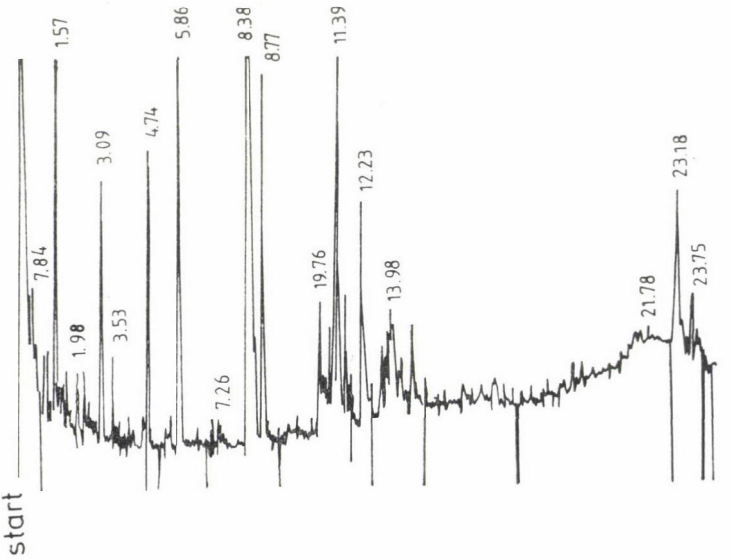

Fig. 3. Chromatogram of pepper sample KD 6
Column: 14 m long, 0.25 mm I.D. glass capillary wetted with SE 52; thermostat: 200–260 °C at 3 °C/min; injector: 260 °C; detector: 260 °C; carrier gas: H_2; pressure of carrier gas: 40 kPa; amplification: 10^{-11} A.

As it can be seen from the chromatogram, the purification procedure proved sufficient, since no peaks were found near enough to interfere with the zearalenone peak, due to the efficiency of the separation technique employed. It was thus concluded that the zearalenone content of red pepper samples could be reliably determined using this method. Considering the sensitivity of the analyzer and the concentration ratios applied, the sensitivity of the method was taken to be 10 μg per kg. In other, mainly cereal, samples zearalenone contents of this magnitude have been successfully demonstrated using this method.

Twelve randomized samples were examined during 1981. The selection was made on the basis of microbiological analyses: from the samples taken for official inspection, those containing the largest number of mould fungi were chosen, on the assumption that the formation of mycotoxin was most probable in the samples which were of the poorest quality from a microbiological point of view.

In the twelve samples examined no zearalenone was found above the 10 μg per kg level. This statement is confirmed by the chromatogram of sample KD 6, shown in Fig. 3. It can be seen from the chromatogram that no appreciable peak can be observed in the region of the zearalenone, which has a retention time of 17.57 min.

3. Discussion

The results show that the investigated red pepper samples did not contain a detectable quantity of zearalenone by that method. Since the samples contained a greater or lesser quantity of Fusarium fungi, there was a potential danger of infection with zearalenone. Further investigations are required to determine whether the Fusarium fungi produce secondary metabolites on red pepper as a substrate. Since a positive answer can be expected to this question, it will definitely be necessary to compare the proclivity of one and the same strain of fungus to produce mycotoxine on a cereal substrate and on a pepper substrate.

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A MODIFIED METHOD FOR HYDROXYPROLINE (HOP) DETERMINATION IN MEAT AND MEAT PRODUCTS

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A simplified method for HOP determination based on the PDAB (p-dimethyl-amino-benzaldehyde) reaction has been developed and compared with the ISO method and Szeredy's method (modified Hurych and Chvapil's method) using analysis of variance and Deming's regression analysis. The mathematical analysis of the data showed no significant difference and the methods proved to be equally sensitive. For the oxidation of HOP and colour development the method of Bergman and Loxey was applied with some modifications. The new procedure resulted in more colour yield compared with the ISO and Szeredy's method. Instead of the time-consuming manual neutralisation of the acid hydrolysate the aliquots are buffered by the application of automatic dilutors and the reagents can be added in a similar way.

Owing to this modification, the procedure for HOP determination appears to be applicable in an automatic analyzer.

Keywords: hydroxyproline (HOP)-determination, HOP in meat products, spectrophotometry

Since 1950 several authors have recommended methods for the determination of connective tissue content by means of spectrophotometric determination of HOP. All these methods were based on the reaction of PDAB with pyrrol or pyrrol derivatives, but the conditions of oxidation and colour development were different.

According to SZEREDY[a] (1970) the oxidation of HOP with hydrogen peroxide, used by GRAU (1969), is somewhat less reproducible than oxidation with Chloramine-T (the sodium salt of p-toluene sulphon-chloramine). ARNETH and HAMM (1971) obtained the same result. Chloramine-T was also used by STEGEMANN (1958) and NEUMAN and LOGAN (1950). These methods could be reproduced well but the circumstances of the colour reaction were not clear, as reported by BERGMAN and LOXLEY (1963). In their study they investigated the optimal reagent concentration, time and temperature of the reaction. STEGEMANN and STADLER (1967) investigated the effect of the pH on the optical density of the coloured solution.

Hurych and Chvapil's method, which was modified by SZEREDY (1970), is somewhat less sensitive, but simpler than that mentioned above (BERGMAN & LOXLEY, 1963).

[a] In Hungary this method is generally considered as a reference procedure concordant with the ISO method.

The ISO method (1978) uses 6 *N* hydrogen chloride containing 0.75% $SnCl_2$ for hydrolysis under reflux. The hydrolyzed samples have to be neutralized using a pH-meter, which is a very time-consuming step. The neutralized solution must then be filtered again and washed free of HOP. This step is also laborious and a considerable source of error.

Although the spectrophotometric determination of HOP can be carried out in a reasonably short time, in practice the hydrolysis and neutralization of the samples involves time-consuming manual work.

In the present work an attempt was made to avoid this neutralization procedure and achieve a high colour yield. For the oxidation of HOP and colour development Bergman and Loxley's method was chosen with some modifications.

1. Materials and methods

1.1. Materials

1.1.1. Photometers: Coleman 6/20 Junior II (Perkin Elmer) and VSU-2 P (Karl Zeiss, Jena).

1.1.2. Oxidant solution: 1.5 g Chloramine-T dissolved in 100 cm^3 water and made up to 250 cm^3 with n-propanol. This solution is stable for two weeks at +4 °C in a dark bottle.

1.1.3. Colour developing solution: 37.5 g PDAB dissolved in 42.5 cm^3 70% perchloric acid and immersed in cold water. After chilling it is made up to 250 cm^3 with n-propanol. This solution is stable for two weeks if stored in a dark bottle at +4 °C.

1.1.4. Buffer: This is an acetate–citrate buffer, the pH value of which is 6.0. Two hudred and forty g sodium acetate (3 H_2O) is dissolved in a small amount of water and 68 g sodium hydroxyde, 100 g citric acid (H_2O) and 24 cm^3 acetic acid (96%) are added. After cooling it is made up to 2000 cm^3 with water.

1.1.5. Blank: 40 cm^3 6 *N* sulphuric acid containing 0.75% $SnCl_2$ is freshly made up to 250 cm^3 with water. Thirty cm^3 buffer is added to 1 cm^3 of this solution to avoid precipitation. This latter solution is very stable and is treated in the same way as the samples with oxidant and colour developing solution.

1.1.6. List of the samples: minced trimmed tendon (miscellaneous), bovine Achilles tendon, boneless beef neck (miscellaneous), sausage emulsion, boneless beef round (miscellaneous), neck-band (ligamentum nuclea) dried sausage (Hungarian salami), pork skin and boneless beef head.

1.2. Methods

The samples were minced in a cutter. Fat and moisture were extracted with a trichloromethane–ethanol mixture (4:1), followed by drying at 80 °C and powdering in an IKA apparatus (Janke and Kunkel KG).

Fat and moisture were removed by extraction and/or drying in order to lower the scatter caused by inhomogeneity. This is inevitable when making comparative tests with different methods. This method can also be used, of course, with the original sample without defatting and drying.

Depending on the expected HOP content, 0.1–2 g of powder was weighed and 40 cm^3 of 6 *N* sulphuric acid–$SnCl_2$ solution was added. The hydrolysis was carried out in a drying oven for 16 hours at 110 °C.

One cm^3 of the hydrolyzed solution (with an HOP concentration not exceeding 180 $\mu g\ cm^{-3}$) was pipetted into an Erlenmeyer flask (100 cm^3) and 30 cm^3 of buffer were added. One cm^3 of this neutralized solution was pipetted into a test-tube, 2 cm^3 oxidant solution were added and the mixture was shaken. After 5 minutes 2 cm^3 colour developing solution were added, after which the mixture was shaken and immersed for 25 minutes in a water-bath at 60 °C. Thereafter it was cooled in running tap water. The optical density was determined within an hour at 565 nm against a blank.

Preparation of the calibration curve: the standard solution had an HOP concentration of approximately 50 $\mu g\ cm^{-3}$. From this solution 0.5, 1, 2, 3 and 4 cm^3 were pipetted into Erlenmeyer flasks (100 cm^3) and 1 cm^3 blank was added, from which the buffer was still missing. The 30 cm^3 of buffer was added and mixed. 1 cm^3 of each solution was pipetted into the test-tubes and 2 cm^3 oxidant solution was added and shaken. After 5 minutes 2 cm^3 colour developing solution were added and the mixture was shaken again. The covered test-tubes were immersed in a water-bath at 60 °C for 25 minutes. After chilling in running tap water the optical density was measured within an hour.

2. Results and discussion

So far, the effect of pH 6 values on colour development has not been investigated and indicator paper or a pH-meter have been used for the neutralization. STEGEMANN and STALDER (1967) used 6 *N* HCl for hydrolysis and removed the HCl in an evaporator, so neutralization was not necessary. However, as Fig. 1 shows, if the pH value decreases, the colour intensity decreases, too. It was also established that the colour intensity between pH 6 and 7 did not differ from the colour intensity at pH 6. STEGEMANN and STALDER (1967) investigated the effect of pH on the colour at above pH 6 and found that pH 8 was also suitable for the test. In this modified test the pH was adjusted to 6 using an acetate–citrate buffer.

The absorbance curve of the coloured solution is shown in Fig. 2. The absorbance maximum is at 565 nm, which differs from the values obtained by other authors (SZEREDY, 1970; BERGMAN and LOXLEY, 1963).

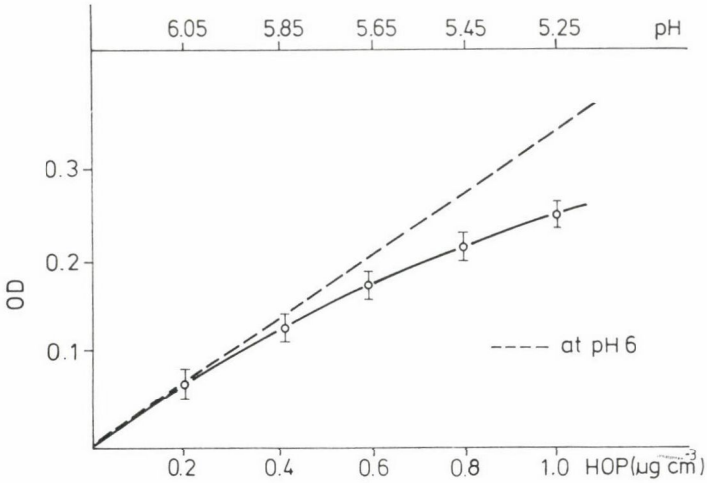

Fig. 1. Relationship between pH value and colour intensity using the modified method for HOP determination

As it was found by PINDUR (1978), thed ifferent pyrrol derivatives may vary. This is presumably the cause of the differences in the absorption curves.

The calibration curves are shown in Fig. 3. Comparing these curves, it can be seen that they have different slopes, the modified method having the steepest one. It means that the appearance of the deviation (σ_δ) deriving from measuring errors in HOP content deviation (σ_m, σ_{ISO}, σ_{sz}) is different in the various calibration curves and it is the lowest in the modified method.

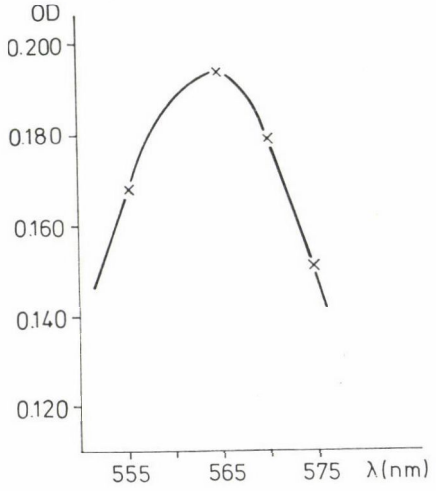

Fig. 2. Absorbance curve of the coloured solution using the modified method for the determination of the absorbance maximum of HOP

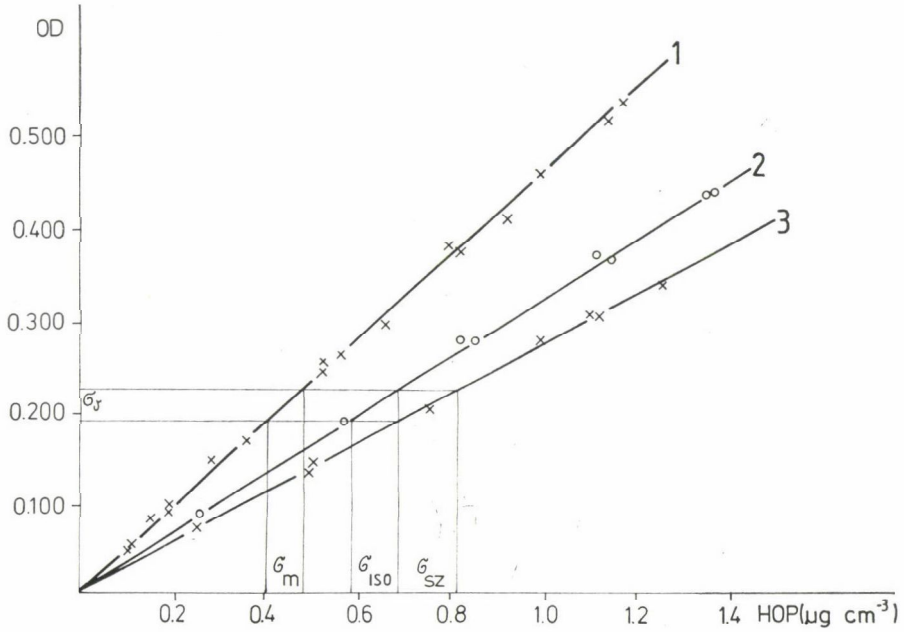

Fig. 3. Relationship between optical density and HOP concentration
1: modified method, $y = 0.0069 + 0.04493x$, $r^2 = 0.9999$; 2: ISO method, $y = 0.0043 + 0.2813x$, $r^2 = 0.9998$; 3: Szeredy's method, $y = 0.01 + 0.2358x$, $r^2 = 0.9984$

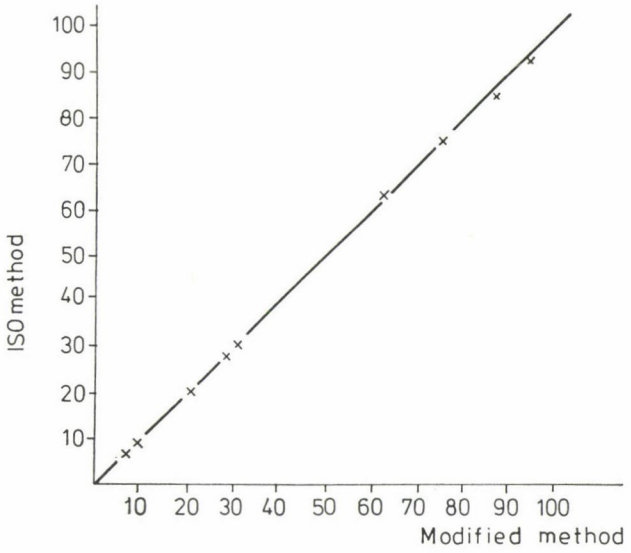

Fig. 4. Relationship between the ISO method and the modified method for HOP determination
(The values are expressed in $8 \times g$ HOP per 100 g dry powder)

Table 1 shows the recovery of HOP added to 22 different samples (beef with various contents of fat and connective tissue). As can be seen, there was no significant difference at the 5% probability level between added and recovered HOP. This confirms the reliability of the proposed modified method.

Nine different samples were compared by the ISO method, Szeredy's method and the modified method (Table 2). The results show that the methods give no significant difference, and no significant interaction (method × sample)

Table 1

Recovery of added HOP in different meat samples by the modified method

Samples	HOP added (μg)	HOP recovered (μg)	HOP added (μg)	HOP recovered (μg)
1	1.1089	1.093	2.1819	2.185
2	1.1089	1.093	2.1819	2.289
3	1.1089	1.104	2.1819	2.116
4	1.1089	1.081	2.1819	2.139
5	1.1089	1.139	2.1819	2.254
6	1.1089	1.058	2.1819	2.197
7	1.1089	1.035	2.1819	2.208
8	1.1089	1.127	2.1819	2.174
9	1.1089	1.070	2.1819	2.254
10	1.1089	1.116	2.1819	2.277
11	1.1089	1.081	2.1819	2.162
12	1.1089	1.070	2.1819	2.162
13	1.1089	1.104	2.1819	2.116
14	1.1089	1.093	2.1819	2.139
15	1.1089	1.081	2.1819	2.162
16	1.1089	1.127	2.1819	2.128
17	1.1089	1.070	2.1819	2.105
18	1.1089	1.070	2.1819	2.162
19	1.1089	1.047	2.1819	2.070
20	1.1089	1.127	2.1819	2.070
21	1.1089	1.093	2.1819	2.105
22	1.1089	1.127	2.1819	2.082
$\bar{x}$	1.1089	1.0925	2.1819	2.1620
$\pm s$	—	0.0391	—	0.0642
CV	—	3.58	—	2.97
t		1.89		1.45

$\bar{x}$: mean values
$\pm s$: standard deviations
CV: coefficient of variation
t: Student's parameter

Table 2

Means ($\bar{x}$) *and standard deviations* ($\pm s$) *of samples*

Samples	ISO method		Szeredy's method		Modified method	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Minced trimmed tendon	63.18	1.061	61.12	0.775	62.65	1.907
Bovine Achilles tendon	93.83	0.714	93.96	2.206	92.63	2.684
Boneless beef neck	19.51	1.091	19.82	0.864	19.67	1.091
Pork skin	77.51	1.139	74.16	1.108	75.01	2.474
Boneless beef head	30.34	1.037	28.07	0.599	28.19	1.434
Sausage emulsion	8.92	0.410	8.55	0.325	8.47	0.384
Boneless beef round	6.07	0.445	6.03	0.235	5.89	0.255
Neck-band	30.36	1.016	30.23	0.814	30.07	0.061
Dried sausage	8.18	0.397	8.58	0.423	8.46	0.581

(The mean values and the standard deviations expressed in $8 \times \mu g$ HOP per 100 g dry powder)

occurs (Table 3). The methods were compared by analysis of variance with two-way classification. The analysis was made with the logarithm of the data; the standard deviation was non-homogeneous but the coefficient of variation was nearly constant.

A comparison of the ISO method and the modified method was carried out using regression analysis (Table 4, Fig. 4) according to Deming (MANDEL, 1964; ZUKÁL et al., 1970). As Fig. 4 shows, the regression curve can be approx-

Table 3

Analysis of variance on the base of data in Table 2

Factors	SSQ	DF	MS	F
Total	74686.83	80		
Methods	12.16	2	8.081	4.389
Samples	75574.35	8	9321.794	6728.150***
Interaction (method × samples)	25.50	16	1.594	1.150
Residual	74.82	54	1.385	

*** : very highly significant at $P \geq 99.9\%$ probability level
SSQ : sum of square sum
DF : degree of freedom
MS : means of square
F : mathematical variant

Table 4

Regression constants, value of standard errors and ratio of sensitivity for the relationship shown in Fig. 4

a	b	S_a	S_b	$E_{y/x}$
—0.0996	0.983	0.447	0.0092	1.01665

$y = a + bx$
$E_{y/x}$: ratio of sensitivity
S_a and S_b: standard errors of "a" and "b"

imately described with the equation y = x. Thus, no systematic deviation can be established between the two methods. The ratio of sensitivity is nearly 1, showing that both methods are equally sensitive. The disadvantage of the other methods mentioned in this paper is the numerous manual steps of which the neutralization procedure in particular is not suitable for automation.

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BOOK REVIEW

Food Irradiation Now

Martinus Nijhoff/Dr. W. Junk Publishers, The Hague, 1980, 157 pages.

On October 21, 1981 at the occasion of its 10th anniversary the GAMMASTER Irradiating Enterprise (Ede, Holland) organized a symposium under the title: Food Irradiation Now. The main part of the book consists of the papers read at the symposium. The book contains 157 pages, including 17 tables and 18 figures. The Preface and Introduc tion are followed by the eight papers, the questions and the answers heard at the panel discussion, the report of 1980 of the FAO/WHO/IAEA Joint Expert Committee (Wholesomeness of irradiated food) and finally by a small glossary and the addresses of the authors. The authors and their papers are the following:

D. A. A. Mossel and P. van Netten (University of Utrecht, Faculty of Veterinary Medicine, Utrecht): Whither protection of the consumer against enteropathogenic bacteria on fresh meats and poultry by processing for safety. Food poisoning frequently occurring even in the most developed countries too, is usually transmitted by foods of animal origin. These enteric infections cannot be eliminated even by the highest slaughterhouse hygiene in itself. Only a terminal processing is capable of protecting the health of the consumer, similar in its aim and effect to the pasteurization of milk. The authors find only two processes suitable for this purpose: treatment of the meat surface with a diluted lactic acid solution or radicidation, the treatment of the enteral pathogens with irradiation.

E. H. Kampelmacher (University of Agriculture, Wageningen): Public health aspects of food irradiation. In the USA about 25 000 people come under medical attendence per year because of salmonellosis, but the true number is considered to be about 2 million. In addition to salmonellosis lately the campylobacteriosis is spreading, too. A radiation dose of 2–5 kGy after processing and packaging is very efficient in destroying the Salmonella and Campylobacter strains and eliminates the danger of reinfection.

D. Is. Langerak (ITAL, Wageningen): Irradiation of foodstuffs — Technological aspects and possibilities. The author gives an account of the fields of application where radiation treatment in itself or in combination with other known preserving technologies was found suitable to extend the shelflife of foods of plant origin.

J. G. Leemhorst GAMMASTER, Ede): Industrial application of food irradiation. Describes the equipment (container plant) used earlier in food irradiation and in the sterilization of therapeutical instruments and the pallet facility put into operation in 1982 (capacity, investment, operation and irradiation costs).

J. Ch. Cornelis (Ministry of Health and Environmental Protection, Rijswijk): The government and food irradiation — National and international rules and regulations. Contains the national and international Food Acts. The author considers the main problem that preservatives may be detected while radiation treatment is nondetectable. This involves the necessity of much more detailed international agreements than on other matters.

R. M. Ulman (Food Irradiation Scrutinizing Body, The Hague): 14 years clearing irradiated foods in the Netherlands. Story of food irradiation in the Netherlands.

J. G. van Kooij (International Atomic Energy Commission, IAEA, Vienna): International aspects of food irradiation. An account is given of the clearances, provisional or unconditional, granted by various countries and for various food items. The activities of the different international organizations are described. The result of these activities is the, after several modifications finally accepted Recommended International General Standard for Irradiated Foods and the Recommended International Code of Practice for the Operation of Radiation Facilities. These are distributed to all the countries concerned to adapt them to their national Food Acts and food control systems without changing the basic principles. It is the precondition of the free international trade of irradiated foodstuffs to co-ordinate national legislation. In this case every country has faith to buy irradiated foods from the other country.

F. Defesche (Young & Rubicam Koster B. V., Amsterdam): How does the consumer react to irradiated food? In the consciousness of the consumer irradiation

is in close association with cancer and the atom bomb. The author gives useful advice how to apply psychology in convincing the consumer.

Ionizing radiation is one of the most modern tools against bacterial spoilage and contamination of foods. The research works carried out all over the world in the last twenty years have shown this technique to be effective while being wholesome and safely applicable. This book will aid in convincing the experts and officials of the countries who doubt the benefice of this technique and hesitate to grant clearance to its use.

GY. ZACHARIEV

ANNOUNCEMENT

INTERNATIONAL CONFERENCE ON PHTHALIC ACID ESTERS

In conjunction with the IX International Congress of Pharmacology (London), a Post-Congress Satellite Symposium on the Phthalate Acid Esters will be held on August 6–7, 1984, at the University of Surrey, Guildford, England. For further details please contact Professor J. W. Bridges, University of Surrey or Dr. John A. Thomas, Travenol Laboratories, Inc., Morton Grove, Illinois

RECENTLY ACCEPTED PAPERS

Toxicological studies on jute (Corchorus olitorius) seed protein
GHOSH MAJUMDAR, S., LASKAR, S., BASAK, B. & MAITY, C. R.
Quantitative determination by computerized spectrum analysis of the pigment components in ground paprika
VARGA, L., FEKETE, M. & KOZMA, L.
Correlation analyses of the SDS test and valorigraph values of autumn wheat varieties
PALLAGI-BÁNKFALVI, E. & MATUZ, J.
Investigation of plasteins by SDS polyacrylamide gel electrophoresis
DELINCÉE, H. & HAJÓS, GY.
Symposium on Food Microbiology, Budapest, 1983

NOTICE TO CONTRIBUTORS

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

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

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

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

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

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

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

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

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

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CONTENTS

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ACTA ALIMENTARIA

An International Journal of Food Science

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TOXICOLOGICAL STUDIES ON JUTE (*CORCHORUS OLITORIUS*) SEED PROTEIN

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Jute (*Corchorus olitorius*, family: *Tiliaceae*) seed protein was isolated from matured jute seeds. The protein is tolerable to albino rats as evidenced from biochemical studies (haemoglobin percentage, blood sugar, blood urea, blood cholesterol, plasma protein and albumin–globulin ratio).

Keywords: Jute seed protein, toxicology, non-edible seeds

Attempts have been made to utilize proteins, fats and oils of non-edible seeds — such as sal (GANDHI et al., 1975a; 1975b), mowrah (MULKY & GANDHI, 1977) and teak (GHOSH MAJUMDAR et al., 1980) for human and animal consumption. It is a very urgent necessity to meet the increasing demand of traditional food materials. The seeds which are rich in either proteins or oils or rich in both may be utilized after thorough investigation of the toxic principles present therein.

In the present paper we want to report the toxicological studies on jute seed protein in an attempt to utilize it for human and animal consumption.

1. Materials and methods

1.1. Isolation of protein from jute (C. olitorius) *seed*

Extraction of protein was carried out from deoiled jute *(C. olitorius)* seed by 10% aqueous sodium chloride solution (pH was maintained between 10 to 12 by adding 0.5 *M* sodium hydroxide solution). Protein was then precipitated with a 10% TCA (trichloroacetic acid) solution, isolated by centrifuging at 5000 r.p.m. for 10 minutes. The process was repeated twice with the same protein; ultimately it was washed well, dried under vacuum and stored in a refrigerator.

The protein (nitrogen content 15.24% estimated by micro-Kjeldahl method) gives violet colour readily when subjected to Biuret reaction. It was hydrolysed with 6 *N* HCl for 24 h and the following amino acids were detected from the hydrolysate with the help of two dimensional paper chromatography using butanol–acetic acid–water (4 : 1 : 5) and phenol–water (80 : 20) systems.

The amino acids detected from the hydrolysate are: lysine, valine, histidine, aspartic acid, glycine, serine, alanine, tyrosine, glutamic acid, proline, leucine, isoleucine.

As the protein firmly responds to the Molisch test, a glycogen part may be present as non-proteinous residue in the protein.

1.2. Feeding experiment with the isolated protein

Eighteen local inbred albino rats (age 105–120 days; reared in the Department of Bio-Chemistry, Burdwan) were divided into two groups, control group (six rats of an average weight of 130.67 g) and experimental group (twelve rats of an average weight of 129.45 g). All the rats of both groups were housed individually in wire net cages.

Fifteen gram of normal diet — the diet was prepared from flour and milk powder(6 : 1 ratio by weight), common salt (1.0 g per 100 g diet), sugar (5 g per 100 g diet) and multivitamin tablets (4 tablets per 100 g diet) of Glaxo, India — was given daily to each rat of the control group. The above normal diet (nitrogen content 2.19%), chemical composition of which is given in Table 1, mixed with the jute seed protein in the ratio of 9 : 1 (by weight) and 15 g of this protein enriched diet (nitrogen content 3.49%) was given to each rat of the experimental group. This protein enriched diet was used in the experimental group with a view to study as to whether such diet possesses any toxic substance which might cause any deleterious effect on the test animals.

Before feeding, blood sugar (SOMOGYI, 1952), blood urea (SOM et al., 1958), total cholesterol (NATH & GHOSH, 1962), haemoglobin percent (COHEN & SMITH, 1919), plasma protein (REINHOLD, 1953) of each rat in both groups were determined. Total food intake of each rat was recorded daily and the body weight of each rat was taken before and after the experiment. After 30 days the above biochemical parameters were determined again for each rat in both groups.

2. Results

It was observed that except haemoglobin percentage, all other biochemical parameters (i.e. blood sugar, blood urea, total cholesterol, plasma protein) and body weights of the rats in the experimental group increased in comparison

Table 1

Chemical composition of normal diet for 100 g

Protein (g)	Fat (g)	Carbohydrate (g)	Crude fibre (g)	Salt (g)	Multivitamin[a] (g)	Moisture and trace elements (g)
13.68	4.06	69.95[b]	1.34	1.00	2.43	7.54

[a] Manufactured by Glaxo, India (multivitamin tablets, each contains Vitamin A I.P. 5000 I. U, Vitamin D_3 1000 I. U, Vitamin B, I.P. 3 mg, Riboflavin I.P. 2 mg, Nicotinamide O.P. 20 mg, vitamin C I.P. 30 mg, Calcium dibasic phosphate I.P. 250 mg and appropriate averages added as given by manufacturer)

[b] including 56.09 g starch

Table 2

Body weight, total food intake and FER-values of the control and the experimental groups

Parameters	Control group		Experimental group	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
No. of rats	6		12	
Body weight before feeding (g)	130.67	1.51	129.45	2.04
Body weight after feeding (g)	148.50	2.43	161.70	3.24
Weight gain (g)	17.83	1.17	32.25	1.66
Average food intake (g)	324.33		351.25	
Average food efficiency ratio	0.0549		0.0918	

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

Table 3

Values of the different biochemical parameters in the control and the experimental groups

Blood parameters	Control group (n = 6)				Experimental group (n = 12)			
	Before feeding		After 30 days feeding		Before feeding		After 30 days feeding	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Haemoglobin (g%)	14.97	1.26	15.17	1.18	14.66	0.57	14.96	0.63
Urea (mg%)	24.17	1.03	25.55	0.88	36.04	1.57	40.58	2.17
Sugar (mg%)	100.00	10.49	105.00	10.49	101.25	3.11	115.42	3.34
Plasma protein (g%)	6.25	0.22	6.42	0.13	6.15	0.25	7.17	0.19
Cholesterol (mg%)	56.67	3.78	57.50	3.89	67.25	3.68	74.40	3.68
A:G ratio	1.73	0.09	1.88	0.12	1.89	0.18	1.68	0.19

n = number of rats
$\bar{x}$ = mean value
$\pm s$ = standard deviation

Table 4

Statistical comparison of the biochemical parameters and body weights in the experimental and the control groups after 30 days

Parameters	Inrease				Probability level
	Control group		Experimental group		
	$\bar{x}$	σ	$\bar{x}$	σ	
Blood haemoglobin (g%)	0.20	0.05	0.29	0.04	$P > 0.1$
Blood urea (mg%)	1.38	0.45	4.54	0.61	$P < 0.001$
Blood sugar (mg%)	5.00	1.29	14.17	0.56	$P < 0.001$
Blood cholesterol (mg%)	0.83	0.54	7.14	0.001	$P < 0.001$
Plasma protein (g%)	0.17	0.05	1.07	0.05	$P < 0.001$
Body weight (g%)	17.83	0.48	32.25	0.48	$P < 0.001$

$\bar{x}$ = mean value
σ = standard error

to those of the control group (Tables 2, 3). The increase in the last four of the above biochemical parameters and the body weights in the experimental group was highly significant (Table 4). In the control group the albumin – globulin (A : G) ratio increased while it decreased in the experimental rats (Table 3).

3. Discussion

In order to ascertain whether jute seed protein was suitable for human and animal consumption or not, some biochemical parameters including body weight were chosen.

No doubt, the significant increase in body weight in the experimental group during experiment was due solely to protein administration. Moreover, increase in body weight is directly related to increase in cholesterol. This fact was observed in the present investigation. It is a well-known fact that urea is formed solely in the liver from the catabolism of amino acids. The concentration of urea in the blood represents mainly a balance between urea formation from protein catabolism and urea excretion of the kidneys. As there was no detectable renal disease (monitored through pathological experiments), urea level increase in experimental rats was due to the protein-rich diet which was also responsible for significant increase in plasma protein compared to that in the control group.

Significant increase in blood sugar was due to the non-nitrogenous residue of the protein. This indicates a further confirmation of the presence of glycoprotein (LASKAR et al., 1983) in the jute seed. It is well known that the A : G ratio has no value in toxicological studies but the determination of the ratio after the experiment clearly indicates the formation of more globulin than albumin in the experimental group. This was further supported by estimation of

the albumin and globulin separately. The formation of more globulin in the experimental rats was another proof of the presence of glyco-protein in jute seed. Higher F E R-values (food efficiency ratio) in experimental rats showed that the protein-enriched diet was more growth promoter than the normal diet.

Although a significant increase of all parameters excepting haemoglobin percentage, was observed in the case of the experimental group, they remained within the normal pathological limits and also there was no mortality. Therefore it may be concluded that jute seed protein appears to be non-toxic for the parameters investigated.

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THE ENZYMIC IN VITRO DIGESTIBILITY OF METHIONINE-ENRICHED PLASTEIN

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Incorporation of limiting amino acids into proteins by enzymic modification seems to be a suitable method for increasing the nutritive value. By application of plastein reaction, L-methionine has successfully been incorporated into proteolytic casein partial hydrolysate.

The significantly higher value of methionine in plasteins, in comparison to the substrate, is explained by incorporation of L-methionine methyl ester during the enzymic reaction. In vitro experiments carried out under physiological conditions (with gastrointestinal enzymes) showed that digestibility of the methionine-enriched plastein product is similar to that of the intact casein. Liberation of methionine was found very high.

We have shown that the methionine-enriched plastein product does not involve bonds less digestible than the intact casein.

Keywords: plastein reaction, in vitro digestibility, amino acid analysis, enzymic hydrolysis

During the two last decades the utilization of non-conventional protein sources for human and animal nutrition became one of the most important problems of research on food technology and food science.

Unfortunately, the amino acid composition of such novel proteins like plant and single cell proteins do not cover the physiological need of some essential amino acids. Protein fortification for nutritional purposes by amino acid supplementation, however, is not quite suitable because amino acids supplied to the organism in free form are not as effective for metabolic processes as protein bound amino acids (ALTSCHUL & HARON, 1974).

The enzymic modification of proteins — the so called plastein reaction (YAMASHITA et al., 1970) — seems to be an effective tool to overcome this problem.

The aim of our studies was to increase the nutritive value of protein by incorporation of the limiting essential amino acid into the protein by application of plastein reaction. The experiments were carried out with proteolytic partial hydrolysate of casein, whereas L-methio nine methyl ester was used as source of amino acid. In our earlier studies we found that essential amino acids form covalent linkage to the protein under conditions of the plastein reaction (HAJÓS & HALÁSZ, 1982).

The fact that the protein undergoes a modification during the plastein reaction raises the question of how and to what extent the digestibility suffers modification due to the structural changes.

For this reason, in vitro digestibility of the methionine-enriched plastein and of the starting protein was studied. The enzymic in vitro digestion is a suitable method for testing proteins with regard to structural alterations and possible nutritional important protein damages as result of technological processes like heating and/or pressure and others. Due to the method used here proteins are digested by a combination of gastrointestinal enzymes. The amount of the enzymically liberated amino acids should be a measure of the overall digestibility by gastrointestinal enzymes as well as an indicator of the extent and the position of the protein damages and therefore a measure of the nutritional quality of the protein sample examined. The aim of the enzymic in vitro hydrolysis of the methionine-enriched plastein was to characterize its enzymic digestibility by physiological proteases and peptidases of the gastrointestinal tract compared to the digestibility of the intact casein and to estimate the availability of incorporated methionine. The enzymic amino acid liberation indicates whether the plastein reaction results in the formation of less digestible bonds in the molecules.

1. Materials and methods

1.1. Enzymes and materials

Commercial quality of pepsin (Merck), papain (Sigma), α-chymotrypsin (Sigma) and L-methionine methyl ester hydrochloride (Riedel de Haen AG) were used.

1.2. Protein hydrolysate

Casein was hydrolyzed with pepsin and papain, respectively (HAJÓS & HALÁSZ, 1982).

1.3. Substrate

The substrate of the plastein reaction was a soluble, non-dialysable fraction of the casein hydrolysate (HAJÓS & HALÁSZ, 1982).

1.4. Plastein reaction

The plastein reaction was carried out with α-chymotrypsin. The substrate concentration was 35% (w/v); the enzyme–substrate ratio was 1:100; pH: 5.5; incubation temperature: 37 °C; incubation time: 65 h.

A methionine methyl ester hydrochloride-substrate ratio of 5:100 was used for the methionine incorporation process. The enzymic resynthesis was carried out without shaking or mixing.

1.5. Enzymes for the in vitro digestibility test

Pepsin and pancreatin were commercial products from VEB Berlin-Chemie, GDR.

Isolated brush borders from the small intestinal mucosa of rats served as source for the intestinal peptidase activity. For each enzymic incubation they were freshly prepared according to the method of NOACK and SCHENK (1965).

The leucine-aminopeptidase activity (substrate leucine-β-naphthyl--amide) was used as indicator for the aminopeptidase activity of the isolated brush borders. The aminopeptidase activity of the isolated brush borders amounted to 0.116 μmole per mg protein per min. The incubation was carried out by the use of a brush border suspension in (0.2 M sodium phosphate buffer, pH: 8.0). One cm^3 brush border suspension contained 4.5 ± 0.8 mg protein.

1.6. Amino acid analysis

Determination of the amino acid composition has been carried out after a 24 h hydrolysis of the protein samples with the reflux of 6N HCl.

The S-containing amino acids, methionine and cystine, have been determined after performic acid oxydation of the protein samples followed by acidic hydrolysis (KRAMPITZ, 1957).

For the determination of the enzymically liberated amino acids the deproteinized enzymic digest has been analysed directly without acidic hydrolysis. The amino acid analysis has been carried out by column chromatography according to SPACKMAN and co-workers (1958) using Hd 1200 E amino acid analyzer (ZSNP Ziar nad Hronom, CSSR).

1.7. Method of the enzymic hydrolysis

The enzymic digestibility of the methionine-enriched plastein and of casein (the protein source for the plastein synthesis) has been determined by the use of a standardized in vitro hydrolysis method (UHLIG, 1974).

According to this method the protein samples have been digested successively by pepsin, pancreatic enzymes (pancreatin) and by peptidases of the brush border region of the small intestinal mucosa of rats.

The procedure was as follows:

A plastein sample equivalent to 60 mg protein was suspended with 5.0 cm^3 of (0.2 M sodium citrate buffer, pH: 1.8) and incubated in the following manner:

	Incubation steps		
	1	2	3
Enzyme	pepsin	pancreatine	brush border suspension
Amount of enzyme	0.36 mg equivalent to an activity against casein of $0.0479 \times 10^{-2} E_{280}^{1cm} min^{-1}$	1.2 mg equivalent to an activity against casein of $0.175\ E_{280}^{1cm} \times min^{-1}$	3.0 cm^3 equivalent to an activity of 1.56 μmole β-naphthylamine $\times min^{-1}$
Temperature (°C)	37	37	37
Time of incubation (h)	3	3	24

After finishing the peptic digestion the pH value of all samples was adjusted to 8.0 (addition of about 1.9 cm^3 1*N* NaOH + 5.0 cm^3 Na-phosphate buffer).

Inactivation of the pancreatic enzymes (after finishing the second incubation step) was carried out by heating the samples for 15 minutes at 100 °C (boiling water bath).

During enzymic digestion the samples were shaken. The rate of enzyme autolysis was tested for each enzymic digestion by incubation of blank samples (enzyme solutions + buffer instead of substrate).

After the last incubation step (with brush border suspension) was finished the samples were deproteinized by trichloroacetic acid (10% w/v). The enzymatically released free amino acids have been determined by column chromatography.

2. Results

2.1. Plastein reaction

The most important factors in plastein reaction are:

– the type of the enzyme and the pH value used,

– molecular mass distribution of the substrate, namely the size of proteins and peptides.

Besides of these main factors, the reaction is also influenced by the amino acid composition and conformation of the protein. Our experiments were carried out with proteolytic hydrolysate of casein. The cleavage was carried out with two enzymes: pepsin and papain, for 24 hours in each case. This method, however led to a relatively high ratio of small peptide fractions. The value of degree of hydrolysis (α_H) determined according to ARAI and coworkers (1975), was found to be 85% (HAJÓS, 1979; HAJÓS, 1981). Therefore the proteolytic hydrolysate of casein obtained as described above did not seem to be a suitable substrate for plastein reaction.

The insoluble, highest molecular mass fraction was separated by centrifugation. The amino acids and the small peptide fractions, which are also not suitable for the enzymic resynthesis were removed by dialysis. The remaining peptide solution was lyophylized and by this method, a suitable substrate for the plastein reaction was obtained.

Into this substrate, methionine was incorporated in the presence of α-chymotrypsin, at 37 °C. The essential amino acid was brought into reaction in activated form as methionine methyl ester. After completing the reaction the excess amount of amino acid ester was removed by dialysis.

The molecular weight characteristics of polypeptides synthesized by the plastein reaction, thereby leading to the formation of new bonds between the substrate peptides in a protein hydrolysate, are of considerable interest. Therefore, an attempt was made to separate plasteins with the aid of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. By this method polypeptides can be fractionated according to their molecular weight. The plastein patterns indicated that mainly transpeptidation took

Table 1

Amino acid composition of a methionine-enriched plastein, of its substrate and of casein (g amino acid per 16 g N)[a]

Amino acid	Plastein	Substrate	Casein
Lys	9.2	9.0	7.9
His	3.9	3.8	2.5
Arg	2.7	2.9	3.3
Asp	6.1	6.6	7.2
Thr	3.6	3.9	4.1
Ser	6.4	6.3	5.7
Glu	21.1	22.9	24.6
Pro	12.0	11.8	11.8
Gly	1.9	1.8	1.8
Ala	3.0	2.9	3.1
Cys	0.8	0.4	0.4
Val	6.8	6.7	6.3
Met	3.9	2.3	2.4
Iso	6.0	5.9	5.3
Leu	8.5	8.2	10.2
Tyr	4.2	4.1	5.8
Phe	5.7	5.4	5.3

[a] The measuring error is about 10%

place during the enzymatic resynthesis reactions. In certain cases, (e.g. in the presence of papain) however, condensation reactions also occur (DELINCÉE & HAJÓS, 1984).

2.2. *Amino acid composition*

The amino acid composition of the methionine enriched plastein compared to that of the substrate and casein is given in Table 1.

The amino acid compositions of casein and substrate are evidently not the same, because the substrate was obtained after centrifugation and dialysis of the proteolytic hydrolysate of casein.

The difference between the amino acid composition of the methionine enriched product and of the substrate does not materialize in methionine content only, because at the end of the reaction, dialysis was used for removal

Table 2

Enzymically liberated amino acids from plastein and from casein[a]

Amino acid	Liberated amino acid (g amino acid per 100 g protein)		Percentage of the total amount	
	plastein	casein	plastein	casein
Lys	6.7	5.0	72.8	63.3
His	2.6	2.0	66.7	80.0
Arg	2.3	3.0	85.2	90.9
Asp	0.5	0.8	8.2	11.1
Thr	1.1	1.3	30.6	31.7
Ser	1.7	2.3	26.6	40.4
Glu	1.8	1.6	8.5	6.5
Pro	1.9	2.1	15.8	17.8
Gly	0.4	0.7	21.1	38.9
Ala	1.3	1.6	43.3	51.6
Val	2.8	2.8	41.2	44.4
Met	3.9	2.2	100.0	91.7
Ile	2.8	2.1	46.7	39.6
Leu	4.9	5.7	57.6	55.9
Tyr	3.2	4.8	76.2	82.9
Phe	3.9	4.6	68.4	86.8
Total amount of free amino acids	41.8	42.6		

[a] The measuring error is about 10%

of the excess amino acid ester. Thus, some amount of small peptide fractions or amino acids formed by transpeptidation during the reaction (YAMASHITA et al., 1973) could have been removed by dialysis.

In comparison to the substrate we found only a difference up to 10% in amino acid content, which lies within the error of the technique applied. The value of methionine content represents an exception, which was found to be the same in the substrate and in casein.

Compared the amino acid composition of the plastein product to the amino acid composition of casein used as the source for the enzymic hydrolysis, the methionine enrichment amounts to about 63%.

At the same time the plastein formation results in a twofold increase in the cystine content. The higher cystine content of the plastein is also advantageous nutritionally because under physiological conditions cystine is able to partly replace methionine.

2.3. Enzymic amino acid liberation

The results of the enzymic in vitro hydrolysis of the plastein and of casein are summarized in Table 2.

The enzymic liberation of amino acids is expressed in two different ways: as the absolute amount of enzymatically liberated amino acids (g per 100 g protein) and as the percentage of liberation, i.e. the proportion of the total amount of each amino acid which could be enzymatically released as free amino acid (total amount see Table 1).

3. Conclusions

Results of the enzymic hydrolysis of the plastein lead to the following conclusions:

The total amounts of the enzymatically liberated amino acids (g per 100 g protein) from plastein and from casein, are equal. Former digestion studies with casein and other proteins (UHLIG, 1974) have demonstrated that a total liberation rate of 42 g amino acids per 100 g protein is maximal under the experimental conditions used.

Similarly to the total amount of free amino acids, the liberation rates of the single amino acids from plastein and from casein do not show large differences.

The different liberation rates especially of three amino acids (serine −34%, glutaminic acid + 30% and glycine −46%) in plastein compared with casein could be the consequence of alterations in the amino acid sequence as a result of the plastein reaction.

The reduced release of glycine and serine as reflecting protein damages in the course of the plastein reaction, can be excluded. From previous experiments it is known that under such conditions less digestible peptides are formed consisting of more than two amino acids and leading to decreased liberation rates for the majority of different amino acids.

Considering that the enzymic total amino acid liberation as well as the liberation of all the essential amino acids are similar compared to casein, it can be concluded that such structural alterations induced by the plastein reaction exert no negative effect on the nutritional value of the methionine-enriched plastein.

The enzymic availability of methionine from the methionine-enriched plastein and from casein is equal. Compared with the liberation rates of all the other amino acids from both protein substances the methionine liberation is very high. These results are in agreement with results of previous enzymic digestion studies which have demonstrated the high liberation rate of methionine from native proteins by the gastrointestinal proteolytic enzymes used here.

In conclusion it can be said that the methionine-enriched plastein is digestible by physiological proteases and peptidases just as well as casein. Casein is known as a well digestible protein under physiological conditions.

Present results favour the view that the plastein reaction does not reduce the enzymic digestibility of the bonds in the molecule.

In further studies it is intended to examine the behaviour of methionine-enriched plastein under in vivo conditions in view of nutrition.

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THE DESIGN AND PRACTICAL USE OF AN OVERALL QUALITY INDEX FOR FOOD PRODUCTS

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Within the framework of the adaptation and continued development of the qualimetric method applicable to any product, the different pattern recognition procedures (such as cluster and discriminant analysis) can be readily used to select properties decisive for quality, to determine weighting factors and to develop limit values for classification. In case linear transformation and well founded weighting factors are used, the error in the complex quality index is very simple to determine through the method of error spread. The selected example (black currant nectar) illustrates the calculation procedure of the complex quality index and its suitability to evaluation.

Keywords: Quality index for food products, qualimetric methods, pattern recognition procedures, weighting factor

1. Materials and methods

At the present time the issue of product quality is a major subject of food research and production throughout the world. Quality is a concept that is based on a number of product properties that basically determine their level of suitability to a concrete and predetermined use. The food articles play a particular role in the supply of population and cannot be classed at all into the general category of consumers' goods. Foodstuffs satisfy basic biological requirements and they are interchangeable only to a limited extent at the most.

Unlike other consumers' goods, their particular role is featured by their satisfying immediate needs. It can be gathered from these few hints as well that food quality is a special case of quality determination.

In order to formulate an evaluation pattern, the concept of food quality is outlined as follows (MOLNÁR et al., 1979):

The quality of food products, in conformity with consumer requirements, is determined by the following criteria: sensory properties expressing their hedonic value, composition featuring biological nutritive value and useability, physical status, microbiological and other properties determining the keeping quality as well as packaging method and labelling. Another peculiar trait of food quality is the hierarchical and dynamic interrelation of almost all of its

properties. For this reason, in formulating an evaluation system for food products, the analytical investigation of interrelations should not be disregarded.

From the outlined quality definitions it also follows that quality is a convention and it may therefore be considered as constant over a shorter period only, which — beyond the absolute level of product characteristics — is also dependent on the base values specified in various descriptions (like standards). The variations of product characteristics determine the change in quality only if the specified base values and the conditions of their determination (test, measurement) are unaltered. Evaluation is in fact a comparison with an "étalon", which means the location of the parameters of product properties along a "standard" scale.

For the quantification of quality, qualifying methods with so-called quality indices have been introduced. The research and development of evaluation methods with quality index have recently been included into the research programme of several research institutes and of establishments concerned with the development of quality control. A reason for this lies in the fact that by the research and development of methods necessary for complex food evaluation results are acquired which are very important in the process of innovation and quality control. Another principal reason for doing so may be concluded from the fact that in recent years food research has revealed a lot of new knowledge, interdependency and compositional and physical product features whose synthesis and rational presentation are very timely as a task. In addition, there are high performance computers available for the analysis and processing of the existing or issuing data.

The calculation of the overall quality index is based on the transformation by a predetermined method, of the parameters of product features obtained by measurement or scoring into dimensionless numbers, and these numbers are then adequately summarized. For the differentiated numerical evaluation of quality, the following tasks should be resolved:

- selection and determination of properties, — if necessary — their quantification and grouping,
- transformation of property parameters,
- weighting of properties,
- computation of a complex quality index,
- determination of the error of a complex qualifying method,
- classification on the basis of the complex quality index,
- application of pattern recognition methods for the further development of complex qualification and evaluation.

1.1. Selection and grouping of properties decisively determining quality

The following basic principles are to be considered in making the selection:

— Depending on the requirements for information, the range and number of properties needed for food evaluation should be selected to include features which are in effect decisive for quality, paying, at the same time, attention to the average amount consumed. It readily appears that for complex research programmes, new food products or storage experiments the investigation of considerably more characteristics is required than in case of routine testing in food control.

— The knowledge of interactions among the properties is necessary, with particular reference to interdependences between various sensory properties, sensory, chemical and physical features. In view of the results from correlation and regression analyses, the number of properties considered in the evaluation procedure can be reduced, and only those should possibly be retained which can be determined at a desired level of accuracy, reproducibility and at minimum costs.

— Food evaluation calls for an accurate and adequately reproducible testing or investigative method. Random error in the method can be found by collaborative test while the systematic error should be ruled out. Random error appears in the form of repeatability and comparability or by a collective term: reproducibility.

Taking the definition of food quality as a starting point, generally the following property groups are considered in evaluation:

— sensory properties,
— chemical (compositional) and physical properties,
— microbiological contamination and other features of infection,
— keeping quality and level of processing,
— packaging and labelling.

Naturally, not all the food products involve every property group. Within the property group, it is mainly the type of the product that determines the properties to be selected for consideration. This selection is mostly based on prior consultation with specialists, in accordance with the following criteria:

— its importance and function in nutrition,
— the level of its processing,
— properties associated with storability.

This technical judgement is of importance even if mathematical methods are used to select properties. It is advisable to link the mathematical methods used in selecting properties (e.g. principal component analysis) on to the determination of the weighting factors of properties.

1.2. Possibilities of transforming measured data

In accordance with the qualimetric evaluation method, the range of parameters involved in the evaluation should in any case be divided between 0 and 1. It is recommended to choose the limit values so as to have a "sudden jump" in product quality at these values. The parameter ascribed to zero indicates a food product greatly differing from specifications and of reduced quality. The optimum value of the product parameter is ascribed to 1. The theoretical transformation model of measured parameters is as follows:

Parameter scale (P_i)		Scale of dimensionless numbers (k_i)
P_{opt}	optimal parameter limit	1.00
P_{min}	parameter limit acceptance . . .	0.00

Relationship between the dimensionless numbers and values of the parameter interval: $k_i = f(P_i)$, which may be linear or non-linear.

For the evaluation methods formulated so far, the linearity of the relation was assumed, that is: the variations over the dimensionless numerical scale were assumed to be proportional to changes in quality. The linear relation is expressed in the continuous and in the sectionalized scale. In case of a continuous scale, linear transformation may be undertaken in accordance with the following mathematical equations:

$$k_i = \frac{P_i - P_{min}}{P_{max} - P_{min}}$$

if $P_{max} > P_i > P_{min}$ and P_{max} stands for the best parameter

$$k_i = \frac{P_{max} - P_i}{P_{max} - P_{min}}$$

if $P_{max} > P_i > P_{min}$ and P_{min} stands for the best parameter

$$k_i = \frac{P_{max} - P_i}{P_{max} - P_b}$$

if the base value P_b between P_{max} and P_{min} stands for the best parameter and $P_i > P_b$

$$k_i = \frac{P_i - P_{min}}{P_b - P_{min}}$$

if the base value P_b between P_{max} and P_{min} represents the best parameter and $P_i < P_b$

The greatest difficulty in the practical application of these formulas is presented by the scientific determination of the parameter range and of the optimum (best) parameter. Linearity is also only an assumption because it is difficult to prove and its existence, particularly around the limit values, is questionable. If non-linearity is unambiguous and the equation is known, the dimensionless numbers can then be taken from a table or a nomogram.

1.3. Determination of weighting factors for properties

The method of consulting specialists (Delphi-method) is suitable to specify weighting factors for the properties selected and can be carried out in a relatively simple and inexpensive manner. The major steps of the algorithm for preparing and performing the Delphi-method are illustrated in Fig. 1.

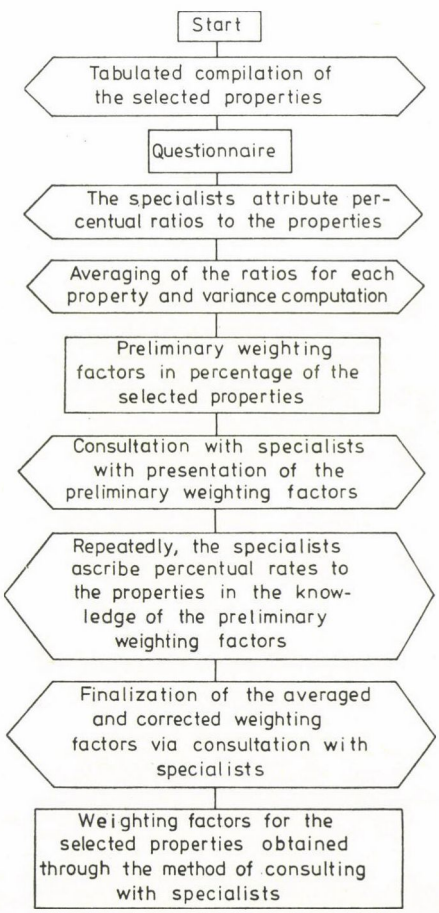

Fig. 1. Determination of weighting factors by consulting a specialist

Particularly in the case of a large number of properties, this consultation method frequently presents requirements on the specialists' preference ability which can only be met with difficulties and inaccurately. For this reason, the inaccuracy of the weighting factors assessed by this method or by direct ranking is often greater than the admissible level.

The random uncertainty is reduced if one proceeds not by A) but by B), i.e. when the determination of the weighting factors

A)	B)
is conducted for all the properties simultaneously, and the weighting factors of the property groups and those within the property groups are computed from the results.	is carried out within the property groups, and those of the property groups are determined separately.

It is more favourable if the specialist does not rank the properties all at the same time but in pairs and if he decides which of the two is more important. From n characteristics $\frac{n(n-1)}{2}$ pairs can be formed.

For example, in case of three characteristics — assuming consistency — from the comparisons of A and B as well as of B and C and from the preferences follows the preference of A and C.

$$\text{If } A \rightarrow B, \text{ and if } B \rightarrow C, \text{ then } A \rightarrow C.$$

The Guilford-method makes it possible to measure the specialists' logical consistency in the procedure; this is indicated by the consistency indicator (GUILFORD, 1936). The measure of judgement by several specialists is shown by Kendall's concord coefficient (KENDALL, 1970).

Based on the judgement of specialists attaining or exceeding the previously specified concord coefficient, one can compute the weighting factors featuring the importance of the different properties.

In the course of the Guilford procedure, the data are transformed from a sequential scale into an interval scale by means of the standardized normal distribution; over this scale, the difference between properties measured on the basis of preference are characteristic of their importance, whereby one can obtain values for the weighting factors of properties.

In certain cases, characteristic values are obtained for the weighting factors even in case if the weighting factors calculated by specialist consultation and the class limit values are rechecked by using measured, concrete data series and some method of pattern recognition e.g. discriminant, cluster or factor analysis (MOLNÁR et al., 1981).

1.4. Computation of overall quality index

Over and above the individually constructed mathematical equations applied to the various products, as a general rule, that qualimetric equation may be applied to any food product in which the complex quality is determined as a function of several variables of the different properties (MOLNÁR et al., 1979).

$$K = M_a \sum_{i=1}^{i=1} m_{ai}\ k_{ai} + M_b \sum_{i=1+1}^{i=h} m_{b1}\ k_{bi} + M_c \sum_{i=h+1}^{i=q} m_{ci}\ k_{ci} + \ldots$$

where K = overall quality index;

M_a, M_b, M_c . . . . = weighting factors of the property groups, featuring the importance of each property group; their sum being equal to 1 for every food product;

m_{ai}, m_{bi}, m_{ci} . . . = weighting factors of properties, representative of the importance of properties within the property group, their sum being equal to 1 for any food product within the property group;

k_{ai}, k_{bi}, k_{ci} . . . . = dimensionless numbers ranging from 0 to 1; they are the transformed values of the parameters of the features investigated within a certain range;

l. h q . . . . = number of properties whose sum is n.

The product-specific application of the basic qualimetric equation should be prepared through several steps. The first task is to select and group

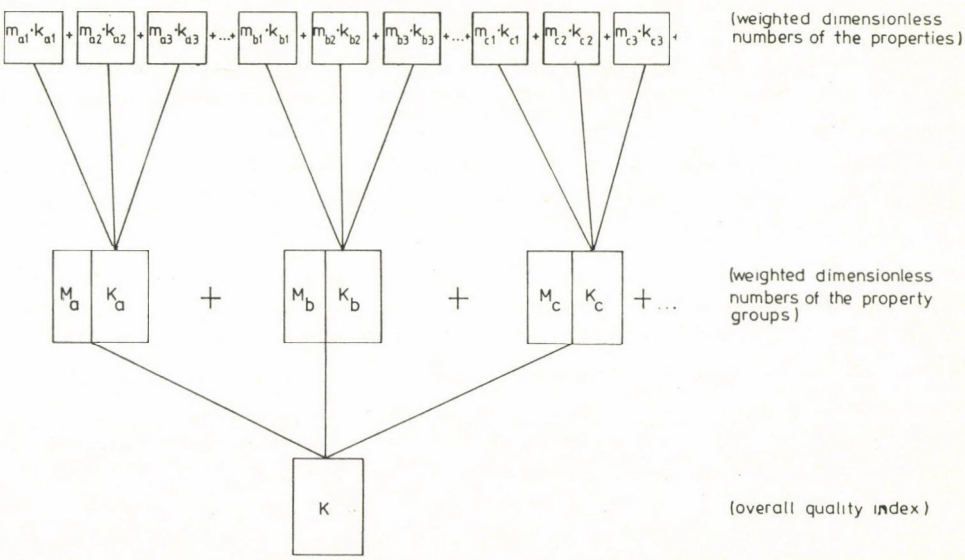

Fig. 2. Scheme for computation of the overall quality index

the properties basically determining quality. The next step is to determine the adequate transformation formulas for the parameters of the property groups, other possibilities of solution as well as the determination of their weighting factors. The reliability of results obtained by the valuation method is further enhanced if error determination is also performed. The qualification procedure must be made "viable" for peculiar cases also. The evaluation method, therefore contains other additional constraints also in the form of critical characteristics.

These "critical" characteristics supplement the transformation rules. Should the value of the "critical" property be zero, the transformed dimensionless value is eliminated from the property group, and the K_j value of the property group is also zero. A "critical" property can be termed as one whose importance is outstanding or stressed (its weighting factor) or which indicates a correlational link to the other characteristics of the property group. The "eliminating" function of the "critical" property fortifies this latter one.

It is also a general rule that the zero value of the "primarily critical" feature "eliminates" the calculated value of the overall or complex quality index (K). For food products, all characteristics are termed as "primarily critical" whose zero value indicates their harmfulness to health or their unfitness for human consumption. The sensory and microbiological properties are generally ranged with the group of "primarily critical" ones.

1.5. Error of the complex evaluation method

The random error involved in the investigation methods designed to measure properties pertaining to the complex evaluation also gets "transformed", which affects the accuracy of the overall quality index. By taking into consideration the weighting factors, these errors can be summarized through the variances, and thus the confidence interval of the overall quality index can be approximately computed. As a first step, the variance of the index component of the property groups are calculated:

$$\sigma K_a = \sqrt{\sigma k_{a1}^2 \, m_{a1}^2 + \sigma^2 k_{a2} \, m_{a2}^2 + \sigma^2 k_{a3} \, m_{a3}^2 + \ldots}$$

$$\sigma K_b = \sqrt{\sigma k_{b1}^2 \, m_{b1}^2 + \sigma^2 k_{b2} \, m_{b2}^2 + \sigma^2 k_{b3} \, m_{b3}^2 + \ldots}$$

$$\sigma K_c = \sqrt{\sigma k_{c1}^2 \, m_{c1}^2 + \sigma^2 k_{c2} \, m_{c2}^2 + \sigma^2 k_{c3} \, m_{c3}^2 + \ldots}$$

where: σK_a, σK_b, σK_c represent variance for the index component of the property groups;

σk_{ai}, σk_{bi}, σk_{ci} are the transformed *scatter* of the dimensionless values of the properties;

m_{ai}, m_{bi}, m_{ci} are weighting factors for the properties.

The resultant variance (σ_K) of the overall quality index is computed from the above data by the following relation:

$$\sigma_K = \sqrt{\sigma^2 K_a M_a^2 + \sigma^2 K_b M_b^2 + \sigma^2 K_c M_c^2}$$

where M_a, M_b, M_c are weighting factors of the property groups.

Based on σ_K at the chosen probability level (generally $P = 0.95$), the uncertainty (error) of the complex quality index can be given in the form of a confidence interval.

The consideration of the complex quality index as a composite probability variable enables its various values to be compared on the basis of various statistical trials.

1.6. Application of the overall quality index to classification

The classification of food products into different quality classes is an important primary task of evaluation. In order to make classification possible, it is necessary to have an adequate division of the complex quality index ranging from 0 to 1. Our experiences indicate that the specialists' judgements provide a right basis for the determination of class boundaries definitely within the range between 0 and 1. In the knowledge of the data series of several samples, the work of the specialists consists in performing the classification without prior computation of the overall quality index. Review and command over five quality classes may generally be expected from a specialist.

The five quality classes are as follows:

— excellent,
— good,
— mediocre,
— satisfactory,
— unsatisfactory.

Between the average values of the quality classes given by the specialists and the complex quality index values calculated with the relevant weighting factors linear regression equations were computed by the method of least squares. The class boundaries expressed in the complex quality index (K) were obtained by substituting the values 1.5, 2.5, 3.5 and 4.5. In the course of preliminary calculations it could be proved that contrary to theoretical assumptions, within a certain range the class limits hardly depend on the magnitude of the weighting factors (MOLNÁR et al., 1981) in the case of the scoring method.

On the basis of our experiences obtained thus far and of the calculated results, the following rounded up limit values are suggested for classification by the complex quality index:

— 0.875–1.000 excellent,
— 0.750–0.874 good,

— 0.600–0.749 mediocre,
— 0.450–0.599 satisfactory,
— < 0.450 unsatisfactory.

The linkage of the limit values to the supplementary limit values related to the "critical" properties of property groups continues to be a requirement; in case of a negative deviation from this, the sample, independently of the value of the complex quality index, is to be relegated to the proper lower quality class.

2. Results

2.1. *Use of pattern recognition methods for further development of complex evaluation*

For the founded determination of the limits of classification, pattern recognition is useable (HOLLÓ et al., 1977). Its application in formulating qualification classes is outlined in the following schematic diagram (Fig. 3).

The basic principle of the direct classification is that in the appropriate quality range the products belonging to the same class by their selected qualities are located close to one another, whole products pertaining to different classes are more widely spaced.

As outlined already, pattern recognition and qualifying classification are in each case preceded by the promotion of features and the determination of the essential properties or features. There are several mathematical means known to carry out the promotion of features, in addition to those already reviewed above. As a generally valid rule one may state that the property vector is of importance as regards classification if its variance is sufficiently small within the various classes, but at the same time, its averages, taken over the different classes are adequately divergent. This is indicated, e.g. by the

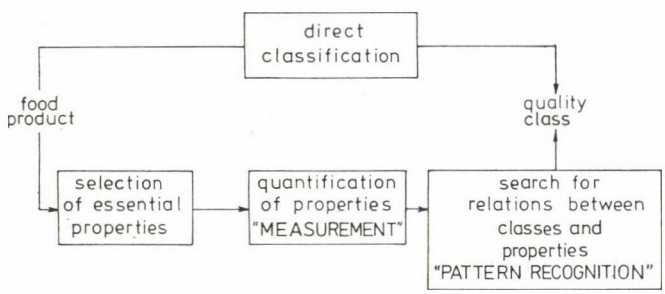

Fig. 3. Direct and "pattern recognition" based classification by quality of food products

Fischer-ratio. The combined transformation of property vectors is designed primarily to reduce interdependences between properties, redundacies and their number. Such procedures are, among others, the Fourier-, Haar- and Walsh-transformations and the factor analysis. As far as I know, there is still only little known about the efficiency of these methods in the field of food evaluation. Concerning measurement results, only the importance of

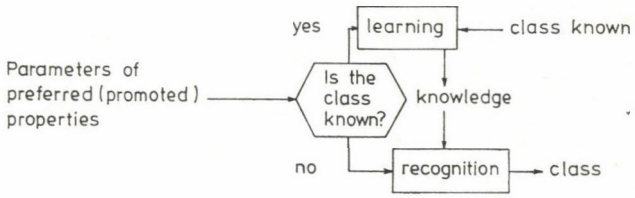

Fig. 4. Scheme of controlled classification

normalization should be stressed through which the property vectors are rendered commensurable.

Two basically different types of classification are conducted by the following schematic diagram (Fig. 4).

The first phase is "learning" to which the empirically developed class limits are applicable. In case enough knowledge is available, the quality class can be determined with sufficient reliability by pattern recognition.

An important special type of the controlled classification methods is the Simca method (HOLLÓ et al., 1977) through which the model of "hyperboxes" enclosing the points of products belonging to the various classes is determined in the course of learning. Recognition itself means the investigation of whether the unified points of the property vector of a product of unknown class is located or not (outliner) in a certain hyperbox of some class in the principal component space.

Based on the quality index system currently used in food evaluation, this method is supposed to be directly utilizable in rendering class limit values more exact.

The other large group of pattern recognition methods is uncontrolled classification. In these methods, the classes are not known beforehand, that is there is no product for which the quality class is known a priori. The essence of cluster analysis applied in this case is that the possible classes (clusters) are inferred from the location of the product points (parameters) in the space of essential properties. Those products whose points (parameters) are located "closely" together in the space of essential properties (or features) pertain to the same cluster whereas those whose points are widely "spaced" belong to different clusters. By applying this method, the suitability of the food

testing method by quality index can be basically checked and a controlled classification can be formulated.

There are preparations under way in Hungary to directly use pattern recognition for the further development of food evaluation methods; by means of this procedure there is a possibility to enhance the accuracy of the current evaluation based on overall quality index and to rule out subjective elements.

2.2. Application of the overall quality index to black current nectar

In addition to consultation with experts, findings and results from storage tests were put to use in order to work out an evaluation method for black currant nectar (MOLNÁR, 1972). For this reason, certain features were assigned a greater weight (like vitamin C content) in the evaluation method because their variation over the storage period is appreciable and more unambiguous than that of other, equally important features (e.g. ratio of sugar – acid). In addition to the qualitative characteristic, its relative value (weighting factor), the value 1.00 ascribed to the optimum parameter (P_{opt}), the value 0.00 corresponding to the defective parameter (P_{min}) and the relevant transformation formula, the table comprises, by way of example, two measured parameter series and the calculated dimensionless numerical data. The selected 16 qualitative features may be subsumed into two property groups. From those, the sensory properties are illustrated in detail since profile analysis was applied as method of investigation. All the features included in this property group have indicated a close correlation with the various sensory properties. Thus, instead of sensory scoring the colour value is used which had been obtained photometrically from the absorbancy measured at two different wavelengths. Consistency, which appears as a gustatory sensation, can be well specified by the visual judgement of the fruit parts (Table 1).

The number of compositional properties may be extended, although the importance of eligible properties like ash, ash alkalinity, native metal content, etc. is insignificant if one rules out the possibility of adulteration. On the other hand, their number might perhaps be rationally reduced if consideration is given to the correlation existing between the alcohol and volatile acid contents featuring the microbiological status of the processed fruit.

The importance of the features associated with keeping quality and level of processing (K_d) is generally less appreciable for this product: they were, therefore, ignored in the storage experiment. The omission of packaging (K_e) is questionable but this otherwise important element of product evaluation did not play a major role in the storage test series.

Specialists suggest that parameter series No. I. signifies a nectar of good quality, and No. II indicates a lower quality nectar. The overall quality index and its major components for product No. I may be computed as follows:

$$K_a = m_{ai}\, k_{ai} + \ldots + m_{a9}\, k_{a9} =$$
$$= 0.050 \cdot 0.76 + 10.064 + 0.020 \cdot 0.68 + 0.010 \cdot 0.84 + 0.05 \cdot 0.96 +$$
$$+ 0.05 \cdot 0.90 + 0.20 \cdot 0.96 + 0.20 \cdot 0.71 + 0.05 \cdot 0.96$$
$$K_a = 0.797$$
$$K_b = m_{b1}\, k_{b1} + \ldots + m_{b7}\, k_{b7} =$$
$$= 0.50 \cdot 0.71 + 0.00 \cdot 0.68 + 0.10 \cdot 0.67 + 0.05 \cdot 1.00 + 0.05 \cdot 1.00 +$$
$$+ 0.010 \cdot 1.00 + 0.10 \cdot 0.76$$
$$K_b = 0.766$$
$$K = M_a\, K_a + M_b\, K_b = 0.60 \cdot 0.797 + 0.40 \cdot 0.766$$
$$K = 0.785$$

Based on the complex quality index black current nectar No. I. is rated into quality class "good" if due consideration is given to the above classification limit values.

The quality index of product No. II is as follows:

$$K_a = m_{a1}\, k_{a1} + \ldots m_{a9}\, k_{a9} =$$
$$= 0.05 \cdot 0.50 + 0.10 \cdot 0.36 + 0.20 \cdot 20 \cdot 40.0 + 0.10 \cdot 0.56 +$$
$$+ 0.05 \cdot 0.72 + 0.05 \cdot 0.60 + 0.20 \cdot 0.62 + 0.20 \cdot 0.07 + 0.05 \cdot 0.76$$
$$K_a = 0.439$$
$$K_b = m_{b1}\, k_{b1} + \ldots + m_{b7}\, k_{b7} =$$
$$= 0.50 \cdot 0.55 + 0.10 \cdot 0.59 + 0.10 \cdot 0.00 + 0.05 \cdot 0.87 + 0.05 \cdot 0.32 +$$
$$+ 0.10 \cdot 1.00 + 0.10 \cdot 0.24$$
$$K_b = 0.518$$
$$K = M_a\, K_a + M_b\, K_b = 0.60 \cdot 0.439 + 0.40 \cdot 0.518$$
$$K = 0.471$$

3. Conclusions

Further development of scientifically founded and supported complex evaluation methods is a most timely task for synthetizing food research. This attempt is enhanced, among other things, by the general and increasingly pronounced need for evaluation and classification by quality also on the part of the quality control and test practice.

Over recent years food research has extended and expanded the amount of information related to different food products with several new scientific items of knowledge. On the other hand, for the measurement of many really important features and some other factors of evaluation sufficiently accurate, reproducible and conveniently feasible methods are not available. There is, therefore, an urgent need to work out so-called complex evaluation programmes for products or product groups, which comprise a review of the scale of quality characteristics, the elaboration and testing of missing investigation methods by collaborative tests, and the determination of the scale and size of the data ranges for evaluation of transformation formulas and of weighting factors.

In the course of implementing evaluation programmes, the error determination of the evaluation method is also subjected to further study. Calculations thus far indicate that the Gauss-type error determination will provide adequate information on the error of the complex quality index, whose magnitude imposes a limit on the maximum number of the classes as well. Assumably further calculations and studies will provide generalizable orders of magnitude for the errors pertinent to the various product groups if the error spread procedure proved to be suitable for error determination.

In relation to some of the products only preliminarily estimated limit values of classification are available. Should the general qualimetric equation be applicable to all products, it is also important to decide whether the limit values of classification can be established in a unified way or the use of separate limit values for each product group or even product is more advisable. Experiences to date indicate that the limit values depend only to a minor extent on the magnitude of weighting factors and on the number of properties or property groups decisive for quality. For this and other practical reasons it appears to be more advisable and feasible to apply the alternative of unified limit values.

To develop and apply the complex evaluation method to various food products requires and presupposes multivarious and profound continued research. A significant progress can, therefore, be expected only if this synthesizing development work gains ground in international research and if the complex evaluation methods obtained for certain products, by using methods of pattern recognition, result in extendable experiences as far as a further development and practical application of the qualimetric equation is concerned.

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INVESTIGATION OF ASH CONTENT AND ASH COMPOSITION OF FOOD SAMPLES AS A FUNCTION OF ASHING TEMPERATURE

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Investigations were carried out to determine the effect of ashing temperature (500–1000 °C) on the ash, K and Ca quantity in milk and sorrel. The relative K concentration in ash increases to 700 °C and decreases above 700 °C. The change in Ca content shows an inverse trend, the relative Ca concentration decreases (or does not increase) up to 700 °C, and increases above 700 °C. Ashing at 500 or 600 °C gives practically the same result, therefore the application of ashing at 600 °C for only 5–6 h is of advantage.

Keywords: ash composition of foods, ash determination, flame-photometry

The Hungarian standard for ash determination in foods specifies 500–554 °C as the ashing temperature. It is known that as a function of the temperature there is an important loss of certain components (in consequence of thermal dissociation of carbonates, volatility of alkali chloride and some oxides). PETER (1967) found that the Cs loss may be eliminated only if the ashing temperature is below 400 °C. KOVÁCS and NEDELKOVITS (1965) found a considerable K loss above 600 °C. BOPPEL (1973), MENDEN and co-workers (1977) and ROWAN and co-workers (1982) deal also with the questions of asting conditions.

In this paper we report on investigations of ash and K and Ca content of food samples as a function of ashing temperature.

1. Materials and methods

Investigations were carried out in the Institute for Food Control and Chemical Analysis (Győr), studying various samples of plant and animal origin. The samples were collected — with other food and fodder samples– systematically in County Győr-Sopron for the determination of their radioactive contamination level (fall-out contamination). In this publication we give an account of the results as obtained in milk and sorrel samples.

The ashing temperatures applied were 500, 600, 700, 800, 900 and 1000 °C, duration of ashing at each temperature 8 h. The sorrel samples (about 2 kg) were dried at 105 °C, crashed and ignited in a Pt crucible prior to ashing.

The milk samples (about 3 litre) were evaporated under an infrared lamp and ignited prior to ashing. The burnt samples were wetted with 10% NH_4NO_3 solution as an ashing aid. 8 hours were enough at 500 °C to obtain nearly white ash samples.

To determine the K and Ca contents we used a flame-photometrical method. The K concentration was measured at 770 nm, and of Ca at 554 nm, from HCl solutions of the ashes in a Flaphokol (Zeiss, Jena) photometer.

2. Results

The results of investigations are shown in Tables 1, 2, 3 and 4. Tables 1 and 2 give the weights and K and Ca contents as a function of temperature in comparison to the data obtained by ashing at 500 °C. The standard deviations ($\pm s$) were calculated on the basis of 3 parallel measurements. The actual values of the analytical measurements at 500 °C are shown in Table 3. The average K and Ca concentrations in the ashes ($\bar{x}$), as compared to the values obtained by ashing at 500 °C are given in Table 4.

Table 1

Change in the ash, K and Ca contents of milk as a function of ashing temperature
(in percentage of the value as measured at 500 °C ashing temperature)

Ashing temperature (°C)	Weight of ash $\bar{x} \pm s$	K content $\bar{x} \pm s$	Ca content $\bar{x} \pm s$
500	100.00	100.0	100.0
600	99.46±0.19	99.8±1.0	98.5±0.9
700	98.51±0.82	99.5±1.3	97.5±2.0
800	95.31±1.02	91.8±2.8	95.9±1.8
900	76.50±0.98	49.1±2.0	80.3±2.9
1000	76.38±1.15	49.0±1.8	80.1±1.7

Table 2

Change in ash weight, K and Ca content of sorrel as a function of ashing temperature
(in percentage of weight as measured upon ashing at 500 °C)

Ashing temperature (°C)	Weight $\bar{x} \pm s$	K content $\bar{x} \pm s$	Ca content $\bar{x} \pm s$
500	100.00	100.0	100.0
600	98.91±0.31	99.4±1.1	98.7±1.2
700	94.22±1.80	98.5±1.9	93.5±2.1
800	92.45±1.01	92.7±2.6	91.1±1.9
900	79.47±2.03	74.4±3.1	82.9±2.7
1000	75.03±2.35	57.6±4.1	79.5±3.0

Table 3

Ash, K and Ca content in milk and sorrel as percentage of the dry matter upon ashing at 500 °C

Sample	Ash content $\bar{x} \pm s$	K content $\bar{x} \pm s$	Ca content $\bar{x} \pm s$
Milk	0.730±0.004	18.6±0.4	14.3±0.5
Sorrel	17.02 ±0.08	23.4±0.6	9.5±0.6

Table 4

Average relative K and Ca concentration of ash (in percentage) as compared to the data of 500 °C ashing temperature

Temperature (°C)	Relative K content in		Relative Ca content in	
	milk	sorrel	milk	sorrel
500	100.0	100.0	100.0	100.0
600	100.3	100.5	99.0	99.8
700	101.0	104.5	99.0	99.2
800	96.3	100.3	100.6	98.5
900	64.2	93.6	105.0	104.3
1000	64.1	76.8	104.9	106.0

Table 5

Ash-weight in percentage as a function of the ashing time at 600 °C

Ashing time (h)	Milk $\bar{x} \pm s$	Sorrel $\bar{x} \pm s$
8	100.00	100.00
7	100.21±0.37	100.39±0.19
6	100.43±0.28	100.81±0.35
5	100.75±0.41	101.90±0.79
4	101.40±0.62	106.41±2.41
3	109.05±2.10	128.25±2.97

3. Discussion

As seen in Tables 1 and 2, the difference between ash weights as obtained at 500 °C and 600 °C, is very low. But above 700 °C the weight-loss is already important and the ash-content at 1000 °C is only about 70–80% of the quantity obtained at 500 °C.

At high temperatures the K loss is high, at 1000 °C the value measured was only about half of the original (as determined at 500 °C) K content. Taking into consideration the standard deviations of determination, the K contents, as measured at 500 and 600 °C are practically the same.

The quantity of ash and K change as a function of temperature in a different way. It can be said that the relative K concentration of ash increases up to 700 °C, and above 700 °C decreases.

The change in Ca shows an inverse trend. The relative Ca concentration slightly decreases to 700 °C and it increases above 700 °C. The loss in Ca is much lower than for K. The ash content of the sample ashed at 1000 °C is about 80% of that obtained by ashing at 500 °C.

We would like to mention, that the losses — at high temperature — are great in the case of other elements, too. At 1000 °C the loss in Na is 45–55%, that of Fe 30–50% in plant samples (KIST, 1973). In consequence of different volatilities of the ash components, the chemical composition of the ash changes with different temperatures, the relative concentration of some elements decreases, while other increase.

By changing the ashing temperature from 500 °C to 700 °C, the relative concentration of B and Al increases, and the relative concentration of Cu and Mn decreases (PAIS, 1980).

Table 5 shows the dependence of ash weight on the ashing time at 600 °C. The results are given on the basis of 10 parallel measurements. Data in Table 5 prove that 5–6 h ashing at 600 °C gives a satisfactory result, therefore the application of this temperature instead of 8 h at 500 °C is of advantage.

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HALF A CENTURY OF MICROBIAL ECOLOGY OF FOODS

What we gained and whom we lost

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The year 1933 marks, to a certain extent the renaissance of the microbial ecology of foods. In that year a series of papers by Dr. R. B Haines, Low Temperature Research Station at the University of Cambridge, appeared which, for the first time in the history of food microbiology, separated the three cardinal determinants of the microbial ecology of foods: population, proliferation and dissimilative metabolism. Admittedly, 75 years earlier Pasteur had observed that the fate of a mixed inoculum in a given niche depended on the response of the various components of the community structure to the physical and chemical determinants of the habitat. By this mechanism attributes of the "seed" as well as those of the "soil" affect the type of microbial association that will ultimately become the most abundant one in a given environment. Pasteur's pioneer's work fascinated and inspired the so-called Delft school: Beijerinck, Kluyver and Baas Becking, who, between 1905 and 1935 substantiated and extended Pasteur's work. Professor Johanna Westerdijk at

* The abstracts are published as submitted.

Utrecht University found that Pasteur's principle also applied to the mycological colonization and attack of foods. However, Haines was the first making attempts to quantify such phenomena and he was very successful in his efforts.

Haines' most brilliant student was Maurice Ingram, who graduated as a Ph. D. in 1937 on physiological aspects of the attack of cured meat products by salt tolerant bacteria. He would never since loose interest in the mechanisms by which micro-organisms adapt to essentially hostile extrinsic and intrinsic conditions. After Haines' tragic death in 1944 Ingram succeeded him as chief microbiologist at the L.T.R.S., continuing the original line of research: the microbial ecology of fresh meats, meat products, eggs and egg products with special reference to control of deterioration by proper choice of intrinsic and extrinsic parameters. In that capacity teaching the microbiological part of post-doctoral courses in food science which the L.T.R.S. organized soon after World War II was entrusted to him. This British initiative probably shaped the future of the microbial ecology of foods more than anything else.

Amongst the many students at Cambridge were dozens of young food microbiologists who, later, became celebrities in their own countries as well as internationally. As customary in academic education, the benefit of these courses was for students and tutor alike. From about 1946 Ingram organized his research and that of his many associates around ecological principles rather than specific commodities, though the latter continued to get attention – from orange juice concentrate and its osmophilic yeasts to *Cl. perfringens* and its fate in whalemeat. Control of spoilage relying most frequently on ecological intervention, one finds Ingram and his students studying the effects of heat processing, food irradiation and the incorporation of chemically safe, antimicrobial food additives.

The latter choice once more exerted a tremendous influence on the development of the microbial ecology of foods. First and foremost it brought Dr. K. Vas to Cambridge. His main field became the molecular basis of the antimicrobial effects of sulphur dioxide, a preservative of crucial significance in the fruit products industry. However, as most of Ingram's overseas' students Vas was impressed by other facets of the research going on at Cambridge as well. The quantification of all techniques, the attention being paid to proper taxonomy, fundamental aspects of microbial cell death and the intricacies of endospore formation and germination all became part of Vas' research programme upon his returning to Hungary. But probably the predominant influence of Ingram was that in the field of the use of ionizing radiation, particularly gamma rays for the decontamination and preservation of foods. In 1958 an international conference on this subject had been organized in Harwell and Oxford: the Americans brought an update of the facts, Ingram precipitated on the fundamentals, especially the changes in the microbial association of foods secondary to irradiation. This was another area of research he was impressively

faithful to. His most talented disciple in this area was no doubt Vas, since 1959 Professor of Food Microbiology at the University of Horticulture, Budapest. He eventually became the Director of the United Nations' Food Irradiation Programme, stationed at the International Atomic Energy in Vienna. He made admirable efforts to promote the application of the most beneficial ionizing radiation to improve the safety and quality of very many foods. Like Ingram being a real savant, he sensed that the — purely emotional — opposition to the use of gamma rays in food processing could only be overcome by comprehensive studies on the biological effects on food constituents ("radiolytic changes") and subsequent reassurance of consumers and governments alike. Moreover, Vas encouraged continuing studies on the ecological repercussions of food irradiation, the most recent results being reported in several papers to this 12th International Symposium on Food Microbiology and Hygiene.

Shortly after the liberation of France a young bacteriologist-gastro-enterologist at the Pasteur Institute, Lille, Dr. René Buttiaux, became interested in the fundamental aspects of infectious diseases transmitted by foods. A brilliant scientist he soon reached the conclusion that the conventional isolation procedures for pathogens occurring in foods were inadequate for a reliable diagnosis of food-borne gastro-enteritis, let alone for achieving its control. Consequently, assisted by a very large group of future celebrities he set out to study reliable methods of detection of, and the fate of relevant pathogenic agents in, foods and drinking water. A notable achievement of the Lille group was a fundamental study on the bacterial association of canned, large size hams, which resulted in a famous international symposium and was of substantial value to the meat industry. No doubt, a turning point in Dr. Buttiaux's research was meeting Sir Graham Wilson in Cambridge during an international symposium on food transmitted disease of microbial aetiology, held in 1955. At that meeting Sir Graham expounded his level-headed approach to the assurance of food safety: relying on measures of intervention rather than on acts of final product inspection. Buttiaux immediately sensed the wisdom of his fellow-physician and some time later founded the CERTIA, Center for Training and Research in Food Technology — the latter synonymous with what Dr. Betty C. Hobbs, Sir Graham's superb student had termed "processing for safety". One of the assignments of the CERTIA became the organization of annual postgraduate courses in food microbiology and hygiene, generously supported by the World Health Organization. Over a thousand young microbiologists were trained at Lille between 1956 and 1975 and many of them carry the Pasteur torch on in Greece, Italy, Latin America, the Maghreb and Spain.

Our American descendents had their own centers of excellence in food microbiology since about 1930. Professor F. W. Tanner at Urbana, Ill. and Professor W. C. Frazier at Madison, Wisconsin are no doubt the pioneers of

our specialty, with Professor G. M. Dack at Chicago as the leading medical specialist of world-wide renown. Particularly Professor E. M. Foster at Madison became the inspirator of ecological research on organisms like *Cl. botulinum* and *Staph. aureus* and foods of all sorts, from smoked fish to cheese. In addition our American colleagues discovered the phenomenon of sublethal damage in microbial populations exposed to microbicidal effects, or microbistatic intrinsic modification of foods. Injury of this type results in gross underestimation of the numbers and types of survivors, unless special precautions are taken, and thereby invalidates ecological studies not relying on these special measures. Impressive work has been carried out in this area and is being continued presently, because at the molecular level details of some types of injury and their repair have not yet been fully established.

Ingram died in 1977, Vas in 1981 and Buttiaux in 1982. Not in all instances is their superb research continued by academic successors sensu strictu. Fortunately, given the vast numbers of second generation scientists trained by these giants in their respective countries it may be assumed that continuation of the main lines of ecological research will be assured. A serious desideratum for the future remains the dissemination of the results of research, particularly attracting the attention and recruiting the invaluable assistance of microbial ecologists working in the field of general microbiology. It is quite striking that at international congresses of microbial ecology the most extravagant niches and the most peculiar types of organisms come to the fore, whereas virtually no papers or reports are devoted to equally fascinating niches and lower forms of life in an area vital to mankind: food. It is an ethical duty of food microbiologists and a fitting tribute to the pioneers in that field commemorated in this essay that the coming 50 years will be equally profitable for the food industry and contributory to general microbiology as the era initiated by Haines. They should be even more productive, because the most shameful attribute of mankind 1983: lack of food is not showing any tendency to loose numerical importance, while essential contributions to resolving the problem could result from markedly increased insight in the properties of microorganisms threatening or favouring the microbial ecology of food.

MICROBIAL ECOLOGICAL PRINCIPLES IN CONTROLLED ATMOSPHERE STORAGE OF FRUITS AND VEGETABLES

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The storage of foods in a controlled atmosphere (CA) makes use of extrinsic parameters of microbial ecology, i.e. temperature, humidity and composition of the gaseous atmosphere. The advantage of CA storage comes of

its simultaneous retarding effects on both the respiration of plant tissues and the growth of microorganisms.

The microbiological grounds of CA storage have been investigated on three levels: on laboratory scale, in experimental chambers and in commercial storehouse.

The laboratory experiments were made with representative strains of *Penicillium expansum*, *Aspergillus versicolor*, *Aureobasidium pullulans* and *Flavobacterium* sp. isolated from stored plant products. Their growth rates were studied at different temperatures (5, 10, 15 °C), equilibrium humidities (92, 95, 98 and 100%), and composition of atmospheres (normal air, O_2 decreased to 5%, CO_2 increased to 10%). Particular emphasis was laid on the evaluation of the interactions among factors.

In the experimental chambers products could be stored on pilot-plant level (30–50 kg) at various CA conditions. It was found, that optimal CA condition for a given product did not necessarily correspond to the strongest inhibition of microbial growth.

The commercial CA storage of apples resulted in good product quality, prolonged shelf-life and definite growth inhibition of bacteria, while the development of moulds was only partially retarded.

INTESTINAL PATHOGENS AND THE ENVIRONMENT

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The mechanism of adaptation of organisms to their environment is considered in relation to the survival of intestinal pathogens. Products such as pyocins and colicins are used as tools for typing, but they may be substances used in defence by the bacteria.

The toxins of *Clostridium perfringens* A and C may be used to engineer circumstances to increase the outflow and dispersion of spores. The type C organism takes advantage of enzymic lack in certain diets to prevent the destruction of β-toxin in the intestine.

Viruses may survive in an inert form for preservation in food and water; the value to organism of plasmids carrying R factors against antibiotics may be self-preservation.

Temperatures of optimum growth, generation times and length of lag phase may all play a part in the struggle for existence and perpetuation.

The production of special enzymes under certain circumstances, for example the growth of *Salmonella typhi* in canned corned beef, could be relevant to survival.

The Eltor biotype of *Vibrio cholerae* has learned to adapt to the environ-

ment better than the classical biotype. The phage type 60/122 of *Salmonella typhimurium* has swept over the Indian continent with invasive efficiency and ability to develop multiple R plasmids.

The key to these adaptive habits can be assumed to be survival.

VEGETABLES AS AN ECOLOGICAL ENVIRONMENT FOR MICROBES

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The microflora of vegetables relfects their chemical composition and the cultivation conditions.

The high average water content (88%), the low protein content (approx. 2%) and a neutral or slightly acid pH combined with aerobic conditions favour certain gram-negative microorganisms as well as mold. Unharvested vegetables have an indigenous microflora on the surface as well as in the deeper tissues, similar to the microflora of the soil. After harvesting, propagation of microbes increases as a result of release of nutrients from injured vegetable cells. Those microorganisms, especially "soft rot" organisms, which e.g. have the ability to decompose pectin and also have a low protein demand, are favoured.

Vegetables are not protected by a natural defense mechanism like fruit, such as a thick skin or a natural content of preservatives like benzoic acid and essential oils, for which reason post harvest microbial propagation takes place rather easily.

MICROFLORA OF EDIBLE OFFAL WITH PARTICULAR REFERENCE TO SALMONELLA

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Earlier studies have shown increasing frequency of salmonellosis among pets, particularly dogs, in the city of Berlin (West). Feeding of raw offal has been suspected to be a major source of infection. The present study revealed 225 (56.6%) of more than 400 samples of edible offal (liver, lungs, heart, bovine rumen, porcine oesophagus) to contain 24 types of salmonella. *S. typhimurium* prevailed (142 samples = 63% of the positive samples = 35.5% of the total) including 8 strains of *S. typhimurium* var. copenhagen. Three types had an incomplete seroformula. The investigations covered a period of 26 months. The percentage of positive findings did not significantly differ during various

seasons. Positive findings were most frequent in porcine oesophagus (40/49) and least frequent in imported swine liver (15/18) and bovine rumen (13/45). Presence of salmonellae was not correlated with other microbiological criteria, in particular aerobic plate count and number of enterobacteriaceae. All samples were sold at West-Berlin's central wholesale meat market and originated from slaughter animals judged as "fit for consumption" which means that they were also intended for human consumption and not only as animal feed. Repeated isolation of the same salmonella type from different samples drawn on the same day indicated rapid spreading during transport and storage. This contamination did not persist for a longer period but was replaced by other strains which subsequently appeared. Since raw offal for retail sale represents a considerable health hazard, it is recommended: Raw offal should not be offered in retail unless in hermetically sealed packages. Packages should have labels with directions for proper handling of the contents in the household. Warning should also be given that viscera should neither be eaten raw nor fed to pets inadequately heat processed.

BACTERIA PATHOGENIC TO MAN IN FOODS OF PLANT ORIGIN

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Although a number of outbreaks of food borne infections and intoxications have been reported following the consumption of foods of plant origin such foods do not figure prominently in the disease statistics published in various parts of the world. Fruits and vegetables, either eaten raw or after varying degree of processing, have been incriminated in episodes of typhoid fever (watercress, lettuce, celery), paratyphoid fever (desiccated coconut), cholera (raw vegetables, dates, cooked rice), shigellosis (tomatoes), food poisoning (celery, watercress, lettuce, cabbage, endive, water melon, cereals, spices) and botulism (underprocessed vegetables and fruits). Some vegetables such as watercress have also been implicated in cases of hepatitis and fascioliasis.

In general, vegetables and fruit crops which have not been exposed to irrigation and fertilization with human and animal waste should be free of pathogens other than those which form part of the natural soil flora such as *Clostridium perfringens* and *Bacillus cereus*. However, in countries with warm climates, where irrigation, often with polluted water, is necessary to maintain an adequate water supply to growing crops, and where sanitation is primitive, night soil is used as fertilizer, animals are allowed to roam amongst crops and the general standard of hygiene is poor there is a risk of transmitting disease via foods of plant origin.

A number of reports have been published on the incidence of organisms pathogenic to man in vegetables, fruits and various food ingredients prepared from plant material (herbs, spices, soya protein, fruit drinks). Salmonellae and shigellae have been isolated in some surveys but in a study carried out in the author's laboratory the incidence of these organisms as well as of *Staphylococcus aureus*, *Vibrio* spp, *Campylobacter* spp, and *Yersinia enterocolitica* in vegetables and spices was very low, although small numbers of *C. perfringens* and *B. cereus* were frequently found.

Various studies have also been carried out on the survival of enteric pathogens on a range of foods of plant origin. *Vibrio cholerae* may survive up to 7 days on fresh vegetables and can grow to levels of 10^6 per g on some. Shigellae may survive up to 10 days and salmonellae up to six weeks depending on the vegetable.

There are many opportunities for foods of plant origin to become contaminated with organisms of public health significance. However, the numbers present are likely to be low so it is important that these organisms are prevented from multiplying to levels at which they can cause infection or intoxication and from contaminating other foods and the environment in which foods are prepared.

THE EFFECT OF ALCOHOL PERCENTAGE IN EGGNOG ON SURVIVAL OF *BACILLUS CEREUS* AND SALMONELLA

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Egg nog is a very important product of the Dutch distillery industry. The domestic product, "advocaat", contain 14–16% of alcohol, the export products contain at least 18% of alcohol. Because of the presence of alcohol the product was believed to be well protected against bacterial spoilage and growth of potentially pathogenic bacteria.

However in recent times many cases of bacterial spoilage of egg nog, as shown by the decomposition of lecithin and the separation of whey, have been reported. The bacterium involved was mostly *Bacillus cereus*, which has often been isolated from eggs and egg products.

There is a trend perceptable that egg nog producers lower the alcohol percentage to below 11% in products, which for instance are used in the bakery industry. The lower alcohol percentage, but also the bacterial contamination of the egg product, used as a starting material, could have been reasons for the spoilage of egg nog.

Therefore, the effect of the alcohol percentage on survival of *Bacillus cereus* in these products has been investigated.

At the same time experiments were carried out to study the effect of alcohol percentage on the survival of salmonellae, as there was concern about the presence of these bacteria in egg nog with low alcohol percentage. *Bacillus cereus*, *Salmonella heidelberg* 5- and *Salmonella worthington* were inoculated into freshly prepared egg nog. The final concentration was between 10 000 and 1 000 000 colony forming units per cm^3 product. The alcohol percentages ranged from 0 to 22% by volume. The products were stored at room temperature for 90 days.

Alcohol percentages of 17.5% and 21% reduced the survival of *Bacillus cereus*. A total destruction could not be obtained within 30 days of storage. After 18 days storage no colony forming unit of salmonellae was found in the product containing 14% alcohol. Five days after preparation of the egg nog containing 18% alcohol no salmonella cfu could be detected in this product.

No danger has to be expected from salmonellae in this type of product, provided the alcohol percentage is above 10%.

Bacillus cereus survived the alcohol treatment. Optimum hygienic conditions during the processing of eggs may result in a very low contamination level of the egg products with these bacteria.

SALMONELLA IN A PRECOOKED MEAT PRODUCT AND CONDITIONS OF ITS PREPARATION[a]

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The meat product studied is a sliced one containing at least 50% beef or pork, about 20% poultry and some vegetable protein, precooked industrially and to be distributed in frozen condition.

The investigation aimed to determine the warming conditions permitting to destroy salmonella inoculated experimentally at massive numbers: 10 000 per 1 g.

Sixty 100 g samples were cooked, inoculated, packed in an aluminium sheet and heated in a laboratory grill to attain 80 °C in the center. Strains used: *S. typhimurium* and *S. saint-paul*, freshly isolated. Warming times: 5 and 10 minutes.

Bacteriological examination. Pre-enrichment in peptone water (18 h at 37 °C) of 25 g, 10 g, 1 g → 10^{-4} g; enrichment in tetrathionate broth (24–48 h

[a] Translated from French by the first author

at 37 °C) and selenite broth (24–48 h at 43 °C); planting onto D.C.L. medium (desoxy-cholate—citrate—lactose, 24–48 h at 37 °C); picking and purification of suspect colonies; biochemical and serological identification.

Results

N° 1 series — 10 assays — 5 m at 80 °C —
S.t.m. 1 sample + (10^4/g)/10
N° 2 series — 10 assays — 5 m at 80 °C —
S.t.m. 4 samples + (ds 25 g and 10/g)/10
N° 3 series — 10 assays — 5 m at 80 °C —
N° 4 series — 10 assays — 10 m at 80 °C —
S.t.m. 0 sample +/10
N° 5 series — 10 assays — 10 m at 80 °C —
S.t.m. 0 sample +/10
N° 6 series — 10 assays — 10 m at 80 °C —
S.t.m. 0 sample +/10

Conclusions. The precooked meat preparation studied when artificially contaminated with 10^4 *S. typhimurium* or *S. saint-paul* per 1 g was not satisfactory from the bacteriological point of view, when the warming-up time was limited to 5 minutes.

On the contrary, warming-up at about 80 °C in the heart during 10 minutes was satisfactory. The organoleptic properties of the product were not affected by the mode of warming-up.

REFERENCE SAMPLES FOR THE DETECTION OF SALMONELLA

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Results of experiments carried out over more than 15 years have shown that because of the great number of variables involved, the isolation of salmonella from food and feed, even using the standard method, will not be equally successful in all laboratories. It is suggested to check the performance of the standard method by the examination of reference samples. It has been demonstrated that differences in laboratories become especially apparent when samples are used containing low numbers of evenly distributed salmonellas. An even distribution by artificial contamination can only be achieved in liquid substrates. However, the reference samples will be transported and stored and

therefore, should be microbiologically stable. A liquid sample cannot fulfil this requirement. Based on previous experiments milk powder was chosen as substrate. The salmonella contamination in milk powder was stable for at least 6 months at 4 °C, but was unstable at room temperature. Disadvantages of milk powder are that it is not routinely examined by all laboratories and it contains lactose that may be fermented by coliforms and cocci and thus acidify the pre-enrichment medium. Therefore, the amount of milk powder used in the reference sample should be kept to a minimum. Moreover, competitive flora, may interfere with the isolation of salmonella, and should, therefore, be added. It should be representative for the foods that are examined. In conclusion: reference samples consist of a small amount of artificially contaminated milk powder and are added to a mixture of buffered peptone water and food sample. When applying reference samples results suggested that the isolation of salmonella is influenced by yet unknown factors. Results will be demonstrated and discussed.

IMPROVING THE MICROBIOLOGICAL QUALITY OF CHICKEN BY RADIATION ENERGY

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The microbiological quality of chicken is stabilized by freezing and storage at low temperature. The number of microorganisms causing spoilage and that of the pathogens do not change under such conditions. After defrosting the meat becomes a potential source of infection.

The antimicrobial effect of radiation energy was investigated. Eviscerated whole chickens were packed in polyethylene and Saran foil-bags, deep-frozen, then irradiated with ^{60}Co radioactive isotope. 40–50 chickens per dose-level were stored at –18 °C for 58 weeks. A dose of 3 kGy reduced the mesophilic aerobic colony count by 2–3, the number of psychrophylic bacteria by 3 logs at least, and that of the enterobacteria by 3–4 log cycles in both types of packaging. 3 kGy eliminated the *S. aureus*, being present in low count, and freed the chicken of Salmonellae, too. The number of enterococci was reduced only by 1 log cycle. In case of clostridium contamination the spores survive the 3 kGy dose, after treatment with 5 kGy their number was less than 1×10^1 g^{-1}. A dose of 5 kGy is more effective than 3 kGy, however, the effect is not linear. The number of salmonellae was reduced by 5 log cycles in chicken inoculated with *Salmonella derby* and *S. panama* and irradiated with 3 kGy. The organoleptic quality of the treated chicken did not differ from that of the unirradiated ones.

SENSITIVITY OF *CAMPYLOBACTER FETUS* SUBSP. *JEJUNI* (CFJ) TO GAMMA RADIATION

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CFJ has only recently been recognized as one of the most common causes of acute bacterial gastroenteritis in humans. Today the ubiquitous presence of the microbe in wild and domesticated animals and birds is evident. At present poultry are regarded as the main reservoir of the bacteria and the improperly heat-processed poultry foods constitute the most frequent cause of human campylobacteriosis.

Both literary data and the results of our investigations indicate that CFJ will not be destroyed if stored frozen at –15 °C for a lengthy period of time (16–30 weeks) in case of substantial initial CFJ contamination.

Our aim was to study the resistance of CFJ against irradiation in culture media and in chicken pulp as well as to determine whether radurization doses employed to destroy salmonellae are sufficient to kill campylobacter.

We found that CFJ survived for as long as 16 weeks while stored at –15 °C after irradiation with 0.2 kGy. When the radiation dose was increased to 1 kGy, no CFJ was detected at all, even if the initial count had been $10^9\,cm^{-3}$. In chicken pulp (instead of culture media) the destruction was of a smaller degree. As a result of lower irradiation doses (0.1, 0.2, 0.5 kGy respectively), CFJ count decreased in proportion to rising dosage. When we increased the dose to 1 kGy, the initial $10^8\,g^{-1}$ count was reduced to 0 after one week following irradiation.

To sum up: *Campylobacter fetus* subsp. *jejuni* survived a 1 kGy radiation treatment neither in culture media, nor in chicken pulp. Consequently, the 3–5 kGy dose generally used for meat products to kill salmonellae will also certainly destroy campylobacters.

EXAMINATION OF THE HYGIENIC QUALITY OF CREAM FILLED PASTRY IN FRANCE

RESULTS OF MICROBIOLOGICAL EXAMINATIONS PERFORMED IN 1981

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A survey was carried out including 3200 samplings and 4586 samples of cream filled, fresh pastry throughout France. The official French methods of examination, published in 1980 were used. These included:

— aerobic colony count at 30 °C;

— enumeration of cfu of the coli-aerogenes group at 30 °C, faecal types

of the coli-aerogenes group at 44 °C, *Staph. aureus* at 37 °C and sulphite reducing anaerobic bacteria at 46 °C;

— detection of salmonella in 25 g aliquots.

The reference values employed were 3×10^5, 10, 1, 10, 10 per 1 gramme, and a negative result of the test, respectively, for the criteria listed above. With the exception of the salmonella detection test, results up to three times the recorded levels were considered acceptable. However, when, among 5 samples of the same lot of pastry one sample exceeded the pertinent reference values by a factor of 10 or more the consignment was considered unsatisfactory.

Aerobic colony counts varied from 10^2 to 3.2×10^9: excessively high counts were only observed infrequently. Colony counts of the coli-aerogenes group varied between less than 1 to over 10^6; they were the second most frequent reason for rejection. Faecal types of the coli-aerogenes group also showed a wide variation: from less than 1 to 5×10^6; the reference value was exceeded by 75% of the samples and in 30% of the consignments. The other criteria were only exceptionally found to be slightly exceeded.

The results of this survey prompt the question whether the current low acceptance level for the faecal types of the coli-aerogenes group should be maintained. An answer to this question can only be derived from a thorough investigation on the preparation and storage practices of pastry in France which will reveal which levels of these bacteria are attainable when acceptable manufacturing and distribution practices are followed.

MICROBIOLOGICAL INVESTIGATION OF DRIED WHOLE EGGS

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115 egg powder lots produced for dried pasta products were analysed. The microbiological survey included the followings: aerobic colony count, MPN of enterobacteriaceae, number of moulds and *Staph. aureus* as well as the detection of salmonella. In case of salmonella examination, 13×25 g sample units were analysed per lots; the investigations of indicators were generally carried out by random sampling approach.

Microbial contamination of egg powder was generally very low (total count: $< 3.0 \times 10^3$–10^4; moulds: $< 1.0 \times 10^1$–10^2). In all 115 egg powder lots examined only 3 proved to be salmonella positive. There is no close correlation between the presence of the indicator microorganisms, their numbers and the presence of salmonella. In the cases of salmonella positive samples the number of enterobacteriaceae was very low. In contrast in cases of high enterobacteriaceae counts the samples were negative for salmonella. Thus detection of salmonella should have an emphasized priority.

In spite of it, as the microbiological condition of egg powder may be influenced by the processing technology, it is essential to monitor processing hygiene by the use of indicators. According to the results the egg powder from the point of view of occurrence of indicators is not homogeneous. Thus the use of a three-class plan for sampling is recommended as a necessity.

OCCURRENCE OF *CLOSTRIDIUM PERFRINGENS* IN BOLOGNA AND FRANKFURTERS

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26 samples of six types of Bologna and 14 samples of frankfurters obtained from retail network in Prague were investigated for the presence of *Cl. perfringens* using tryptose-sulfite-cycloserine-agar (TSC) in the modification of Hauschild and Hilsheimer for colony counts and rapid perfingens medium (RPM) of Ericksson and Deibel for the enrichment of this microorganism. Confirmation tests for both methods used the production of lecithinase on nutrient agar with lactose, egg-yolk and neutral red and its inhibition by specific antitoxin. Using TSC and confirmation tests, *Cl. perfringens* was found in 69 per cent of the analysed samples of Bologna and in 43 per cent of frankfurters. 67 per cent of positive samples contained less than 100 cells of *Cl. perfringens* per g. The value of 10^3 cells of this microorganism per g was found in one sample of Bologna and in another sample of frankfurters. Enrichment in RPM yielded weakly positive results also in further 6 samples of Bologna and 5 samples of frankfurters, but the presence of *Cl. perfringens* was not confirmed by lecithinase-antitoxin tests. There was no relationship between the number of total mesophyllic bacteria and of *Cl. perfringens*. No cells of *Cl. perfringens* were found in spice and spice-mixtures obtained from the manufacturers of the analysed products and the used spice, therefore, does not contribute to the occurrence of *Cl. perfringens* in these products.

BEHAVIOUR OF HUMAN PATHOGENIC STRAINS OF *YERSINIA ENTEROCOLITICA* IN ENVIRONMENTAL MEDIA: SURVIVAL AND VIRULENCE

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Clinical strains of *Yersinia enterocolitica* have been characterized by known pathogenicity in vitro tests like Ca^{++}-dependency and autoagglutination at 37 °C. These virulence factors have been associated with a plasmid of the size of 40–46 mega dalton.

In the present study similar plasmid-bearing strains have been examined. The survival of these strains was tested in different media at 4 and 25 °C. At the same time the pathogenicity factors were tested.

Although the Ca^{++}-dependency was lost, no changes were found in the size of the virulence plasmid. No information about deletions was gained by digestion with different restriction enzymes.

These results show the behaviour of human pathogenic strains in environmental media and the changes in the pathogenic factors.

THE ROLE OF FOODSTUFFS IN TRANSMISSION OF LISTERIA

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Listeria monocytogenes is an ubiquitous, opportunistic microorganism. Human listeriosis has been classified by WHO and FAO Expert Committee into bacterial zoonoses. On the basis of the newest epidemiological data at least 50% of these infections have originated from human sources.

As to the food hygienic aspect of the question the picture is not clear yet. WHO and FAO Expert Committee, rediscussed microbiological aspects of this theme in 1976. *L. monocytogenes* was not listed among bacteria which could cause food-borne disease, however, it could frequently be isolated from different kinds of uncooked food. It is a fact which can be explained in different ways:

- *L. monocytogenes* bacteria are harmless as causative agents transmitted by food.
- There was not enough information about this bacterium for the committee.
- Exact investigations have not been performed to study food hygienic importance of listeria in food hygiene.
- Nobody knows exactly what is the clinical picture of an acute or chronic food-borne infection caused by *L. monocytogenes*.

The main aim of this paper is to focus attention on listeriosis as well as to deal with different aspects of this question on the basis of literary data and the results of the author's own studies.

PSEUDOMONAS AERUGINOSA PRODUCT INHIBITION OF *SALMONELLA THOMPSON* AND OTHER ORGANISMS

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S. thompson and *P. aeruginosa* grew independently of each other in mixed culture. When *S. thompson* was grown in *P. aeruginosa* culture filtrate, not only was the lag phase extended, but the cells also became more sensitive to selective media. *S. aureus* and *E. coli* strains were more sensitive than the salmonella. The pseudomonas-filtrate contained at least two different factors that effected an inhibition of growth. These were not pyocyanase or the pyo compounds of Hays. The major inhibitory factor was the blue pigment: pyocyanine. Gamma irradiation of the pyocyanine solution resulted in the loss of the blue colour and all of the inhibitory activity.

Pyocyanine appeared to act on *S. thompson* by causing a shift to a slower growth rate rather than by destroying a segment of the population. This was concluded when it was observed that the number of viable *S. thompson* cells remained constant during exposure to pyocyanine and that the viable counts were reduced more rapidly in control cultures exposed to penicillin than in pyocyanine treated cultures exposed to penicillin. *S. thompson* cells eventually reduced the pyocyanine in the culture and commenced growth.

Pyocyanine was not taken up by or bound to the cells. EDTA treatment of *S. thompson* cells did not render them more sensitive to the pigment. The uptake of several different compounds by cells exposed to pyocyanine was not altered from that in control cultures. Protein synthesis was disrupted and oxygen consumption during metabolism of various substrates was reduced. Pyocyanine oxidized NADH in vitro and cells exposed to pyocyanine had reduced internal levels of NADH. The level of cytochrome *b* in pigment grown cells was also lower than that in control cultures. The inhibition caused by the pigment could not be overcome by growing the culture on succinate in order to allow for electron flow to the electron transport chain at a site other than NADH. Pyocyanine was also found to inhibit the growth of organisms that have no functional or detectable cytochrome system.

In the test systems studied, the critical site of action of pyocyanine is not known but it is evident that cell wall synthesis is disrupted in some way resulting in delayed growth of the challenged organism. Cultures recover from this challenge by reducing the pyocyanine.

DATA TO THE MICROBIOLOGICAL SPOILAGE OF COMMERCIAL MILK

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A study was made to find out the combined and individual effects of the cell count of the fresh milk, the processing hygiene and the storage temperature on commercial milk. The initial cell count of the milk was established in samples taken immediately upon pasteurization. The effect of the workshop was established by samples taken from packaged milk. The effect of temperature was studied by keeping both kinds of samples at 5, 10 and 15 °C, respectively. In addition to sensory evaluation the main microbial groups were determined, too. In the samples taken upon pasteurization reinfection was established by Kleeberger's method.

It was established that the storage stability of non-reinfected pasteurized milk is inversely proportional to the initial cell count. If a cell count of 0.5 million per cm^3 is considered 100, the shelf life of the pasteurized milk made of fresh milk containing 0.5–3.0 million germs is shorter by 11–22%, while a cell count above 3 million reduces the storage stability by 33–50%. A reinfection above 10^2 per 100 cm^3 modified the above relations. An increase of 5 °C in the storage temperature reduced the keeping quality by about 25–40%. The role of reinfection was inversely related to storage temperature and the cell count of fresh milk.

GROWTH OF STAPHYLOCOCCI AND OF STREPTOCOCCI IN RAW MILK

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More than half of the samples of raw milk from the contaminated udder quarters contains *Staphylococcus aureus*. Raw milk of poor hygienic quality often contains *Streptococcus agalactiae*. The aim of our research was to verify the inhibitory activity of milk from the aseptically stimulated udder on the growth in vitro of strains chosen at random of staphylococci and of streptococci.

Three strains of staphylococci and three of streptococci were cultivated aerobically and anaerobically in raw milk, normally collected from a healthy udder quarter, and actively collected from another healthy udder quarter but aseptically stimulated. The number of bacteria was checked with a plate method on blood agar. Milk samples for plating were taken before the incubation

and after 1, 2, 3, 6, 9 and 12 h of the culture at 37 °C. At the same time redox potential of the culture was measured.

Under conditions of full access of air the redox potential (Eh_7) of raw milk fluctuated from +225 mV to +240 mV. The growth of staphylococci and of streptococci under aerobic conditions influenced the potential to a small degree. In anaerobic conditions Eh_7 of raw milk immediately after milking was several tens millivolt lower than under aerobic conditions. The growth of staphylococci in these conditions decreased the potential evidently, the growth of streptococci caused very fast and profound decrease of the potential. In redox potential similar to that inside the udder streptococci grow better in raw milk than staphylococci. Under these redox conditions in active milk containing 10^6 leucocytes per 1 cm^3, both streptococci and staphylococci survive, some strains grow. Under aerobic conditions active milk inhibits the growth of streptococci but not that of staphylococci.

INDICATION OF AGRICULTURAL POLLUTION IN MILK PRODUCTION

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The occurrence of myxobacteria in the raw and pasteurized milk was followed in connection with indications of primary contamination by excreta of milking cows. The authors examined 152 samples of raw milk from farms of the area of the districts Olomouc, Přerov and Prostějov. The frequency of occurrence of myxobacteria in raw milk was found to correlate with the quality class, determined on the basis of resazurine test and mechanical impurities.

The milk contaminated with myxobacteria has been found to be produced by 50% of the observed farms. The milk contaminated in this way comes into the milk-processing plant and batches from all sources are mixed together. Since the resting myxobacteria cells resist the temperature of pasteurization, the percentage of positive findings of myxobacteria in the distributed milk increases up to 80%.

The authors are of the opinion that myxobacteria are a highly specific and sensitive indicator of farming pollution in milk.

INVESTIGATION OF THE ORIGIN OF COLIFORM AND *E. COLI* CONTAMINATION IN MILK GAINED WITH MILKING MACHINE

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Fecal contamination in foods is judged very severely. The problem remains, however, whether the bacteria often detected in fresh milk, which form a part also of bovine intestinal flora, signal a direct contamination by excrement or get into the milk from the environment.

Results have shown that in spite of non-satisfactory milking and cow-shed hygiene the milk obtained with expertly cleaned and desinfected milking machine was of a low cell count ($\log \bar{x} = 3.0 \times 10^3$ cm^{-3} of which coliforms were below 1.0 cm^{-3}). In the control section of the same cow-shed on the inner surface of the milking machine and milk tanks the number of coliforms was very high and *E. coli* was also found among them. Milk obtained by this equipment had a count of coliforms $\log \bar{x} = 2.4 \times 10^3$ cm^{-3} and that of *E. coli* was $\log \bar{x} = 3.2 \times 10^1$ cm^{-3}.

In the excrement of the cows investigated the total cell count amounted to $\log \bar{x} = 1.7 \times 10^6$ g^{-1}, the coliform count to $\log \bar{x} = 1.3 \times 10^5$ g^{-1} 46% of which was found to be *E. coli*.

A coliform count of 10^3 to 10^5 per cm^3 shows a highly contaminated milk originating from not properly cleaned equipment.

GRADING OF RAW MILK

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A new system for receipt of raw milk based on the hygienic level and paid for accordingly is being introduced in Hungary. In the first milk qualifying laboratory, Veszprém, random samples are taken of milk coming from state farms since 1980. Samples are analyzed for acidity, pH, physical purity, colony count, fermentation inhibiting additives, chemical composition and adulteration (watering). Data are fed back to dairy industry, milk producer and animal health service.

The short film gives an outline of the laboratory work carried out with up-to-date equipments (Petrifoss, Biomatic, Milkoscan, Cryoscop, etc.).

DETECTION OF STAPHYLOCOCCAL THERMONUCLEASE (TNase) IN NATURALLY CONTAMINATED DRIED PASTAS

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Staphylococci are the most common bacteria causing food poisoning. Presence of viable *S. aureus* in food initially and their subsequent growth during processing and/or storage could lead to presence of various enterotoxins in food. *S. aureus*, also including enterotoxigenic strains produce an extracellular nuclease that is heat-stable. Toxic properties of staphylococci strains are considered to correlate with TNase production. Nuclease as well as the enterotoxin can survive processing procedures and/or storage that kill the organisms; therefore, both are better indicators of staphylococcal growth than the viable counts. Due to the difficulty and time-consuming nature of demonstrating staphylococcal enterotoxins in food, TNase analysis has recently been proposed as a rapid means of screening for growth of *S. aureus* and potential enterotoxin production.

A new method for extraction and concentration of staphylococcal TNase of dried pasta is discussed. The procedure involves extraction, centrifugation, heat-treatment of supernatant 15 min at 100 °C, extraction of TNase from water layer with CG-50 Amberlite ion exchange resin (pH 5.8), eluation with 1.0 *M* ammonium acetate buffer of pH 9.1, concentration by evaporation. The dried matter is dissolved in a minimal amount of proteose pepton — yeast extract solution. Detection of TNase activity is performed in ethidium bromide-deoxyribonucleic acid agar plate. Specificity of the reaction was proved by specific antibody inhibition.

The presented assay method was apparently suitable for detection of staphylococcal TNase in egg-containing dried pastas.

COMPARISON OF FOUR LIQUID MEDIA FOR RAPID ENUMERATION OF FECAL COLIFORM BACTERIA IN FOOD

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The number of fecal coliforms in 41 samples of raw meat products and 14 samples of various processed food products were determined by an MPN-procedure using four different media: lauryl sulphate-tryptone broth (LST), minerals modified glutamate medium (MMGM), A-1 medium, and MacConkey broth. The inoculated tubes were incubated directly at 44 °C and gas production was recorded after 24 and 48 hours. From positive tubes, representative cul-

tures were isolated and identified. Estimated from the number of positive tubes, A-1 medium and LST had the highest recovery rate. With these two media the maximum yield was obtained after 24 hours of incubation — in contrast to MMGM and MacConkey broth, in which gas production was delayed.

Recovery of sublethally injured indicator organisms in food is discussed with reference to selective media.

COMPARISON OF SINGLE-TUBE MEDIA FOR COLIFORM DIFFERENTIATION TESTS AT 44 °C

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Simple techniques are of paramount importance in water and food bacteriology. A single-tube medium, the lactose-tryptone-ricinoleate broth (LTRB) was used since 1972. A slight modification resulted in better indole production and a comparison during routine water examination with the two-tubes classical formulation (brilliant green-bile broth and peptone water BGBB/PW) gave identical results and minor differences (statistically not significant) with Fennell's medium. In a series of 2350 differential tests during milk cream examination, LTRB was again found in complete agreement with BGBB/PW. As sodium recinoleate is not easily found in the market it was substituted by sodium lauryl sulfate and the new formulation, lactose-tryptone-lauryl sulfate broth (LTLSB) was compared not only with the two-tubes classical method (BGBB/PW) but also with the commercially available media a) lauryl-tryptose-lactose broth (LTLB), b) LTLB plus tryptophane (LTLBT) and c) lauryl-tryptose broth prepared with mannitol instead of lactose (LTMB).

The new formulation is as follows: Tryptone 20 g, Tryptose 10 g. K_2HPO_4 3 g, KH_2PO_4 1 g, Lactose 1 g, Sod. lauryl sulfate 0.1 g, Sod. formate 5 g, Dist. water 1000 cm^3. Final pH 7.5 $\pm$ 1. The comparisons were made during routine water examination.

Results: The use of sodium lauryl sulfate did not change the performance of the medium and complete agreement with the old formula as well as with BGBB/PW was always observed. In a series of 453 tests, LTLSB gave 279 positive reactions (gas and indole), LTLB 125 and LTLBT 199. The superiority of LTLSB medium over the 2 other single-tube media is obvious ($P < 0.001$). More extensive comparison was made with LTMB which was recommended by the Joint Committee. In a series of 1079 tests at 44 °C, LTLSB gave 610 positive reactions and LTMB 576. The difference between the two media is statistically significant (Paired x^2, $P < 0.001$). All negative tests of LTMB were due to negative indole reactions although indole producing organisms were present.

Generally LTLSB medium gives more strong and clear indole reactions than the other lauryl media, prepared even with mannitol. It is concluded that the new medium LTLSB is a useful simplification for the coliform differentiation test at 44 °C.

A NEW SELECTIVE AGAR PLATE MEDIUM FOR SALMONELLA ISOLATION

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A new method has been introduced by the authors for the basis of a very selective agar plate medium.

This plate is inhibitory to all genus of enterobacteriaceae but salmonellas, inhibitory to *Ps. aeruginosa* and to all other bacteria.

The plate medium has a strongly marked feature namely in case of inoculation with faeces being negative for salmonellas remains sterile after a specific incubation.

Checking substances, some other material and an indicator system are incorporated into the culture medium.

The method has been used for years by the authors for their own routine and scientific purposes.

Up to now about 50 000 agar plate media have been inoculated. Their method has been tested with more than one thousand straines belonging to the various genera of enterobacteriaceae family, with numerous *Ps. aeruginosa* strains and with hundreds of salmonella strains isolated from various epidemiological specimens.

Results of investigations including 200 salmonella positive food samples are reported in a form of comparative examination.

This method is available also in other fields of public health and epidemiology for salmonella isolation.

In addition to the routine examinations which are mostly qualitative depending on the case, this method can be used as a semiquantitative method for counting salmonellas which is of great value to scientific purposes. It seems these results will advance the possibility of salmonella examinations with their simplicity, reliability and quickness.

The medium is available for industrial production. At present the authors are applying for a patent.

COMPARISON OF THE ORIGINAL RAPPAPORT MEDIUM (R30) AND THE RAPPAPORT-VASSILIADIS (RV) MEDIUM IN THE ISOLATION OF SALMONELLA

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The original Rappaport medium (R30/37 °C) was modified twice in 1970 (formula R25/37 °C) and in 1976 (formula R10/43 °C, RV medium) by Vassiliadis and his colleagues. These two modified media have been used very successfully as enrichment media for the isolation of salmonellae, after pre-enrichment in buffered peptone water (P medium) for 18–24 h at 37 °C. However, the original Rappaport medium is still used by some investigators at 43 °C (R30/43 °C) and for this reason a comparative evaluation of Rappaport enrichment media of different compositions was done in this study.

A total of 135 samples, including 55 chicken carcasses, 42 samples of bovine minced meat, 28 specimens of pork sausages, 6 samples of animal feeds, and 4 samples of sewage on Moore's swabs were examined for the isolation of salmonellae. All samples were preenriched in P medium and were subcultured into Rappaport media of different compositions as given in the footnote of the Table. The results are summarized below.

It can be concluded that the Rappaport-Vassiliadis enrichment medium (RV/43°) was found more effective in isolating salmonellae than the R30/37° ($P < 0.01$), the R30/43° medium ($P < 0.001$) and the R25/37° ($P < 0.01$). It also revealed a greater number of salmonella serotypes and strains. The RV medium inhibited the lactose and sucrose negative competing organisms much more strongly than the R25/37° medium.

Enrichment methods[a]

	R30/37° (10 cm³)	R30/43° (10 cm³)	R25/37° (10 cm³)	R25/37° (5 cm³)	RV/43° (10 cm³)	RV/43° (5 cm³)
Samples exam.	135	135	135	135	135	135
Samples posit.	40	34	41	41	47	44
Positive (%)	29.6	25.2	30.4	30.4	34.8	32.6
Serotypes isol.	12	13	12	14	17	13
Strains isol.	44	39	47	48	60	50

[a] R30/37° = original Rappaport medium, incubation at 37 °C for 48 h; R30/43° = incubation at 43 °C for 48 h; R25/37° = medium containing 25 cm³ of malachite green solution in 1125 cm³ of final medium, incubation at 37 °C for 48 h; RV/43° = medium containing 10 cm³ of malachite green solution in 1110 cm³ of final medium, incubation at 43 °C for 48 h.

All tubes containing 10 cm³ of Rappaport medium were inoculated with 0.1 cm³ of P medium, while all tubes containing 5 cm³ of Rappaport medium were inoculated with a 3 mm loopful of P medium.

GROWTH AND THERMONUCLEASE PRODUCTION BY *STAPHYLOCOCCUS AUREUS* IN VEGETABLES

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The thermonuclease produced by enterotoxic *Staphylococcus aureus* in foods has been suggested as an indicator of *S. aureus* growth and potential food intoxication caused by staphylococcal enterotoxins. To serve as a suitable indicator, however, detectable amounts need to be produced in all kind of foods in which growth is possible.

The present study was designed to determine growth and thermonuclease production by *S. aureus* in vegetables. In unheated fresh vegetables e.g. peas, beans, lettuce, turnip and celery, inoculated with 10^3 cells of *S. aureus* per gram, there is no growth of *S. aureus* and hence no production of thermonuclease at 37 °C due to antagonistic activities of the normal microflora.

In heated samples of these vegetables artificially contaminated with 10^3 cells of *S. aureus* per gram and incubated at 37 °C, an abundant growth of the bacterium was observed, however without thermonuclease production.

Experiments with heated vegetables mixed with a detectable amount of thermonuclease have shown that there is a recovery of 10 to 40%. This recovery can be compared with the recovery for other foods e.g. meat, poultry and dairy products. Moreover the thermonuclease is not inactivated by filter sterilized vegetable juices. Consequently, it appears that there is an inhibiting factor in vegetables for thermonuclease production. The factor is water soluble and heat stable. These findings were confirmed in laboratory media prepared with the various vegetable juices and also in beef and chicken mixed with the vegetables. It appears, therefore, possible that in such food mixtures the detectable level of thermonuclease is not reached, notwithstanding good growth of *S. aureus*.

Hence, the absence of a detectable amount of thermonuclease in vegetables or foods mixed with vegetables, seems to be no criterium with respect to the potential previous growth and enterotoxin production by *S. aureus*.

GAS-CHROMATOGRAPHIC ANALYSIS OF METABOLIC PRODUCTS OF MICROORGANISMS

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Gas-chromatographic methods are applicable for the analysis of microbial cell components, their metabolic products excreted in growth media and the products produced by the organisms by degradation of a specific substrate.

Gas-chromatographic analysis is useful not only for taxonomic differentiation of the organisms but also for diagnostic purposes.

The paper deals with the possibilities of characterizing lactic acid bacteria by gas chromatography. The method is applied to the analysis of volatile constituents of lyophilized bacterial cultures. The single species may be characterized by the produced amounts of acetaldehyde, acetone, butanone-2, ethanol and diacetyl.

It also reports on the application of gas chromatography for the identification of clostridium species isolated from foods.

IDENTIFICATION OF BACTERIA OF THE FAMILY ENTEROBACTERIACEAE BY GAS CHROMATOGRAPHY

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The identification is based on the isolation of the organism, lyophilization and esterification of the cell biomass of the isolated organism and on the determination of the methyl esters of organic acids by gas chromatography.

Special attention was given to organisms, important in water management and from the sanitary aspect, belonging to the family enterobacteriaceae as well organisms having similar properties from the family vibrionaceae and pseudomonadaceae. Characteristic for each organism of this set is the presence of different organic acids and their mutual quantitative ratios. The evaluation of results from more than 500 strains enabled the preparation of a key for the manual identification as well as a procedure for the numerical identification using a computer. The procedure can be completely automatized.

The superiority of the proposed method compared with currently used procedures (biochemical tests, etc.) is evident: it is several times quicker (the time for determination reduced to 6–8 hours), less demanding as far as manual work is concerned and at the same time it provides more precise and complete information.

The method is so sensitive that it permits to distinguish lower taxonomic units than species (biotype, serotype, etc.). Compared with the presently used classification, certain taxones appear, based on these results, as an inhomogeneous group and split into 2 or more smaller groups.

MODIFIED ANDERSON/BAIRD-PARKER (A/B—P) PROCEDURE FOR THE ENUMERATION OF *ESCHERICHIA COLI* IN FOODS

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The A/B—P procedure is a rapid, novel approach for detection of *Escherichia coli* in food. In limited studies in the USA, equivocal results have been obtained. Our laboratory evaluated the procedure, using pure cultures and slurries of food artificially contaminated with *E. coli*. Problems included reduced productivity among strains, variability of colony counts, slow temperature equilibration, interference from *Aeromonas hydrophilia* and *Enterobacter agglomerans* and background interference from food particles. Suggested improvements include

- reduction of bile salts No. 3 concentration from 0.15 to 0.10%,
- use of 10 plates per sample,
- submersion of plates in plastic bags in a water bath,
- routine tests for suitability of available media, and
- filtration or centrifugation of homogenate.

To minimize interference from other populations in designated foods, one or two confirmatory tests may be required, viz., oxidase and V-P. Collaborative studies using a variety of foods artificially or naturally contaminated with *E. coli* in different nations will be required for evaluation of an improved procedure.

COLLABORATIVE STUDIES FOR THE ELABORATION OF MICROBIOLOGICAL METHODS

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The microbiological criteria of foods as defined by ICMSF (1974) are the standard, the end-product specification and the microbiological guideline. The reference method of high sensitivity and reproducibility should be used as standard. The reference method is the basis of choosing the alternative or routine methods and it may be used in the specification or in the guideline. The precision of the method (repeatability: r value and reproducibility: R value) should be statistically established in collaborative studies of several laboratories.

The collaborative work is carried out in the laboratories of state food control institutions and in the industry. The different media and techniques for the determination of spoilage organisms — first of all the AMC, yeast and mould counts — in several foodstuffs are compared.

The results of comparative studies show that the difference is not significant among the AMC or yeast counts determined by SPC, MPN or membrane-filter techniques. The yeast and mould counts produce identical results when OGY or Rose-Bengal Agar is used.

The precision of the method is strongly dependent on the solubility or homogeneity of the foodstuff. The best and the lowest R value is obtained by investigation of liquid or soluble materials — as dry sugar or syrup — and the highest R value by the examination of dried, hard products, like dried vegetables.

The elaboration of reference methods of other spoilage organisms, such as osmotolerant yeasts, lactic-acid bacteria and sporeformers by ISO or ICMSF, should be useful beside the pathogens.

CULTIVATION OF ANAEROBIC MICROORGANISMS FROM FOOD — A NEW METHOD TO GENERATE AN ANAEROBIC ATMOSPHERE

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A new principle for the cultivation of anaerobic microorganisms which are relevant to food hygiene is presented. It consists of

— Anaerocult A to generate an anaerobic atmosphere in commercially available 2.5 l-anaerobic jars;

— Anaerocult P to generate an anaerobic atmosphere in a single Petri dish;

— Anaerobiosis Indicator to indicate an anaerobic atmosphere.

The microbiological investigations showed good to very good growth of all anaerobic organisms examined that are relevant to food hygiene (especially clostridia species).

Important physical-chemical parameters are:

— Rapid reduction of the oxygen concentration to below 0.5 vol. % within 45 minutes;

— Maintenance of an anaerobic atmosphere even if slight oxygen leaks in the anaerobic vessel occur because of the excess of the oxygen-binding agent and the slight gas over-pressure;

— High carbon dioxide concentrations over 15 vol. % for strongly CO_2-dependent microorganisms are obtained;

— Low hydrogen concentrations and only very slight increase in temperature in the anaerobic vessel guarantee very safe working conditions;

— Eh-values below 200 mV are rapidly obtained;

– Easy to use and very economical because there is no need to use a catalyst;

– Reversible indication of an anaerobic atmosphere by means of anaerobiosis indicator.

It is easy to use and its microbiological and physical-chemical characteristics make the anaerocult system a reliable, practical and economical means of cultivating anaerobic microorganisms from food.

IMPACT OF VARIOUS MODES OF PROCESSING FOR SAFETY ON THE MICROBIAL ASSOCIATIONS OF FOODS

ECOLOGICAL ESSENTIALS AND FUNDAMENTALS OF METHODOLOGY

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Food as marketed to the consumer is in some instances, e.g. meats and poultry, contaminated with pathogenic organisms causing outbreaks of foodborne diseases. Control relies on intervention which often includes a decontamination process to "eliminate" pathogenic organisms from food. This intervention also entails a change in the primary microbial flora of the food thus treated. It is essential to assess these shifts in order to identify the character, but particularly the fate of the organisms surviving decontamination.

When possible, the original microbial community structure after decontamination and its changes during storage are determined by the use of selective media, because this saves much work. It is then essential to apply, first of all, a resuscitation step to ensure that all surviving organisms are indeed included in colony counts. The Solid Medium Repair (SMR) method was found the most versatile technique available for this purpose. The use of rich infusion agar as resuscitation medium may yet underestimate the numbers of survivors, unless catalase and/or $NaHCO_3$ are used to complete the repair of sublethally damaged cells of particular organisms under special conditions. These facts have also to be taken in account when selective plating is less productive, so that primary plating has to be carried out on non-selective media.

It was found that an accurate assessment of flora-shifts had to rely on primary isolation plates showing at least the order of 70 colonies, and that groups of less than 3 colonies of a given type were better ignored. Studying the square root of the total number of colonies of a given type with a minimum of 3 allowed reliable taxonomic grouping. Studies on flora-shifts should never be related to situations wherein anomalous survival curves are obtained.

When irradiation with gamma rays is used for food decontamination, determination of immediate and subsequent changes in the microbial community structure after irradiation is a most essential part of the evaluation of the beneficial effects of the process. When applying the elaborated recovery techniques for this purpose, in some instances bacterial types were isolated that normally are missed because they occur as a very low fraction of the population in untreated foods. Identification, particularly of some catalase positive cocci and coryneform bacteria with described species was not always possible, so that isolates had to be tentatively assigned to matrix groups.

SOME RAPID METHODS IN FOOD MICROBIOLOGY

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Application of rapid methods in food microbiology is much more complicated, than in medical microbiology or when testing isolated strains. The composition of foods prevents the use of e.g. electrical impedance or calorimetry or others. Cultivating in a droplet medium and reading with a simple microscope is often successful. Paperstrips as Microbitests (reg. name) proved to be good tools for routine work in small laboratories. They are impregnated with various media. We compared the Microbitests with official plate methods. Microbitests are useful as a rapid, informative test. Their accuracy does not meet the official plate methods.

Enzymatic tests, based on biochemical destruction of glucose, or nitrate reduction or the presence and quantity of lactate-dehydrogenase of microbes, present in the food, were done. These tests were originally applied to quick-frozen ready-to-eat foods, but they are useful for some other types of foods, too, especially those having a big microbial load. The enzymatic tests are simple to handle and give rapid, informative results.

EVALUATION OF REFERENCE VALUES FOR SPOILAGE MICROORGANISMS IN FOODS OF PLANT ORIGIN

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The microbial criteria of food quality are evaluated in collaborative work of laboratories in state food control inspections and in the food industry under the auspices of Hungarian Scientific Society of the Food Industry, (MÉTE). The principles of the work are defined by ICMSF. The methods described by Mossel are adopted.

The direct stability test is used for the assessment of the keeping quality of foods. The samples of foodstuffs are stored at appropriate temperature and time and are investigated by microbiological and organoleptic methods. In this way the specific microbial group of spoilage association of food products and the minimal spoilage level (MSL) can be determined.

The reference value is based on the surveys performed in factories manufacturing the commodity applying GPM. A distribution curve of microbial count in the samples, determined during 2 or 3 years is then prepared. From this curve the so-called 95% Ø value is computed.

The operating characteristic (OC) curve of sampling plans are drawn by means of calculating the "generalized standard deviation" of microbial count per lot.

The reference value is determined graphically by fitting the distribution curve taking into account the MSL, the Ø value and the stingency of the criterium.

VARIABLES SAMPLING PLANS FOR THE MICROBIOLOGICAL QUALITY MONITORING OF FOODS

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The ICMSF (1974) recommended attributive plans for the microbiological quality control of foods for the purpose of international trade. These plans are independent from the distribution of microbiological count in lots, which are unknown to the receiver.

However, the national inspection agencies and control laboratories of food manufacturers are in the position to get informations about the statistical characteristics of the microbial counts in lots. Consequently the variables plans can be applied in the national food control systems.

Four aspects of the statistical methods for the evaluation of sampling plans are discussed in this paper:

— The evaluation of the variables plans, showing the acceptance or rejection results equated with attributes plans, with the advantage of less laboratory work.

— Analysis of the reality of "m" and "M" values of 3 class attributes plans, recommended by ICMSF.

— The determination of "m" value of variable plans considering the statistical characteristics (mean value, standard deviation) of the microbial counts in lots produced in Good Manufacturing practice.

— Statistical problems of the evaluation of sampling plans of lot showing a "trunk distribution" of the microbial count.

NUMERICAL CHARACTERIZATION OF SPOILAGE BACTERIA

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Isolation of spoilage bacteria. It can be assumed that the significant spoilage bacteria in any situation will exist there in large numbers. They may not proliferate competitively on traditional isolation media used and hence their significance may be underestimated. The Spiral Plater (Spiral Systems Inc: USA) which deposits c. 40 mm^3 of aqueous sample on to a rotating Petri dish in a progressively decreasing amount is a tool for colony counting. It also gives us a method of objectively determining the dominant spoilage flora as we need only locate the outer end of the spiral and work backwards picking off say the last ten colonies.

Numerical characterization. Characterization traditionally attempts to name the organism, using tests which tell us little of its spoilage significance. If we select tests which are relevant to our particular spoilage situation we can construct a numerical profile which will give the organism a meaningful identifying number. The tests can be varied to meet different spoilage situations and are grouped in threes. They must give a negative/positive answer and are scored 0 or 1 for the first test of a group of three, 0 or 2 for the second, and 0 or 4 for the third. A selection of tests could be:

A. rod, B. growth on crystal violet agar, C. motile, D. heat resistant, E. water soluble pigment, F. growth at 42 °C, G. oxidase, H. oxidative on glucose, I. fermentative on glucose, J. growth on cellulose, K. growth on hydrocarbon, L. growth on citrate, M. lipolytic on Tween 60, N. lipolytic on Tween 80, O. lipolytic on tributyrin, P. growth on creatine, Q. hydrolysis of urea, R. liquefaction of gelatin, etc., A hypothetical result of characterization could be as follows:

A+=1;	D−=0;	G+=1;	J−=0;	M+=1;	P+=1;
B+=2;	E+=2;	H+=2;	K+=2;	N−=0;	Q−=0;
C+=4;	F+=4;	I−=0;	L+=4;	O−=0;	R+=4;
Totals 7	6	3	6	1	5

The numerical profile would be 763615. This number conveys information on the potential spoilage role of the organism and is also the label which is used to determine its frequency of occurrence and its "epidemiology".

Characterization of total spoilage flora. We are determining gross spoilage potential of a sample by introducing it as a liquid onto appropriate indicator media. For example, Tributyrin Agar (Oxoid Ltd., Code PM4), Iron Sulphite Agar (Code CM79) and Nutrient Gelatin (Code CM135a containing dispersed carbon particles) are tubed in 10 cm^3 aliquots and about 2 cm^3 of the

sample (milk, cutting oil, etc.) is added. If lipolytic organisms are present, the Tributyrin Agar clears from the top downward; if sulphate reducing bacteria are present a black deposit appears at the interface of the iron sulphite agar and spreads downwards; if proteolytic organisms are present, charcoal is deposited in an obvious band as the gelatin becomes less viscous. Many other indicator tubes have been devised. The rate of reaction appears to be of some significance, but the main objective is to determine the gross spoilage potential of the flora present.

MYCOLOGICAL STUDIES ON DUTCH RYE BREAD

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Spoilage of rye bread by moulds is a major problem, especially in the case of packed, sliced rye bread. Because of requests to enlarge the legal methods to preserve rye bread, the level of contamination with moulds and yeasts as well as the composition of the mycoflora of this product were studied. For this purpose 204 samples of different types of rye bread available on the Dutch market were examined.

A low contamination level was found for industrially manufactured, packed, sliced rye bread (120 samples): only 4% of the samples had mould counts $>10^3$ c.f.u. per g (= colony forming units), also 4% of the samples had yeast counts $>10^3$ c.f.u. per g. The contamination level of packed, sliced bread from smaller bakeries (33 samples) was only slightly higher: 9% of the samples had mould counts $>10^3$ c.f.u. per g and 9% of the samples had yeast counts $>10^3$ c.f.u. per g.

A significant difference in keeping quality was observed between industrially manufactured rye bread and rye bread from smaller bakeries: only 12% of the industrially manufactured products were visibly spoiled by moulds or yeasts after 10 weeks of storage at 24 °C, whereas 33% of the products from smaller bakeries were already spoiled after 4 weeks. This difference in keeping quality seems to be related to differences in pH (pH of products from smaller bakeries was higher) and in amounts of added sorbic acid and propionic acid (in products from smaller bakeries the preservatives were found in lower concentrations).

Penicillium roqueforti and yeasts were isolated most frequently from the products preserved with sorbic acid and/or propionic acid, which were visibly spoiled within 10 weeks of storage at 24 °C.

The contamination level of unpacked, unsliced (whole) rye bread from smaller bakeries (30 samples) was also low: 3% of the samples had mould counts $>10^3$ c.f.u. per g, 10% had yeast counts $>10^3$ c.f.u. per g. The use of

sorbic and propionic acid in this product is not allowed and the keeping quality was low: 74% of the samples were visibly spoiled within 1 week at 24 °C, mostly by moulds. *Penicillium verrucosum var. cyclopium* was isolated most frequently. This mould species was also isolated most frequently from samples with mould counts $>10^3$ c.f.u. per g at direct examination.

The keeping quality of the rye bread can be improved by minimizing the recontamination of the sterile loaves after leaving the oven by applying Good Manufacturing Practices (GMP) in the bakery during cooling and possible slicing and packing.

YEASTS IN UNPASTEURIZED SOFT DRINKS

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According to intrinsic and extrinsic factors of unpasteurized soft drinks saturated with CO_2, yeasts are the microorganism of primary interest, concerning the quality as well as health risk of the product.

In soft drinks we tested most frequently the genus saccharomyces occurring especially *Saccharomyces uvarum*, *Saccharomyces cerevisiae*, *Saccharomyces rosei* and others. The occurrence of torulopsis and candida was not so frequent, and other genera were found but rarely.

The sources of yeasts are usually insufficiently cleaned bottles. In the bottles there are spots with dry residue of drinks, and it is difficult to get rid of them during the washing procedure. A new technology of mixing the syrups with water before they enter the filling machine brings more risks, than the old one, when syrup and water were put into the bottle separately, mixing first in the bottle and not in the filling machine. The numbers of yeasts in syrup is not high and there are nearly no yeasts in potable water.

If the number of yeasts in a soft drink is high enough, that is of the order of 10^4 cm^{-3}, than, may be, organoleptic changes are observed. Some types or genera of yeasts can make the changes more profound, while others produce no changes at all. However, if yeasts are present in the density of 10^5 cm^3, then organoleptic changes are more frequent.

Soft drinks of the cola type can have high densities of yeasts without showing organoleptic change. Even here the type or genus is of importance. Soft drinks highly infected with yeasts, can cause gastrointestinal troubles with a short incubation period.

THE ATTACHMENT OF MEAT SPOILAGE ORGANISMS TO L. DORSI MUSCLE

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The attachment capability of common meat spoilage organisms to meat would determine which organisms would be easily released and cause secondary contamination, would be the predominant species isolated from meat depending on the sampling method, e.g., swab method, and could perhaps also influence the subsequent distribution of organisms in the meat. Seven common meat spoilage organisms were isolated and their attachment to I. dorsi muscle determined.

Attachment and S value were determined as follows:

Treatment A:

Each of two meat samples was put into a 250 cm^3 flask containing 100 cm^3 of 0.1% peptona-water (PW). The flasks were shaken at 200 oscillations per min for 1 min at room temperature. Bacterial numbers were then determined in the liquid fraction.

Treatment B:

Each of two samples was blended with 150 cm^3 of 0.1% PW for 1 min and bacterial numbers determined in the homogenate.

Treatment C:

Each of two samples was placed into a 250 cm^3 beaker containing 100 cm^3 of 0.1% PW. The contents of the beakers were swirled gently for 30 s. Each meat sample was then removed, drained, and placed into another 250 cm^3 beaker containing 100 cm^3 of 0.1% PW. The contents of the beaker were again swirled gently for 30 s. Each piece of meat was subsequently removed drained, and blended with 150 cm^3 of 0.1% PW for 1 min and bacterial numbers in the homogenate determined.

Attachment was defined as the number of bacteria adsorbed to 16.2 cm^2 of meat surface during a 20 min incubation in the presence of 10 bacteria per cm^3 in the attachment bath. Attachment strength

$$(S) = \log C - \log[A - (B - C)].$$

The lowest attachment values were recorded with *Acinetobacter* LD-2 and *M. osloensis;* the highest, with the two motile fluorescent pseudomonads. The S values ranged from 0.19 for *P. putida* to 0.70 for *Acinetobacter* LD-2. Except for *P. fluorescens*, the three non-motile organisms (*M. osloensis*, *Acinetobacter* LD-2 and *B. thermosphacta*) had the highest S values.

Considering the attachment and S values, *P. fluorescens* followed by *B. thermosphacta* appear to be the two organisms best suited for attaching to and remaining on the meat surface. The non-fluorescent pseudomonad appears to be the organism least suited for attachment.

Since several organisms are in contact with the meat surface at any one time, it is possible that the presence of one organism may affect the attachment of another organism. The non-fluorescent pseudomonads, *M. osloensis*, *Acinetobacter* LD-2 and *B. thermosphacta* significantly reduced ($P < 0.05$) the attachment of *P. fluorescens* to the L. dorsi muscle surface. In addition, the non-fluorescent pseudomonads, *M. osloensis*, and *Acinetobacter* LD-2 significantly reduced the attachment of *B. thermosphacta* to meat. *E. agglomerans* attachment to the meat surface was reduced in the presence of *P. fluorescens* and *P. putida* but not in the presence of the fluorescent pseudomonad. *P. putida* and *M osloensis* were unaffected by the presence of other meat spoilage organisms. The attachment of *Acinetobacter* LD-2 and *E. agglomerans* to L. dorsi muscle in the presence of *P. putida* was, however, significantly higher ($P < 0.05$) than that for *Acinetobacter* or *Enterobacter* in the absence of any competing organisms.

Although attachment of several organisms was affected by the presence of other organisms, the degree of interaction is probably not sufficient to greatly influence the eventual distribution of microorganisms in spoiled meat.

INFLUENCE OF TYPE OF TISSUE AND INITIAL CONTAMINATION ON THE DEVELOPMENT OF BACTERIAL FLORA ON VACUUM PACKED BEEF

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Sirloins of beef were deboned and cut into steaks in 2 different conditions so that steaks with a high (experiment 1) and a low (experiment 2) initial contamination was obtained. Each steak was vacuum packed in a polyamide-polyethylene film and stored at 0–+2 °C. At intervals of 1 week, one steak of each sirloin was withdrawn at random for sampling.

In both experiments it was found that the development of the bacterial flora was influenced by the type of tissue. On muscle tissue the growth of pseudomonas spp. and *Brochothrix thermosphacta* ceased by a number of 4 log. On the other hand these bacteria developed very well on superficial fatty tissue.

At the same period of storage tissues contained more bacteria in experiment 1 than in experiment 2. In both cases the growth pattern on fatty tissue was similar. After a rapid growth during the first weeks, most counts remained at a fairly constant level. The number of total colony counts and lactobacillus

spp. on muscle tissue with a low initial contamination increased during the course of the whole storage period. In experiment 1 the growth of these bacteria diminished considerably after 4 weeks of storage.

The keeping quality of vacuum packed meat was clearly influenced by the initial contamination. During storage, however, numbers of bacteria were not correlated with the freshness or keeping quality of vacuum packed meat.

BIOCHEMICAL CHARACTERISTICS OF *STAPHYLOCOCCUS AUREUS* STRAINS OF DIFFERENT FOOD ORIGIN AND THEIR ENTEROTOXOGENECITY

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Investigations were carried out on 100 *Staph. aureus* strains isolated from different foods of animal as well as plant origin. The object of this work was to collect data on the biochemical characteristics of different *Staph. aureus* strains to get information about tests for confirmation, and correlation between the tests and the toxin producing capacity of the strains. During the course of the investigation the following characteristics were tested: coagulase production, phosphoprotein lipase activity, heat-stable DNSase production, anaerobic mannitol fermentation, pigment production, crystal violet activity, haemolysin production, phage type and enterotoxin production. Among the strains a great variety can be observed without a definite answer concerning the origin and toxin producing capacity of them. Twenty-five % of the strains studied have produced toxin, namely A, B, D, A–D enterotoxins without uniform characteristics. Some of them had no phosphoprotein lipase, heat-stable DNSase, haemolysin, etc. but all were positive in coagulase test. According to the results the coagulase test proved to be the most reliable and the judgement of *Staph. aureus* strains in motivation of food poisoning should be based only on enterotoxin detection.

PROBLEMS RELATED TO THE MICROFLORA OF CONFECTIONERY PRODUCTS

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A characteristic feature of confectionery products is their low water activity (a_w). In spite of that, there are groups of microorganisms capable to bring about microbial spoilage in confections.

The authors have been studying the influence of various values of wa (within the range of 0.6–0.9) on the growth and proliferation of 28 yeast strains, 10 mould strains and 4 bacterial strains. The examined microorganisms were isolated from spoiled confections, on one hand, and acquired from the CSSR collection, on the other.

The study was aimed at determining the limit of a_w at which the tested strains would have already stopped to grow.

A direct correlation was established between the product's a_w and its shelf-life when contaminated by yeasts.

Growth of moulds was primarily affected by the humidity of air. The moisture content of the product's surface is influenced by the humidity of the atmosphere.

The examined pathogenic microorganisms did not grow in the medium with a_w corresponding to the a_w of confections, but they perished in the course of 12 weeks.

STAPHYLOCOCCUS AUREUS IN DRY PASTES

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The microbiological quality of dry pastes is determined by the number of viable cells in the raw materials used, by the level of hygiene in the manufacturing process and last but not least by the condition of health of the stuff manufacturing them. Investigations into the hygienic condition of manufacture as well as data in related literature has shown objections to quality to depend mainly on the *Staphylococcus aureus* count in the product beside the salmonella infection. The authors wished to elucidate the source of *Staphylococcus aureus* infection and the conditions of its propagation.

The raw materials, the moist and the dried paste samples were suspended in physiological solution with the Stomacher homogenizer. The solution contained pepton and a dilution range was prepared in the usual way. The *Staphylococcus aureus* was isolated on Baird-Parker selective – salt-bloody and 10% cattle blood containing agar plates. Samples were taken further from the mucous membrane of the naso-pharyngeal tract and hands of the staff and from the air of the work room. To identify the isolated strains the coagulase, heatstable DNase and the lecithinase tests were carried out and the haemolytic, pigment producing and mannite fermenting capacity were also tested. Finally the phags were identified. The multiplication kinetics of staphylococci in dough was investigated by in vitro experiments.

The microorganisms separated from the different sources were found to have similar properties and so were the phag types, too. The following conclu-

sions were drawn: Employees have to be tested for staphylococcus infection, and those infected have to be excluded from the workroom. Workers should be obliged to wear mask during worktime. Germicid lamps should be used to ensure infection-free air. The pastes should be dried as rapidly as possible. Machines, equipments and other implements should be disinfected for every work shift.

STREPTOCOCCUS LACTIS INHIBITION OF AFLATOXIN PRODUCTION BY *ASPERGILLUS FLAVUS*

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Previously it had been shown, that *S. lactis* produces an inhibitory compound(s) which inhibits the production of aflatoxin by *A. flavus*. The inhibition was not attributed to a depletion of nutrients in the mixed culture system nor to the production of either lactic or acetic acid. This report deals with the further characterization of this inhibitory compound.

The inhibitory compound produced by *S. lactis* was excreted into the medium. It is an extracellular compound which does not bind to the cell wall of the cell as the cellular fraction stimulated aflatoxin production. The inhibitor accumulated during the logarithmic phase of growth and was subsequently degraded either because it was utilized as a secondary metabolite or because it is a labile compound. The inhibitor has a molecular weight < 500 and was concentrated in the organic phase after a Bligh and Dyer extraction of the filtrate. It is a polar compound (hexane insoluble) and is not a free fatty acid (sodium bicarbonate insoluble).

The inhibitory compounds, extracted with chloroform-methanol, were acetone soluble (neutral lipids). A major inhibitor compound, separated on TLC reacted with an ammonium molybdate spray, (presence of a phosphate ester). After mild alkaline deacylation, the compound was found to contain elements of carbohydrates (positive Dubois test) and an aromatic ring structure (positive Lowry reaction with deacylated compound and negative ninhydrin reaction with original compound). A scan of the inhibitory compound showed an absorption at 275 nm. Aldehyde and ketone reactive groups were not detected.

The inhibitory compound thus appears to be a small molecular weight (MW < 500) phosphoglycolipid containing an aromatic ring structure.

Although *S. lactis* has been found to inhibit the aflatoxin production by *A. flavus*, the question remains whether the *S. lactis* actually detoxifies the *A. flavus* system.

S. lactis and *A. flavus* were grown in mixed cultures and the cultures extracted with chloroform. The concentrated chloroform extract and several

fractions separated on TLC were found to inhibit the growth of *B. megaterium* around the disc, but did not effect elongation of the bacilli at the periphery of the inhibition zone, as was observed with the aflatoxins.

Using the Ames test, no mutagenic or carcinogenic activity was detected in the extracts of *A. flavus* grown in mixed culture with *S. lactis;* whereas fractions of *A. flavus* grown in mono-culture were highly biologically active in the *Salmonella* mammalian-microsome mutagenic test.

The results suggest that the phosphoglycolipid like inhibitory compound produced by *S. lactis* prevents the formation of the biofuran ring structure, the cyclopentantanone ring, or the methoxy structure of aflatoxin B1.

ENHANCEMENT OF EFFECTIVITY OF CELLULASE ENZYMES OF MICROBIAL ORIGIN

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Because of the current concern over energy and food shortages, much research effort has been spent on the recovery and reuse of waste cellulose. One of the processes for the reuse of cellulosic wastes is enzymatic hydrolysis, which converts cellulosic materials into glucose.

Cellulase is a complex of enzymes containing mainly exo-glucanases and endoglucanases, plus cellobiase. For the complete hydrolysis of insoluble cellulose, a synergistic action between these components is required. Since different cellulase preparations vary widely in the proportions of the different components, the rate of their hydrolysis of cellulosic substrates also vary widely.

Five culture filtrates of different fungal origin *(Trichoderma viride* OKI B-1; *Trichoderma reesei* QM 9414; *Aspergillus terreus* OKI 16/5; *Gliocladium* sp.; *Penicillium verruculosum* WA-30) were produced and tested in laboratory experiments. Pure celluloses (cotton, Solka Floc) and wastes (newspaper, maize stalk) were hydrolysed at 50 °C, pH 4.8, for 24 and 48 hours, respectively.

To enhance the effectivity of enzymatic breakdown, two fungal culture filtrates were mixed in different proportions. During the hydrolysis of the cellulosic materials with the mixtures, a strong synergistic effect was observed.

The best combinations are shown in the Table as follows:

A 24 hour-hydrolysis of cellulosic materials

Culture filtrates	Mixture rates	Substrates	Enhancement of activity (%)	Degradation (%)
T.v. OKI B–1 — P.v. WA 30	1:1	Cotton	180.0	21.8
P.v. WA 30 — T.r. QM9414	1:1	Maize Stalk	23.8	25.0
Gliocl. sp. — A.t. OKI 16/5	4:1	Newspaper	44.0	28.0
T.v. OKI B–1 — P.v. WA 30	1:1	Solka Floc	61.5	63.0

It was concluded from these experiments that the use of different fungal cellulase enzyme supernatants in mixtures can lead to a quicker and more complete conversion of cellulose into fermentable sugars than the use of individual fungal supernatants alone.

This may be because in trichoderma and gliocladium cellulase enzyme complexes the β-glucosidase enzyme concentrations are suboptimal, whereas in aspergillus and penicillium enzyme complexes high.

STUDIES ON THE TECHNOLOGY-HYGIENE OF PIG SLAUGHTERING

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Five skinning and five scalding pig slaughtering lines were checked by microbiological tests. The hygienic level of the slaughter technology was determined on the ground of bacterial contamination of the carcass surfaces. Major conclusions are as follows:

— On the surface of carcasses produced by skinning technology colony counts were as follows: total aerobic mesophilic microbes 3.89×10^2–2.51×10^4, coliforms 0.58×10–3.89×10^2, *St. aureus* 0.77×10–1.94×10, pseudomonas < 10, enterococcus < 10–1.07×10^2, micrococcus 2.16×10^2–8.46×10^3, clostridium veg. < 1–3.02×10^3, spor < 1 per cm^2. Figures representing scalding technology are listed in the above order: 2.13×10^3–1.23×10^5, 0.58×10–9.12×10, 1.20×10–8.3×10, < 10, < 10–1.33×10, 2.87×10^2–5.37×10^3, < 1–2.75×10, < 1 per cm^2.

— Salmonellae were detected in each case on the surface of the skin before slaughter but none in the 700 samples from the surface of carcasses.

— The bacterial contamination of the instruments in contact with the carcass surface (knives, cleavers, saws chain-gloves) are characteristic of the hygienic level of slaughtering. The following counts were obtained in the above order: 8.10×10^2–2.80×10^6, 10–5.00×10^5, 10–3.5×10^2, 10–3.00×10, 10–4.20×10^4, 10–2.00×10^4, < 10–10^3.

— Comparing the hygienic level of skinning and scalding slaughtering the average values of aerobic mesophilic microbes per cm^2 of carcass surface were 1.67×10^3 and 1.63×10^4, respectively. The difference was significant by Student's t test.

COMPOSITION OF MICROFLORA OF CHEESE SAMPLES

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The microflora composition of cheese samples was studied. The analysis of milk-agar cultures for the determination of the total cell counts (colony forming units) gave the following results:

The total cell counts of 500 natural cheese samples were uniformly over 10^3 g^{-1}, out of these the counts of 78 samples (15.6%) exceeded 10^8 g^{-1}. The most frequent members of the microflora were micrococci, which were found in 92% of the samples. Lactic acid producing streptococci were present in 90% of the samples, but their number was higher than that of the micrococci. Lactobacilli were found in 5.2%, sarcina species in 3.2%, and corynebacteria in 1.2% of the cheeses. Spore-forming bacilli, chromobacteria and enterobacteria were present in the products in 42, 28.4 and 26.6%, respectively, of pathogenic bacteria only staphylococci were found in 1%. The occurrence of yeasts and moulds was 9.2 and 28.6%, respectively.

The colony count was less than 10^5 g^{-1} in 96% of the 430 processed cheese samples investigated, being micrococci (in 98.3% of the samples) and bacilli (in 47.4%) the dominant members of the microflora. The lactic acid producing streptococci were present in 36% of the products, only. Moulds were found in 6%, chromobacteria in 7.4%, enterobacteria in 4.2%, sarcina species in 1.2% of the samples. The numbers of micrococci, streptococci and bacilli were higher than 10^4 g^{-1}, those of the others were less. The difference between the flora of the natural and processed cheeses can be attributed to the heat treatment in the technology.

DEBITTERING OF LUPINE SEED BY LACTIC ACID FERMENTATION

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Lactic acid fermentation of dehulled lupine seed followed by substantial washing was found to be efficient method for gaining practically alkaloid-free product of enhanced protein content (approx. 0.1% remaining toxic quinolizidine alkaloids in the fermented samples with 45–47% protein content).

Lupine is a potentially valuable seed protein producing crop for temperate climates. The protein content of seed varies from 30 to 45%. Its protein quality and digestibility is similar to that of soybean. Though "sweet" varieties

have been developed with low alkaloid content, the original "bitter" varieties containing 0.6–1.3% toxic quinolizidine alkaloids have some advantages, namely they can be cultivated on poor soils where other crops would not survive. Moreover, the nitrogen-fixing capacity of lupine is considerable it may be used as a soil ameliorator.

Several methods of treatment are known to remove the bitter alkaloids from the seed, e.g., extraction with acid or alkali followed by substantial washing. These methods result in effluents of high salt content, therefore, disposal is a problem.

Fermented products in food and feed industry, in general, have enhanced nutritional value because of detoxifying effects and enhanced digestibility. However, little work had been done with lupine to see if treatment of seed by lactic acid bacteria would result in more valuable product (feed or food).

In our laboratory experiments, different lactic acid bacteria and experimental conditions were used for fermentative detoxification of dehulled seeds of the white flowering species *(Lupinus albus* L.). The bacteria have been anaerobically propagated at 37 °C in 750 cm^3 Erlenmeyer flasks, where 100 g (d.w.) of dehulled seed and 600 cm^3 of tap water (soaking-fermenting medium) was placed. After 1–2 days of fermentation, the supernatant was decanted and fresh tap water supplement was added for washing.

The fermented and thoroughly washed sample had no bitter taste, the residual alkaloid content was approx. 0.1%. On the other hand, the crude protein content of product was significantly higher than in the original seed (see table below).

Samples	Quantity (g)	Crude protein (%)	Crude ash (%)	Protein recovery (%)	Total alkaloid (%)
Lupine seed (starting material)	100.0	30.6	3.1	—	1.1
Soaked, dehulled, fermented, washed and dried sample	52.0	46.0	0.9	78.0	0.1
Hull (dried)	16.5	8.8		4.7	

The best results were obtained by different *Lactobacillus plantarum* species.

Further work would be needed to demonstrate the feasibility of this process in larger scale pilot-plant operation.

ALTERATION IN THE COMPOSITION OF SACCHAROMYCES YEAST DURING REPEATED BEER FERMENTATION

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Brewer's yeast *(Saccharomyces cerevisiae)* is repeatedly used for beer fermentation in factories. This prolonged holding in the same fermenter causes alterations in the yeast biomass: although a 2–3 times increase is observed during one brewing process, some quality problems arise by using the same yeast 6–8 times for fermenting malt wort to beer. Most attenuation difficulties happen because of physiological problems affecting the performance of the yeast. Attenuation difficulties merely reflect the fact that cells are reaching a resting state or are becoming less tolerant to ethanol.

Under anaerobic conditions brewer's yeast becomes auxotrophic for sterols and unsaturated fatty acids. Lack of lipids i.e. some of the membrane constituents causes auxotrophism (starvation) and degradation of yeast cells. Samples taken directly from the brewery were dried and analysed, lipids were extracted and fatty acid composition examined by gas-chromatographic method. Analysis of 6 consequent brewing processes showed a significant decrease of lipid extract and the ratio of fatty acids.

Lipid extract and ratio of fatty acids in percentage of dry weight of yeast biomass

No. of fermentation	Lipids	Fatty acids
1	2.4%	1.0%
2	1.9%	0.9%
3	1.9%	0.8%
4	1.5%	0.5%
5	1.3%	0.5%
6	1.0%	0.4%

During 6 consequent fermentation processes the percentage of short chain (C_8–C_{14}) fatty acids in membrane composition increased from 10% to 30%, but the ratio of $C_{18:1}$ (oleic acid) decreased from 8% to 4%, $C_{16:0}$ (palmitic acid) from 35% to 18%.

STUDIES ON THE CONDITIONS OF THE FORMATION OF RENNIN-LIKE ENZYME IN SUBMERGED CULTURE OF *ENDOTHIA PARASITICA*

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A procedure for the production of milk-clotting enzyme in submerged culture of *Endothia parasitica* has been developed at the Central Food Research Institute and Phylaxia Company for Veterinary Biologicals and Feedstuffs. In order to increase the enzyme activity in the fermentation broth further experiments were carried out on laboratory scale based on the experiences of the industrial fermentation.

Conditions influencing the quality of the spore cultures, effect of the spore culture quality on the inoculum, optimum state of the inoculum for starting the enzyme fermentation, and relationship between growth and enzyme formation were examined.

The mold was maintained and sporulated on the surface of a solid medium. — Inocula were seeded with spore suspension and the fermentation media by the inoculum of optimum state detected by reductase test using a resazurin solution. — Submerged cultures were characterized by the change of pH, reduction time of the resazurin solution (min), mycelium concentration (g 100 cm^{-3}), and milk-clotting activity (SU). — Relationships between growth and enzyme formation were analyzed by Kono and Asai's method.

Results of examinations are as follows:

— Considerable effect of the humidity in the air above the spore culture could be stated. The sporulation was intensified and the time of it was shortened by certain regulation of the humidity.

— Linear relationship was found between the mycelium concentration and the reciprocal decolourisation time of the resazurin solution.

— In the optimum state of inoculum for inoculation of the fermentation medium, the reduction time of the resazurin solution was between 10 and 5 minutes.

— The milk-clotting activity in the fermentation broth increased together with the mycelium content to a certain degree, then decreased rapidly, down to about the quarter of the maximum activity, in six hours.

— The pH minimum was reached at the maximum of the milk-clotting activity, then increased quickly.

— Kinetic studies had pointed out that the decomposition or inactivation of the enzyme was caused not by stopping of growth, but the rapid increase in pH, as an aftermath of the depletion of the glucose source.

— The maximum of the milk-clotting activity could be maintained for 10–12 hours, if at the maximum either glucose was added to the culture or the aeration of it was discontinued.

MICROBIAL ASSOCIATIONS IN FERMENTED MEAT PRODUCTS AND INTERACTIONS BETWEEN THEM

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Investigations were carried out on the main groups of microorganisms, developing in raw dried sausages during their ripening and the variations of their number, connected with changes of the medium were determined. Interactions between the microorganisms in the sausage mixture were studied as well.

It was established that the main groups of microorganisms developing in fermented meat products were as follows: lactic-acid microorganisms — mainly streptobacteria; representatives of the family micrococcaceae — micrococci and saprophytic staphylococci; the number of yeast and enterococci was lower. There were coli- and proteus-bacteria at the beginning of the ripening but their number decreased during the processing period. This lowering of number of enterobacteria was greater when the number of micrococci and lactobacilli was higher.

The antagonistic activity of individual micrococcal and lactobacillus strains, isolated from the sausages, against some test-microorganisms was determined. The test-microorganisms used, were: *Escherichia coli, Proteus vulgaris, Salmonella typhimurium, Streptococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus.*

APPLICATION OF STARTER CULTURES IN THE PRODUCTION OF NON-COMMINUTED MEAT PRODUCTS WITH THE AIM TO IMPROVE THEIR QUALITY

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The experiments were carried out with raw dried products of non-comminuted beef. Starter cultures consisting of *Lactobacillus plantarum* and *Micrococcus varians*, were used for shortening and improvement of the ripening process.

Ripening during the first 24 hours was done at 12 or at 20 °C. The amount of the usually added nitrate was reduced by 50% in the experimental variants.

It was established that when the ripening took place at 20 °C the amount of lactobacilli and micrococci was greater. On the second day the number of lactobacilli reached 10^5 cells per g of the product at 20 °C, while at 12 °C it remained 10^2–10^3 cells per g. Micrococci reached 10^6 cells per g at 20 °C and 10^3 at 12 °C. The number of lactobacilli remained higher during the whole period of drying after ripening at 20 °C in comparison with the number of these microorganisms at 12 °C. After two weeks of ripening significant differences were not established in the number of micrococci in the experimental variants.

This development of the useful microorganisms ensures faster and more stable colour and flavour formation. The process of drying was shortened, too.

Salmonella was not isolated from any of the experimental variants; coli-bacteria were found only in products made without using starter cultures.

The reduction of nitrate amount by 50% did not change the quality of the product if starter cultures were applied.

THE CHARACTERISTICS OF A NEW DAIRY LACTIC ACID CULTURE

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A new dairy culture (HSC-1) containing lactic acid and aroma producing thermophilic streptococci was developed within a bilateral international cooperation for increasing the physical stability of cultured milk products. Investigations were run on its most important functional characteristics as starter activity during incubation (in the range of 20–60 °C) and storage (at 15 °C). Viscosity, firmness, syneresis and stirability of curds fermented with culture depending on processes of pretreatment (heat treatment homogenization) were determined. Scanning electron microscopic photographs of curds were taken as well. Our most important results are as follows:

The culture HSC-1 has fermentation ability in the range of 30–55 °C and at temperature higher than 35 °C its activity increases sharply, at 45 °C gives its maximum, and above it is falling. As characteristics of curd are turning bad above 40 °C, the optimum fermentation temperature is 37–40 °C, and in this case the coagulation time is 4.5–5 hours.

Culture HSC-1, however, depending on parameters of pretreatment but in all cases increases viscosity to a large extent, decreases syneresis, and improves stirability. At optimum parameters of heat treatment and homogenization with clean HSC-1 and mixed cultures – comparing with yoghurt culture – there is an increase of viscosity of 50 and 20%, decrease of syneresis of 10–15%, and improvement of stirability of 100%. At the same time the new culture

decreases firmness of curds and that is why its use in processing of stirred cultured milk products is advantageous.

Having made scanning electron microscopic comparative investigations on curds made with the new and conventional cultures it may be declared that in gel construction formed by culture HSC-1 there is a more compact casein framework, whey channels are smaller in size, their dispersion is more homogeneous and this explains the differences found in the field of physical characteristics.

UTILIZATION OF STARTER CULTURES TO IMPROVE THE QUALITY OF BREAD

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Over the last 30 years to enhance production the methods of breadmaking have been modified. All the new methods seek to produce baked dough at a faster rate, mainly by mechanical, enzymatical and chemical means. The microbiological processes of breadmaking were not studied intensively.

In some European countries sour dough is used for breadmaking. In that case baker's yeast produces carbon dioxide during the leavening process, while lactic acid bacteria play an important role in changing the structure and texture of the dough and in producing characteristic flavor and taste.

The new method elaborated for the production of sour dough utilizes the suspension of flour in water, inoculated with starter culture of lactic acid bacteria. The strain was isolated from the dough, produced by conventional method. Acidic fermentation is carried out at 35 °C. The fermented suspension can be stored at +5–+10 °C for 3–4 days. Thereafter follows the dough production from the fermented suspension utilizing flour, water, salt and baker's yeast. Utilization of fermented flour–water suspension to the preparation of the dough suppresses the undesirable microflora of the flour, improves the sensory properties of the bread and increases the yield of production by about 2–3%.

EFFECT OF COMBINED TREATMENT ON SOME QUALITY INDICES OF CANNED MEAT

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A study was made on the effects of applying a mild heat treatment plus low dose ionising radiation on the quality of ham-type canned meats manufactured using reduced nitrite concentration, and stored for one year under refrigeration. The results obtained indicate that the microbiological stability

of the ham using 50 ppm nitrite, a mild heat treatment (F = 0.36) and an irradiation dose of 3 kGy, is better than that of the control ham manufactured with 100 ppm nitrite and heat treatment of F = 0.59. The combined treatment of ham-type meat cans opens the possibility to reduce the nitrite concentration by 50% at least.

INFLUENCE OF PHOSPHATE AND GLUCOSE ADDITION ON MICROBIAL GROWTH IN VACUUM PACKED BOLOGNA-TYPE SAUSAGE DURING REFRIGERATED STORAGE

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Six batches of Bologna-type sausage were produced in accordance with normal practice. Batches 1 and 2 were produced without added phosphate, 3 and 4 with sodium tripolyphosphate, and batches 5 and 6 with a commercial phosphate product intended for use in salami production. The product is a mixture of disodiumpyrophosphate, sodium triphosphate and sodium polyphosphate and has an acid pH. Odd numbered batches were also added 1% glucose. The phosphates were added at a level of 0.3% calculated as P_2O_5. The sausage emulsions were stuffed in casings and cooked in a cabinet to a core temperature of 70 °C. Following cooling overnight the sausages were sliced and vacuum packed at a commercial manufacturer. The packaging film consisted of a polyamide + polyethylene lamination. Microbial analyses were done for total aerobic plate count, lactic acid bacteria, *B. thermosphacta*, Gram negative bacteria and yeast. Sensory analyses were done along with the microbial examination using a taste panel who determined odour and freshness. The packages were stored at 8 °C for up to 8 weeks. The salt–water ratio in the sausages ranged from 3.6 to 4.0 and the initial microbial load : total plate count 2.2×10^2 per g, *B. thermosphacta* 52 per g, Gram negative bacteria 19 per g, yeast 5 per g and lactic acid bacteria less than 3 per g. Initial pH in the series without added phosphate or with added sodiumtripolyphosphate was 6.37–6.41 and in the low pH series 5.93. A marked connection between phosphate and glucose addition and total counts was seen. The microbial development was the same in series with tripolyphosphate and glucose as in series without phosphate with or without added glucose, while an inhibition was seen in series of tripolyphosphate without glucose addition. An even greater inhibition was seen in the series with the phosphate mixture added. Counts of 10^7 per g were reached several weeks later in these latter series compared to the series without phosphate or with tripolyphosphate + glucose. The same picture was seen regarding *B. thermosphacta* and the Gram negative bacteria. No inhibition due to phosphate addition or influence of added glucose was observed

with the lactic acid bacteria and yeast. A clear connection between growth of *B. thermosphacta*, the Gram negative bacteria and the organoleptic quality of the sausage was observed.

CARBON DIOXIDE PACKAGING AS A MEANS OF IMPROVING SHELF-LIFE OF DFD BEEF DURING REFRIGERATED STORAGE

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DFD beef (pH $>$ 6.2) was packaged in pure carbon dioxide at two different headspaces (10 dm^3 and 2 dm^3). Corresponding samples were vacuum packaged and in one experiment also normal pH beef was used as a control. All packages were stored at 4 °C.

After 2 and 4 weeks of storage the samples were examined microscopically and judged for appearance and off-odours.

Vacuum packaged samples of DFD and of normal pH beef were all acceptable after 2 weeks but not after 4 weeks of storage. Carbon dioxide packaged samples of both types of beef were still fresh and had an acceptable appearance after 4 weeks of storage. The fast spoilage in vacuum packages was probably due to growth of enterobacteriaceae comprising 78% of the spoilage flora in one of the experiments.

After storage for 2 and 4 weeks DFD beef packaged in carbon dioxide and under vacuum were sliced, retail packaged in oxygen-permeable film and stored at 8 °C for 3 days. Beef previously packaged in carbon dioxide retained a fresh odour and appearence after storage while samples from vacuum packaged beef rapidly spoiled.

EFFECT OF FREEZING STORAGE ON THE AEROBIC MICROFLORA OF VACUUM PACKAGED BEEF

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Beef-trimmings were vacuumpacked in a polyester (Hostaphan$^{(R)}$) film and stored at +2 °C for up to 45 days.

After microbiological examinations at intervals of 3 or 4 days the samples were vacuumpacked again and stored at –20 °C for up to 2, 4 years, respectively.

After a thawing period of 24 h in a refrigerator taste panel evaluations were made followed by bacteriological-cultural examinations using the spiral system.

The mean total viable count, *Lactobacillaceae* and *Brochothrix thermosphacta* remained fairly constant over the whole period of freezing storage. Enterobacteriaceae, yeasts, micrococci and especially pseudomonas decreased by one log. cycle power and more. Resuscitation for 2 h in TSB (Tryptone Soya Broth) at +22°C did not cause a significant recovery of enterobacteriaceae and pseudomonas.

During the freezing storage there were observed neither sensory alterations nor quality loss by microbial-enzymatic effects, which set a 6–12 month limit for the keeping quality of aerobically stored frozen beef. In addition vacuum packing avoided physical problems e.g. dried areas and freezer burn.

Assessment of the sensory and microbiological findings led to the following conclusion:

Vacuum packing improves the keeping quality of beef during freezing storage similar to the conditions of cold storage.

COMPARATIVE INVESTIGATION OF SOME EFFECTS OF GAMMA RADIATION AND ETHYLENE OXIDE ON AEROBIC BACTERIAL SPORES IN BLACK PEPPER

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Ground black pepper samples, the bacterial flora of which consisted predominantly of aerobic spores, were equilibrated to water activities of 0.25, 0.50 and 0.75, resp., then irradiated with a gamma radiation dose of 5 kGy or fumigated with ethylene oxide (600 mg per litre for 6 hours) at room temperature. After decontamination treatments, three to seven days of incubation was needed at 30 °C to obtain the "final" colony counts in Oxoid plate counting agar, while the number of colonies of the untreated samples did not increase significantly after two days of incubation. The survivors of the radiation treatment were much less heat resistant than the untreated population. The ethylene oxide did practically not affect the heat sensitivity of the survivors. In the a_w-range studied, the water activity did not influence notably the radiation sensitivity of the bacterial spore flora of black pepper, while the efficacy of the fumigation was much higher at $a_w = 0.75$ and 0.50, resp., than at $a_w = 0.25$. An increased sensitivity to the reduction of the pH of the recovery medium from pH 6.0 to pH 5.0 was observed with the survivors of the radiation treatment, while the survivors of fumigation did not show in this pH-range appreciable differences in the colony counts.

DECONTAMINATION OF BLACK PEPPER BY GAMMA RADIATION

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Spices are often severely contaminated with moulds and heat-resistant bacterial spores, sometimes they contain in addition pathogenic microorganisms, e.g. Salmonella spp. Spices are used as ingredients in a wide variety of foods and to avoid the contamination of these foods with disease-producing and spoilage organisms the spices are often treated with fumigants. These chemical treatments have disadvantages, both from the health and technological points of view. A promising alternative physical method is a "hygienization" treatment with ionising radiation, in practice already used successfully for many years in The Netherlands.

In this study the effect of ionising radiation on the microflora of black pepper was determined. A radiation dose between 6 and 8 kGy resulted in a more than 6 log cycles reduction of the mesophilic aerobic bacterial spore (1 min at 80 °C) count. The mesophilic anaerobic bacterial spores, and the heat resistant fraction (20 min at 100 °C) of the aerobic bacterial spores were reduced by 7, 6 log cycles, respectively, at a dose of 6 kGy. Moulds were reduced from 4.1×10^4 to 10^2 c.f.u. g^{-1} by a dose of 4 kGy.

Using the technique of solid medium repair, sublethally stressed Lancefield D streptococci were detected in untreated and irradiated samples. The addition of 150 U cm^{-3} catalase and 0.1% bicarbonate to the solid media did not increase colony counts of bacterial spores, moulds, Lancefield D streptococci and enterobacteriaceae.

The Lancefield D streptococci and enterobacteriaceae were reduced by a factor of 10^4 to 10^5 at a dose of 4 kGy.

The results showed that the microbiological quality of customarily contaminated spices could be improved considerably by a treatment with ionising radiation at a level of 4 to 6 kGy. This also applied to elimination of enterobacteriaceae suggesting possibilities for salmonella-radicidation.

THE COMBINED EFFECTS OF IONIZING RADIATION AND SODIUM CHLORIDE UPON THE BEHAVIOUR OF SALMONELLAE IN MEAT

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The samples of meat (M. longissimus dorsi) were collected from pork carcasses 24 hours after slaughter, ground, inoculated with salmonellae and subjected to irradiation with a dose of 1 kGy. Immediately after irradiation 3% or 6% of NaCl was added both to the irradiated and control meats. All samples were aerobically stored at 20 °C for 7 days. Bacteria enumeration (aerobic plate count and number of salmonellae) and organoleptic investigations were conducted after 0, 24, 72 and 168 hours of storage.

The addition of 3% NaCl inhibited the growth of irradiated populations of *S. agona* and *S. choleraesuis* within the first 24 hours of storage, however, did not exert any influence on irradiated population of *S. typhimurium* compared to unirradiated control.

In irradiated meat samples stored with addition of 6% NaCl higher death rate was observed for *S. agona*, *S. choleraesuis* and *S. typhimurium* than in unirradiated samples containing the same level of NaCl.

The irradiation with a dose of 1 kGy did not notably change general appearance, flavour, colour and consistence of pork tissue.

The shelf-life of irradiated samples of meat was almost doubly extended.

PRODUCTION OF ENTEROTOXIN BY SALMONELLA EXPOSED TO X RAYS

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The influence of irradiation on enterotoxin production by *Salmonella enteritidis* and *S. typhimurium* was evaluated using skin test elaborated by Tschäpe and others. Apart from delayed reaction there was determined also the quick reaction taking into consideration Sandefur and Peterson's findings. The strains examined appeared to be sensitive to X rays in the same degree. A dose of 100 Gy decreased the number of bacterial cells at approx. 1 D_{10} and 200 at 2–3 D_{10}. Irradiation with 100 and 200 Gy of X rays did not influence enterotoxin production by the population of microorganisms which were resistant to the process of irradiation.

LETHALITY AND FLORA SHIFT OF THE PSYCHROTROPHIC AND MESOPHYLLIC BACTERIAL ASSOCIATION OF FROZEN SHRIMPS AND CHICKEN AFTER RADICIDATION

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The impact of gamma irradiation on the psychrotrophic and mesophilic microflora of frozen precooked and peeled shrimps originating from Malaysia and from the North Sea, and of frozen chicken was studied. The frozen samples were irradiated with doses between 0 and 6 kGy.

In the Malaysian shrimps the numbers of c.f.u. of enterobacteriaceae were low, i.e. below 10^3 c.f.u. g^{-1}. It was found that in the North Sea shrimps cells of enterobacteriaceae were in an injured state and this applied, in both kinds of shrimps, to the cells of lactobacillus. Radiation with 4 kGy resulted in 3 log cycles reduction of the aerobic psychrotrophic and mesophilic colony counts. In both kinds of shrimps enterobacteriaceae, lactobacillus, Lancefield D streptococci and *Staph. aureus* were sensitive to irradiation and not detected in 1 g aliquots with doses between 2 and 4 kGy.

Addition of 150 U cm^{-3} catalase and 0.12% sodium bicarbonate to the selective and non-selective media did not improve the recovery of enterobacteriaceae, lactobacillus and Lancefield D streptococci in frozen chicken. Radiation with 4 kGy resulted in 3 log cycles reduction of the aerobic mesophilic colony count and more than 4 log cycles reductions of the psychrotrophic colony count, enterobacteriaceae, lactobacillus and *Staph. aureus* counts. Lancefield D streptococci were more resistant to radiation in chicken than in shrimps.

In the initial psychrotrophic flora of the Malaysian shrimps and in the psychrotrophic and mesophilic flora of the North Sea shrimps micrococcus was predominant. In the initial mesophilic flora of the Malaysian shrimps staphylococcus, streptococcus and the coryneform group were the most prevalent organisms. In Malaysian shrimps irradiated with 2 or 4 kGy, psychrotrophic micrococcus and mesophilic micrococcus and staphylococcus were predominant. In irradiated North Sea shrimps micrococcus and the coryneform group were the predominant genera amongst the psychrotrophic and mesophilic flora.

The initial psychrotrophic and mesophilic flora of frozen chicken was more heterogeneous than the initial flora of shrimps. Pseudomonas and the coryneform group were predominant in the psychrotrophic flora and micrococcus, lactobacillus and the coryneform group in the mesophilic flora. In

irradiated chicken micrococcus and yeasts were the most prevalent organisms amongst the psychrotrophic flora and streptococcus, micrococcus and yeasts in the mesophilic flora.

These results support the view that irradiation does not present a hazard resulting from a shift in the microflora, and substantiate that irradiation improves the safety and quality of frozen products.

SHELF LIFE EXTENSION AND THE INFLUENCE OF EXTRINSIC FACTORS ON THE SPOILAGE PATTERN OF GROUND BEEF

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The limited shelf life of ground beef severely restricts all aspects of handling and storage of this product. In view of modern trends towards centralisation in the fresh meat sector, additional precautions should be taken to limit microbiological deterioration of refrigerated fresh meat during storage.

Aerobic, Gram-negative bacteria, especially those belonging to the genus Pseudomonas, have been identified in numerous studies as the major spoilage organisms of fresh meat. One purpose of this investigation was to determine the influence of extrinsic factors, such as vacuum packaging, on this group of bacteria, as well as on the shelf life of ground beef.

Ground beef was prepared at a commercial plant and subjected to different "treatments". The organoleptic quality and microbiology of aerobically packed samples (Resinite-RMF-S wrapping) ("Treat 1") were compared with that of three other treatments during storage at 0 and 7 °C. Treats 2 and 3 were vacuum packed, with Treat 3 receiving 0.5% L(+) ascorbic acid in addition. Treat 4 contained 0.2% of a commercial "colour retainer" (yielding 250 ppm SO_2). Samples were analysed after 1, 2, 3, 4, 7, 11 and 17 days. Standard procedures were used for determination of total numbers of aerobic bacteria, enterobacteriaceae, lactobacilli, psychrotrophic bacteria, cytochrome-oxidase-positive bacteria and *Brochothrix thermosphacta*. Provision was made for internal replicates, and all data on bacterial numbers were statistically analysed. In addition, representative isolates were made and identified according to traditional principles.

Statistically highly significant differences were found between the four different treatments. In terms of total bacterial numbers as well as the dominating bacterial groups, best results were achieved with Treats 3 and 4. However, even vacuum packaging as such (Treat 2) resulted in at least a doubling of shelf-life (as compared with the control), with 10^6 bacteria per g as arbitrary "cut-off" point. Reduction of the storage temperature (in this case from

7 to 0 °C) appears to be a major factor towards improvement of shelf-life of ground beef. Moreover, a synergistic effect seemed to exist between temperature reduction and Treats 2, 3 and 4, leading to increased shelf-life extension (in terms of Treat 1) as compared with 7 °C. This effect was most pronounced for the enterobacteriaceae, and the least (for Treats 2 and 3) for the lactic acid bacteria.

Dramatic population "shifts" could be detected as a result of the four different treatments. The dominating microbial groups for the different treatments were (in order of priority): *Pseudomonas spp.* (for Treat 1), *Lactobacillus spp.*, *Pseudomonas spp.* and enterobacteriaceae (for Treat 2) *Lactobacillus spp.*, *Pseudomonas spp.* and *Streptococcus spp.* (for Treat 3), and yeasts, *Lactobacillus spp.*, *Pseudomonas spp.*, *Hafnia spp.* and *Kurthia spp.* (for Treat 4).

THE EFFECT OF NATURAL SPICES AND OLEORESINS ON *LACTOBACILLUS PLANTARUM* AND *STAPHYLOCOCCUS AUREUS*

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Although the main purpose of adding spices to the food is to develope flavour it has been realized that they may also inhibit or stimulate the growth of certain bacteria. Various specialized requirements of modern food processing have inspired the development of spice extracts and an increased use of extracted essential oils and oleoresins have taken place in recent years. Thus it seems of importance to know if the spices in their extracted form maintain their influence on bacterial growth.

The first part of the present work deals with the effect of natural spices and oleoresins on the fermentation properties of three commercially available strains of *L. plantarum* in liquid medium and in the production of dry sausage.

All the natural spices tested enhanced the growth as well as lactic acid production of the three different strains, while the oleoresins had no effect. One lactobacillus strain was also tested in salami sausage production and it was clearly demonstrated that the natural spice mixture enhanced the fermentation. This was not the case when oleoresin mixture was used as seasoning.

In the second part of this investigation the effect of natural spices and oleoresins on growth and toxin production of *Staphylococcus aureus* was studied.

Two toxin producing strains were used and the tests were performed in liquid media. The results suggest that some spices inhibit or delay the growth of the bacteria as well as toxin production. The oleoresins seemed to be as effective as their natural counterparts.

THE ANTIMICROBIAL ACTION OF SOME FOOD PRESERVATIVES AT DIFFERENT pH-LEVELS

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A microculture technique was employed to study the growth inhibitory capacity of sorbic, benzoic and propionic acid, and of the butyl ester of p-hydroxybenzoic acid (butyl paraben) at different pH levels. Minimum inhibitory concentration (MIC) was determined with *Candida albicans* and *Bacillus subtilis*. The results were used to assess the antimicrobial activity of undissociated and dissociated acid according to a proposed mathematical model. It is suggested that not only undissociated, but also dissociated acid possesses antimicrobial activity. The MIC of butyl paraben showed no pH dependency.

INHIBITORY EFFECTS OF PLANT OLEORESINS ON YEASTS

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It has been recognized for some time that certain microorganisms are sensitive to certain components of plants used as herbs and spices. The bulk of research in this area has been directed towards determining the response of bacteria and, to a lesser extent, filamentous fungi to aqueous extracts and essential oils of these plants. Yeasts have been given less attention, and oleoresins have not been studied extensively. For this reason, oleoresins of allspice, cinnamon, clove, garlic, onion, oregano, thyme and turmeric were tested for their inhibitory effects on eight yeasts (*Candida lipolytica, Debaryomyces hansenii, Hansenula anomala, Kloeckera apiculata, Lodderomyces elongisporus, Rhodotorula rubra, Saccharomyces cerevisiae* and *Torulopsis glabrata*). Cinnamon oleoresin was the most inhibitory oleoresin tested; a reduction in biomass production was noted at a concentration as low as 50 μg cm^{-3} of culture broth for the seven of eight test yeasts. With the exception of turmeric, which exhibited little inhibition, the other oleoresins retarded biomass production by the majority of yeasts at concentrations of 250–500 μg cm^{-3}. Compared with non-heated cells, heat-stressed cells had increased sensitivity to oleoresins at levels ranging from 5–500 μg cm^{-3} depending upon the yeast. These observations indicate that repair of heat injury was inhibited by a component(s) in the oleoresins. Oleoresins either enhanced or inhibited pseudomycelium production, depending upon the yeast under study, and generally had a retarding effect on sporulation. Based on results from these studies, it is suggested that components of oleoresins, when present in foods, may play a role in preserving such foods against deterioration by yeasts.

IN VITRO STUDIES ON THE FOOD PRESERVATIVE ANTIFUNGAL POLYENIC ANTIBIOTIC — PIMARICIN (NATAMYCIN)

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According to the wide usage of this tetraene in the food industry the efficiency and the mechanism of its action as well as the possible resistance against it are all interesting beyond medicine, too.

This substance was compared with other polyenes (nystatin, amphotericin B, candicidin and their methylesters), an imidazole antifungal (miconazole) as well as with well-known membrane damagers (cetyl piridinium bromide = CPB, digitonin, uranyl nitrate) concerning their effects on cell size and shape, growth, overall ion leakage, potassium and sodium movements of nystatin sensitive and resistant (ergosterol less) mutants of *Candida albicans*.

Pimaricin (like miconazol), however, differed from other polyenes in all the above parameters — excluding growth inhibition, where it was only slightly less active. It causes giant cell production at partially growth inhibiting concentration (never seen with the other polyenes), it could not cause any ion leakage in up to 400 μg per cm^3 nominal concentration (although the minimal growth inhibitory concentration was about 8 μg per cm^3), similarly it was unable (at 10 μg per cm^3) to alter significantly the sodium and potassium uptake of fastened, as well as the spontaneous sodium and potassium efflux of loaded cells.

It is, however worth mentioning that physical membrane damage was caused only by the surface active compounds CPB and digitonin and the polyenes did not induce mechanical holes i.e. pores at the plasmamembrane, but altered only some transport parameters (e.g. Vm) of the studied ions.

MICROBIAL INHIBITORS IN SAGE: TRANSLATING DATA FROM CULTURE MEDIA TO FOODS

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There is a growing interest in antimicrobial effects of natural food ingredients stimulated by health considerations and the rigorous testing currently required for new preservatives by regulatory agencies. Of particular interest are substances which can serve a dual function in foods. Among these, spices, which have been known for many years to contain microbial inhibitors, are now being re-examined for their efficacy as food preservatives, in addition to the flavor they impart to foods. Most of the studies reported in recent years

on microbial inhibition by spices were conducted in culture media. Since antimicrobials are more effective in media, their evaluation for practical purposes requires testing in foods. Sage *(Salvia officinalis)* was shown to inhibit growth of several enteropathogenic food-borne organisms in gradient agar plates and in broth. Gram positive bacteria were particularly sensitive to the spice. Comparative studies in culture media and foods were conducted with *Bacillus cereus*, *Staphylococcus aureus*, *Salmonella typhimurium*, and with spores of *B. cereus*. Sterile nutrient broth (NB), rice, strained chicken-noodles and beef containing from 0 to 2.5% ground sage were inoculated with 18 h cultures of the test organisms, incubated at 35 °C for 48 h and growth was evaluated at time intervals by the pour plate technique. Spores of *B. cereus*, grown on fortified nutrient agar, were tested in a similar manner. Factors such as water and salt levels and inoculum size were also studied. Inhibition was highest in broth (MIC of 0.1 to 1.0% of sage for *B. cereus*, *S. aureus* and *S. typhimurium*, respectively). In rice, inhibitory concentrations of 0.4 and 1.0% were required for *B. cereus* and *S. aureus*, respectively, while *S. typhimurium* was only slightly inhibited by 2.5% of sage. Further increases were required for inhibition in chicken-noodles, and no inhibition was produced by 2.5% sage in beef. Dilution of foods with water increased bacterial inhibition. In general, sage caused extended lag phase, decreased growth rate, and decreased total cell numbers of vegetative cells. Lower sage concentrations were required to inhibit smaller inocula sizes, and increased inhibition was observed in meat containing 1.5% salt. Spore germination time was also extended by sage, and growth and multiplication of vegetative cells were inhibited. The observed minimum inhibitory concentrations in foods indicate limited practical preservative effect if sage is used alone. However, combined effects with other spices, or in conjunction with other antimicrobials and processes, suggest growth inhibition of Gram positive bacteria in foods.

CONTENTS OF THE 12th INTERNATIONAL SYMPOSIUM ON MICROBIAL ASSOCIATIONS AND INTERACTIONS IN FOOD, BUDAPEST, 1983

Acta Alimentaria, Vol. 13 (3), pp. 295—295 (1984)

BOOK REVIEW

Economic d'énergie en matière de froid

Institut International du Froid, Paris, 1982; 224 pages, 223 figures, 24 tables

In view of problems related to the energy crisis the International Institute of Refrigeration organized, already in 1976 a work team on Economy in Energy. The task set for this work team was to investigate the possibilities of economy in refrigeration. Actual work started in 1979 under the direction of Mr. Nowotny, the leader of the work team, and the results were published under the title as given above.

The book contains the essays of 14 distinguished scientists representing on the whole 8 countries (Czechoslovakia, France, Poland, GFR, Norway, GDR, New Zealand and the USA). The papers treat subjects related to economization of energy in the field of refrigeration.

In Chapter 1 sources of thermodynamic losses and their causes in the refrigerating circuit and equipments are investigated based on the conception of energy; losses are demonstrated on a refrigerator and a heat-pump as examples. Mathematical methods for the optimization of a refrigerating plant and for the analysis of the results by computer, are discussed.

In Chapter 2 refrigerating processes are compared to the theoretical circuits of Carnot and Lorenz. Possibilities of energy saving in less known refrigerating equipment (cold gas refrigerators, vortex tube, thermoelectric refrigerator, etc.) and the effects of refrigerants and their mixtures on consumption of energy and on the possibilities of energy saving, were investigated. A comprehensive analysis of the sources of energy losses in open and hermetically closed compressors and in their parts is given. The development of highly efficient heat exchangers is discussed in relation to methods of optimization.

Chapter 3 consists of several independent parts: insulation of refrigerating rooms, determination of the optimum insulation thickness and the deterioration of the thermophysical characteristics of insulating materials; practical possibilities of energy saving in refrigeration, freezing, storage and transport of foods; a detailed study of the energy requirement of household refrigerators with compressor.

In Chapter 4 heat-pumps, their industrial and commercial applications are discussed. In addition to the description of the basic principles of heat-pump operation, of the available heat sources, of methods of cooling and heating problems, of heat-pump optimization, are also discussed.

After each chapter an ample list of references permits the profound study of the subject in hand.

The wide range of the scientific and technical knowledge discussed renders the book useful for experts, engineers, university students and whoever interested in refrigeration in the field of food production.

E. Almási

PRINTED IN HUNGARY

Akadémiai Kiadó és Nyomda, Budapest

RECENTLY ACCEPTED PAPERS

Contribution to the nutritional characterization of fenugreek (*Trigonella foenum-graecum* L. 1753)
HIDVÉGI, M., EL-KADY, A., LÁSZTITY, R., BÉKÉS, F. & SIMON-SARKADI, L.

Comparison between the sensorially established and instrumentally measured colour of red wine
KERÉNYI, Z. & KAMPIS, A.

Investigations of different equations predicting moisture, fat and protein content of raw meat by NIR technique
NÁDAI, B. T. & MIHÁLYI-KENGYEL, V.

Assay into the correlation between protein composition and baking quality of wheat flours
PALLAGI-BÁNKFALVI, E. & ÖRSI, F.

NOTICE TO CONTRIBUTORS

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

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

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

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

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

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

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

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Authors will receive one set of proofs which must be corrected and returned at the earliest convenience to the Editorial Office. In this phase major alterations of the text cannot be accepted.

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CONTENTS

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CONTENTS

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QUANTITATIVE DETERMINATION BY COMPUTERIZED SPECTRUM ANALYSIS OF THE PIGMENT COMPONENTS IN GROUND PAPRIKA

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Received: 25 November 1982; revision received: 6 July 1983; accepted: 17 July 1983)

One of the most important quality characteristics of ground paprika, widely used spice and colouring matter, is the pigment content, therefore, it is essential to have a thorough knowledge of the pigment components. Since gas- and thin-layer-chromatographic methods are costly and time consuming an exact computerized method was developed for the evaluation of OD values as measured in the 400–540 nm range.

Keywords: Pigment component determination, ground paprika, spectrum analysis

Hungarian ground paprika is a product well known all over the world. On most of the European markets it used to be classified according to its taste and colour characteristics considered equally important. On overseas markets ground paprika is sold mainly as a natural colouring matter and not as a seasoning. Thus, not only its non-pungent character but also the pigment content became important. At present the pigment content has advanced to the rank of the most important quality characteristic on almost all the markets in Europe as well as overseas, therefore, every attempt to develope a rapid and simple and at the same time exact method for its determination is highly appreciated.

Based on the pioneering work of ZECHMEISTER and CHOLNOKY (1934; 1936; 1938) and CHOLNOKY and co-workers (1955; 1957; 1958), BENEDEK (1958) developed a rapid and simple method for the determination of the total colouring matter content, equally suitable for practical grading and industrial use. In compliance to new requirements and possibilities Benedek's method had to be modified and also new methods had to be developed.

The latest methods approach the common target from two different aspects:

One of the aspects is aimed at improving the simple and rapid method of Benedek to make it more exact. This path was followed by BENEDEK and MÉCS (1971) in eliminating the visual photometer, by ANDRÉ (1973) in selecting the most suitable wavelength, by FEKETE and co-workers (1976), by HASPEL-HORVATOVIČ and HORIČKOVA (1976) in determining the ratio of red to yellow pigment content.

The other aspect is aimed at the simplification and acceleration of the exact but lengthy chromatographic methods. This was carried out by VINKLER and KISZEL-RICHTER (1972) by the thin-layer-chromatographic method and by BARANYAI and co-workers (1982) by gas chromatography. Although, however, both methods are equally suitable to provide exact and detailed data on the pigment components in ground paprika, thin-layer chromatography due to its time consuming character, gas chromatography because of its higher cost did not give the chance of these methods in everyday quality assessment.

The aim of the present study was to show a computerized method based on spectrometer measurements of less time and work requirement than chromatography, giving a feasible degree of instrumentation and permitting of the exact determination of capsanthin, capsorubin, beta-carotene one by one, and of the joint determination of zeaxanthin, beta-cryptoxanthin and lutein.

1. Materials and methods

1.1. Materials

Measurements were carried out in 5 samples of different pigment content, obtained from the Paprika Processing Plant, Szeged. The samples were extracted with benzene or the 1 : 1 mixture of benzene – methanol, according to ANDRÉ (1973), using only 50 cm^3 solvent as specified by JUHÁSZ and co-workers (1976).

1.2. Methods

The thin-layer-chromatographic method of VINKLER and KISZEL-RICHTER (1972) was used in the course of this study. The principle of the method is as follows: the pigment components (carotenoids), present in the ground paprika as fatty acid esters, are released, after appropriate purification, by saponification. The individual components were separated on an activated Kieselgel-G layer with a petroleum ether–benzene–glacial acetic acid–aceton developing agent. Methanol was used to elute the individual bands and the colour intensities were measured at the absorption maxima. In the knowledge of the extinction coefficients of the reference standards the pigment components were expressed in g per kg units.

For the sake of comparison the total pigment content was determined also by the method of ANDRÉ (1973). The optical density of the benzene solution of the pigment was measured at 447 nm. According to this author the molar extinction coefficients have an iso-absorption point at this wavelength. The total pigment content was expressed as capsanthin g per kg by the following equation:

$$B = \frac{k\ f}{1826\ b} 1000$$

where

k = OD of the benzene solution measured in a cuvette of 1 cm;
b = mass of the paprika sample, g;
f = calibration factor of the apparatus;
1826 = extinction coefficient of a 1% capsanthin solution in benzene.

The OD of the benzene–methanol extract was measured in the 400–540 nm range at 5 nm intervals using an Opton spectrophotometer of about 0.1% accuracy. The band width applied was 2 nm. The spectra obtained were analyzed on a R 40 computer using subroutine GELS of the SSP developed by IBM. The block diagram of the procedure is given in Fig. 1.

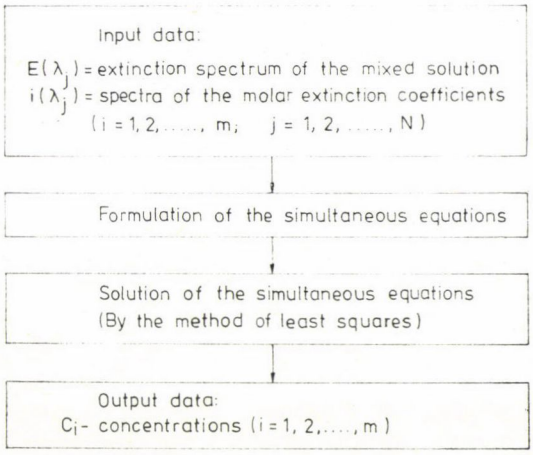

Fig. 1. Block diagram of the computerized program for the determination of pigment components by spectrum analysis

2. Results

The calculation basis of the pigment determination developed by the authors is as follows.

It is known from the related literature (STRAUB, 1961; HAMPEL, 1962; JOSLIN, 1970) that the extinction value of a pigment solution of m component as measured at a given wavelength equals the sum of the extinction values of the components at the same wavelength. On the basis of Beer's law for the solutions of the components, using formula $E_i(\lambda_j) = \varepsilon_i(\lambda_j)\ c_i d$ ($i = 1, 2, \ldots, m$; $j = 1, 2, \ldots, m$) the following simultaneous equations can be set up:

$$E(\lambda_1) = \varepsilon_1(\lambda_1)\ c_1 d + \varepsilon_2(\lambda_1) c_2 d + \ldots + \varepsilon_m(\lambda_1) c_m d;$$
$$E(\lambda_2) = \varepsilon_1(\lambda_2)\ c_1 d + \varepsilon_2(\lambda_2)\ c_2 d + \ldots + \varepsilon_m(\lambda_2)\ c_m d; \qquad (1)$$
$$E(\lambda_m) = \varepsilon_1(\lambda_m)\ c_1 d + \varepsilon_2(\lambda_m)\ c_2 d + \ldots + \varepsilon_m(\lambda_m)\ c_m d,$$

where

E = extinction value of the pigment solution,
E_i = extinction value of the components, measured at the same layer thickness,
λ_i = wavelength,
ε_i = molar extinction coefficients of the component solutions,
c_i = molar concentrations,
d = layer thickness.

In the case of m components the solutions to simultaneous equations (1) is determined by m linearly independent equations. By increasing the number of measurements the effect of the relative errors of measurement appearing at the individual wavelengths may be reduced, therefore, the simultaneous equations were extended over $N(N > m)$ number of equations. Values $E(\lambda_1), \ldots, E(\lambda_N)$, the spectrum of the mixed solution, extends over the 400–540 nm wavelength range with measurements at every 5 nm. The simultaneous equations were solved by the method of least squares.

For the unknown $a_1, a_2, \ldots, a_m$ coefficients (concentrations) in the knowledge of y_i (extinction value of the solution belonging to unit layer thickness) and of $x_{1i}, x_{2i}, \ldots, x_{mi}$ ($i = 1, 2, \ldots, m; N$: molar extinction coefficients) the following linear simultaneous equations of simmetrical matrix were obtained:

$$\begin{aligned} Q_{1y} &= Q_{11}a_1 + Q_{12}a_2 + \ldots + Q_{1m}a_m; \\ Q_{2y} &= Q_{21}a_1 + Q_{22}a_2 + \ldots + Q_{2m}a_m; \\ Q_{my} &= Q_{m1}a_1 + Q_{m2}a_2 + \ldots + Q_{mm}a_m \end{aligned} \qquad (2)$$

where

$$Q_{jk} = \sum_{i=1}^{N} x_{ji}\, x_{ki};$$

$$Q_{jy} = \sum_{i=1}^{N} y_i\, x_{ji},$$

($j = 1, 2, \ldots, m$; $k = 1, 2, \ldots, m$)

By the solution of these simultaneous equations a good approximation of the unknown concentrations ($a_1, a_2, \ldots, a_m$) may be obtained.

To solve simultaneous equations (1), the knowledge of the molar extinction coefficients of the six main paprika pigments, dissolved in the 1 : 1 mixture of benzene–methanol is required. The breaking down into components and the quantitative analysis was carried out according to the thin-layer-chromatographic method of VINKLER and KISZEL-RICHTER (1972). One of the parallel developments was used for the quantitative determination of compo-

Table 1

Pigment content of sample 1 determined by different methods

Components	Pigment content (g kg^{-1})					
	Spectrum analysis by computer		Thin-layer chromatography		Spectrophotometry	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Capsanthin	1.268	0.0711	1.257	0.0681		
Capsorubin	0.388	0.0496	0.401	0.0475		
Beta-carotene	0.122	0.0403	0.133	0.0201		
Zeaxanthin Beta-cryptoxanthin Lutein	0.659	0.0625	0.636	0.0588		
Total pigment content	2.430		2.427		2.633	0.0637

$\bar{x}$ = mean values
$\pm s$ = standard deviation of 5 parallels

nents, while in the other, after eluation with benzene–methanol the OD of the dissolved components were determined.

The molar extinction coefficients were calculated in the knowledge of the extinction spectra, the quantities and molar masses.

The total pigment content of the samples investigated was determined according to ANDRÉ (1973). Capsanthin, capsorubin and beta-carotene were

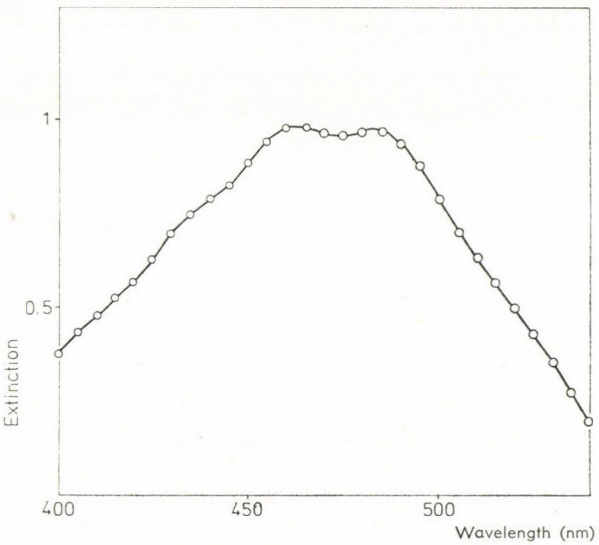

Fig. 2. Extinction spectrum of the sample shown in Table 1. One tenth g ground paprika in 50 cm^3 of the 1 : 1 mixture of benzene–methanol measured in a cuvette of 1 cm width in an OPTON spectrophotometer

Table 2

Pigment content of sample 2 determined by different methods

Components	Pigment content (g kg^{-1})					
	Spectrum analysis by computer		Thin-layer chromatography		Spectrophotometry	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Capsanthin	1.123	0.0654	1.117	0.0721		
Capsorubin	0.402	0.0513	0.395	0.0613		
Beta-carotene	0.110	0.0354	0.118	0.0415		
Zeaxanthin, Beta-cryptoxanthin, Lutein	0.624	0.0597	0.631	0.0719		
Total pigment content	2.259		2.261		2.406	0.0581

$\bar{x}$ = mean values; $\pm s$ = standard deviation of 5 parallels

Table 3

Pigment content of sample 3 determined by different methods

Components	Pigment content (g kg^{-1})					
	Spectrum analysis by computer		Thin-layer chromatography		Spectrophotometry	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Capsanthin	1.219	0.0696	1.228	0.0735		
Capsorubin	0.365	0.0481	0.357	0.0542		
Beta-carotene	0.090	0.0389	0.101	0.0429		
Zeaxanthin, Beta-cryptoxanthin, Lutein	0.692	0.0619	0.702	0.0693		
Total pigment content	2.366		2.388		2.542	0.0675

$\bar{x}$ = mean values; $\pm s$ = standard deviation of 5 parallels

Table 4

Pigment content of sample 4 determined by different methods

Components	Pigment content (g kg^{-1})					
	Spectrum analysis by computer		Thin-layer chromatography		Spectrophotometry	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Capsanthin	1.341	0.0724	1.333	0.0777		
Capsorubin	0.522	0.0457	0.516	0.0582		
Beta-carotene	0.409	0.0367	0.414	0.0483		
Zeaxanthin, Beta-cryptoxanthin, Lutein	0.477	0.0602	0.491	0.0652		
Total pigment content	2.749		2.754		2.768	0.0697

$\bar{x}$ = mean values; $\pm s$ = standard deviation of 5 parallels

Table 5

Pigment content of sample 5 determined by different methods

Components	Pigment content (g kg^{-1})					
	Spectrum analysis by computer		Thin-layer chromatography		Spectrophotometry	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Capsanthin	1.249	0.0678	1.261	0.0755		
Capsorubin	0.418	0.0472	0.407	0.0554		
Beta-carotene	0.254	0.0357	0.265	0.0382		
Zeaxanthin Beta-cryptoxanthin Lutein	0.296	0.0586	0.304	0.0619		
Total pigment content	2.217		2.237		2.522	0.0654

$\bar{x}$ = mean values
$\pm s$ = standard deviation of 5 parallels

determined individually, beta-cryptoxanthin, zeaxanthin and lutein jointly by thin-layer chromatography. The quantities of the components were determined by the mathematical method developed by the authors, based on the spectrum of the benzene–methanol extract of ground paprika. A characteristic extinction spectrum is shown in Fig. 2.

Results are summarized in Tables 1 to 5. Data represent the average values of the samples.

3. Conclusions

As it can be seen from the Tables the quantities of the components can be determined by the computerized mathematical method based on the analysis of their extinction spectra showing a good approximation of the values as obtained by thin-layer chromatography. The numerical values of deviation prove the satisfactory reproducibility of the method.

The time requirement of the method is substantially lower than that of the costly and time consuming gas and thin-layer chromatography.

To the processing of data the ZX 81 small computer containing only 16 k memory units is suitable, thus the method can be used in the everyday grading work.

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CORRELATION ANALYSES OF THE SDS TEST AND VALORIGRAPH VALUES OF AUTUMN WHEAT VARIETIES

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Correlation between the SDS sedimentation test and the valorigraph characteristics in the flour of 50 autumn wheat varieties was studied. A positive correlation was found between the volume of the SDS sediment and the valorigraph values of the samples ($r = 0.779$). The correlation of gluten quality to the volume of SDS sediment was found to be negative ($r = -0.779$) while that of baking quality to the volume of SDS sediment was $r = 0.684$.

On the basis of the analyses it was established that in samples as small as 3.5 g flour the volume of the SDS sediment permit of drawing reliable conclusions in a relatively short time as to the valorigraph quality of the flours.

Keywords: SDS test, valorigraph, wheat analysis

Microanalytical methods of establishing the quality of wheat flours are important in wheat improvement. While their material requirement is low it is possible to determine the quality of the flour of a single plant or even one ear and thus, to select for quality can be started already in generation F_2. Of the methods used for this purpose the Pelshenke and Zeleny tests have been known and widely applied.

The PELSHENKE (1933) meal leavening test is based essentially on the determination of gluten quality. ZELENY's method (1947) is based on measurement of the swelling and sedimenting capacity of gluten in a lactic acid solution. KARÁCSONYI (1956), in the course of a critical investigation of Zeleny's method found a correlation of +0.49 between the Zeleny number and the farinograph value. In his opinion the Zeleny number, possibly complemented by gluten determination, is suitable for the classification of wheat varieties and flours of equal extraction. The SDS test, developed by AXFORD and coworkers (1978) is based on the different volume of the flour or meal sediment of wheat varieties of different quality in the lactic acid solution of sodium dodecyl sulfate (SDS).

A 2% aqueous solution of the SDS anion detergent dissolves about 95% of the protein in wheat flour, therefore, buffers containing SDS are more effective than other solvents, including those containing kation detergents, too (BOTTOMLEY et al., 1982).

AXFORD and co-workers (1979) found a correlation of 0.80–0.84 between the SDS test value of the whole meal of different wheat varieties and the volume of loafs baked from the same meals by the English baking process. In the same experimental series a much lower correlation was found between the loaf volumes and the Zeleny and Pelshenke numbers, respectively. DEXTER and co-workers (1980; 1981) investigated the correlation between the dough and baking characteristics and SDS test values of aestivum and durum wheat varieties. Based on the combined data of 22 durum and 38 aestivum varieties a correlation coefficient of 0.92 was found between the loaf volume and the SDS test value. The correlation between dough formation time (from the beginning of kneading to the peak of the farinogram) and the value obtained by SDS test amounted to 0.72 (DEXTER et al., 1981). A correlation of 0.69 was found between the development time of the pastry in the mixograph made of semolina from durum wheats. MATUZ (1980) states that the SDS test has been used mainly in England for wheat improvement.

The aim of the present study is to investigate the applicability of the SDS test for the classification of wheat varieties and the correlation of SDS test values to valorigraph values.

1. Materials and methods

1.1. Materials

The samples used in this work were autumn wheat varieties obtained from the Cereal Research Institute, Hungary and grown around Kiszombor (County Csongrád, Hungary).

1.2. Methods

1.2.1. Valorigraph tests. The valorigraph tests were carried out according to KARÁCSONYI (1970) on a valorigraph, Type QA-203 (Labor MIM, Hungary).

1.2.2. SDS test. The SDS test was carried out according to AXFORD and co-workers (1978) with certain modifications as described below.

Graduated cylinders of 50 cm^3 with ground glass stopper were filled with 25.0 cm^3 distilled water each. Of the flour samples 2.5 g were weighed on a rapid balance and put in the cylinders. Than the cylinders were stoppered and thoroughly shaken for 15 sec, after 2 min rest the shaking was repeated and after another 4 min again. Then 25.0 cm^3 of the lactic acid SDS solution was added to each of the cylinders (Lactic acid SDS reagent: 1000 cm^3 of a 20 g per dm^3 sodium dodecyl sulphate solution containing 2.5 cm^3 88% lactic acid solution. This solution can be kept for 72 h). The shaker frame was turned

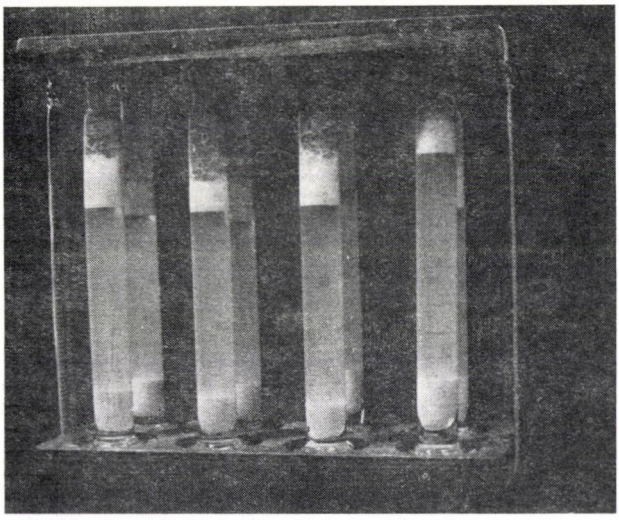

Fig. 1. Shaking frame for the measurement of the volume of the SDS sediment

over 5 times and thereby the sediment was separated from the bottom of the cylinders. This procedure was carried out within 15 sec and was repeated after 2, then 4 and finally 6 min resting periods. After the last turn-over of the frame it was set upright and the sediment was allowed to settle for 15 sec. The volume of the sediment in cm^3 was established.

The tests were carried out at room temperature (20–21 °C).

The frame used for shaking was constructed in the Research Institute with place for 8 cylinders. It is shown in Fig. 1.

The protein content of the samples was determined in a NEOTEC (Near Infrared Research Composition) Analyzer.

2. Results

Results were evaluated and correlations calculated according to SVÁB (1973).

The results of the SDS tests of the 50 autumn wheat varieties and the valorigraph characteristics: dough development time, stability, extendability, softening, water absorption, valorigraph value, average protein content and standard deviations, the minimum and maximum values as measured and coefficients of variation are listed in Table 1.

As it can be seen the quality characteristics of the different wheat varieties are scattered over a wide range. The valorigraph values can be ranged in classes A1, A2, B1 and B2. Flour belonging into quality class C was not found among the samples.

Table 1

Quality parameters of 50 autumn wheat varieties

Attribute	Average	Minimum	Maximum	Standard deviation around the mean	Coefficient of variation (%)
Volume of SDS sediment (cm^3)	35.40	25.0	42.75	4.67	13.20
Valorigraph value (FE)	66.30	46.4	91.2	10.53	15.90
Dough development time (min)	2.01	1.0	3.5	0.746	37.10
Stability (min)	3.73	1.0	8.0	1.990	53.30
Extendability (FE)	18.74	15.0	25.0	2.37	12.64
Softening (FE)	14.73	0.5	27.0	7.46	50.64
Water absorption capacity (%)	64.34	56.0	70.0	3.00	4.66
Protein content (%)	13.23	11.4	15.1	0.87	6.57

Table 2

Correlation coefficients of the volume of the SDS sediment and the attributes as measured with valorigraph and the protein content of 50 samples

Valorigraph value	0.779***
Dough development time (min)	0.189
Stability (min)	0.684***
Extendability	−0.028
Softening	−0.779***
Water absorption capacity	0.317*
Protein content	−0.030

*** Very highly significant at the P = 0.1% probability level
* Significant at the P = 5% probability level

The coefficient of variation (CV) was similar for the SDS test, the valorigraph value and the extendability (12–16%). Higher coefficients of variation were found for dough development time, stability and softening (37–53%). The coefficients of variation for protein content and water absorption capacity were found lower.

The correlation coefficients belonging to the volume of the SDS sediment, valorigraph values and other quality parameters are given in Table 2.

A good correlation was found between the volume of the SDS sediment and the valorigraph value ($r = 0.779$). Somewhat lower was the correlation between the SDS value and the stability of the dough ($r = 0.684$). As expected, the softening value and the volume of the SDS sediment are in a relatively good but negative correlation ($r = -0.779$).

There was no valuable correlation found between the other quality parameters (time of dough development, extendability, water uptaking capacity, protein %) and the volume of the SDS sediment.

The relationship between the volume of the SDS sediment and the valorigraph value is expressed by the regression equation: $y = 4.902 + 1.731x$,

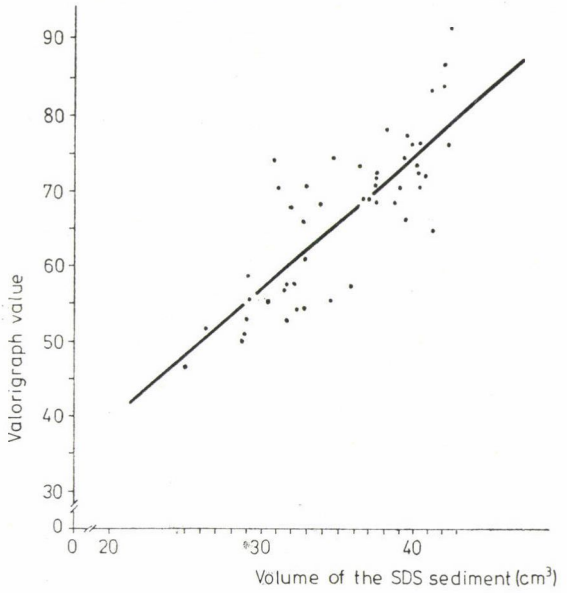

Fig. 2. Correlation between the valorigraph values and the volume of the SDS sediment in wheat flours. Regression equation: $y = 4.91 + 1.73x$; correlation coefficient: $r = 0.779$; number of measurements: $n = 50$

where y stands for the valorigraph value, while x for the volume of the SDS sediment (Fig. 2). For the limits of confidence were found $h_1 = 2.135$ and $h_2 = 1.327$.

As it was shown in the review of related literature, the English and Canadian authors found close correlation between the volume of the SDS sediment and baking quality (loaf volume). As far as we know data on the relation of the volume of SDS sediment and of the valorigraph values have not been published yet.

DEXTER and co-workers (1981) studied bread dough mixing time (similar to dough development time in the Hungarian Standard) and its correlation to the volume of SDS sediment which was found 0.72 for both aestivum and durum wheats.

3. Conclusions

In the course of this study the correlation as observed between the volume of the SDS sediment and the dough development time ($r = 0.189$) was unimportant (Table 2). The correlation coefficient of the stability of dough to the volume of the SDS sediment was found to be $r = 0.684$ and that of the softening of dough and the volume of SDS sediment $r = -0.779$.

On the basis of this study the SDS test was found to be suitable for the estimation of the valorigraph value. It is particularly advantageous where the baking quality of a number of samples of small quantity has to be established in a short time, e.g. in wheat improvement.

*

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INVESTIGATION OF PLASTEINS BY SDS POLYACRYLAMIDE GEL ELECTROPHORESIS

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Plasteins were synthesized from the proteolytic hydrolysate of casein with Pronase, α-chymotrypsin and papain.

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate was carried out in order to detect changes in molecular mass during enzymatic resynthesis.

Protein staining was performed with Coomassie Brilliant Blue R-250 and also by the "silver staining" procedure.

The electrophoretic patterns obtained for the different plasteins showed remarkable changes in molecular mass distribution, which might be ascribed to transpeptidation reactions.

Keywords: Enzymic peptide modification (EPM), SDS-PAGE separation of plasteins, plastein reaction

The molecular mass characteristics of polypeptides synthesized by the plastein reaction, are of considerable interest. Different theories have been proposed to explain the mechanisms. Some results indicate that the plastein reaction is mainly a polycondensation process (DETERMANN & KÖHLER, 1965; YAMASHITA et al., 1973); transpeptidation has also been considered as the mechanism of the plastein reaction (HOROWITZ & HAUROWITZ, 1959; YAMASHITA et al., 1970). Several investigations, however, have shown that the plastein is composed of aggregates held together by hydrophobic and ionic bonds (HOFSTEN & LALASIDIS, 1976; EDWARDS & SHIPE, 1978; SUKAN & ANDREWS, 1982).

In this study an attempt was made to separate plasteins obtained from casein hydrolysate with different enzymes as catalysts, with the aid of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate. By this method polypeptides can be fractionated according to their molecular weight (WEBER & OSBORN, 1975).

1. Materials and methods

Plasteins were synthesized from casein hydrolysate with Pronase, α-chymotrypsin or papain as described previously (HAJÓS & HALÁSZ, 1982).

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS) was carried out with small modifications by the method of

WEBER and OSBORN (1975) using 10% polyacrylamide gels (bisacrylamide–-acrylamide, 1 : 37) containing 0.1 *M* sodium phosphate buffer (pH 7.2), 0.1% SDS and 6 *M* urea. The electrophoresis buffer consisted of 0.1 *M* sodium phosphate, 0.1% SDS, pH 7.2, and the sample buffer was 0.01 *M* sodium phosphate, 1% SDS, 2% β-mercaptoethanol, pH 7.2. Samples were heated in sample buffer for 2 minutes at 100 °C, cooled and 6 *M* urea was added.

Slab dimensions were: 130 mm wide, 100 mm long and 0.7 mm thick. 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 performed with Coomassie Brilliant Blue R-250 (Serva Blau R) according to the procedure of STECK and co-workers (1980), 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). The gels were dried after 15 minutes impregnation in 45% methanol and 3% glycerol with a LKB gel dryer.

2. Results and discussion

Preliminary experiments had shown that plastein concentrations lower than 10% w/v yielded no adequate staining with Coomassie Blue after electrophoresis, the intensity of the stained zones simply being too weak. Although the ratio of SDS to protein should be at least 3 : 1 (WEBER & OSBORN, 1975), in the following experiments with 10% plastein samples the usual sample buffer with 1% SDS and 2% β-mercaptoethanol was employed, so this requirement was not fulfilled. Experiments on both 10% and 15% polyacrylamide gels revealed a better resolution of plasteins on the 10% polyacrylamide gels. The presence of urea greatly improved the separation, only smeared patterns being obtained without addition of urea to the gel.

Figure 1 shows a diagram of the SDS-gel with the plasteins separated. The separation revealed, as expected, little difference between samples 1 and 5, the dialyzed casein hydrolysates with or without incubation. The substrate for the plastein synthesis (sample 5) showed the greatest intensity on staining in the molecular range of about 5000–7000 Daltons. Minor zones were detected at positions corresponding to molecular weights of about 10 000, 15 000 and 30 000 Daltons. The patterns of the three plasteins catalyzed by different enzymes, demonstrated that the catalyzing enzyme has an influence on the distribution of the synthesized polypeptides according to molecular mass. Although staining was very weak for the Pronase-plastein (sample 2), still two zones with relatively low molecular weight, one of about 5000 and another of 7000 Daltons, could be observed. The α-chymotrypsin-plastein (sample 3) showed definite zones, the two major ones embracing the major original sub-

strate zone (see sample 1), and some zones with slightly elevated molecular mass of about 10 000 and 12 000 Daltons. Similarly, the papain-plastein (sample 4) also showed two main zones of about 4000 and 8000 Daltons, embracing the major zone of the substrate, but in contrast to the two other plasteins,

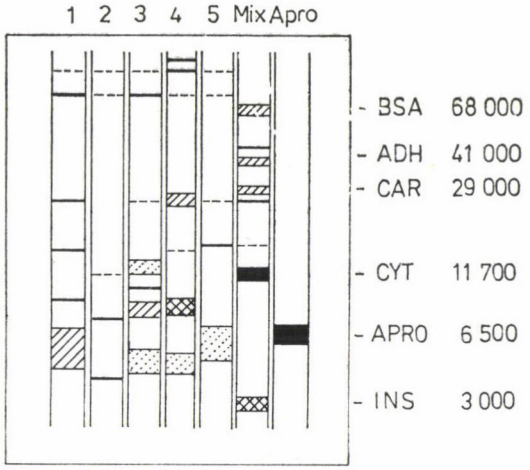

Fig. 1. Schematic diagram of the separation of plasteins by polyacrylamide gel electrophoresis in the presence of SDS. Staining with Coomassie Brilliant Blue R-250. From left to right: 1: dialyzed casein hydrolysate identical with the plastein substrate without enzyme addition, incubated at 37 °C; 2: plastein formed by pronase action; 3: plastein formed by α-chymotrypsin action; 4: plastein formed by papain action; 5: dialyzed casein hydrolysate; Mix: mixture of marker proteins consisting of bovine serum albumin (BSA), alcohol dehydrogenase (ADH), carbon anhydrase (CAR), cytochrome C (CYT) and insulin (INS); APRO — aprotinin

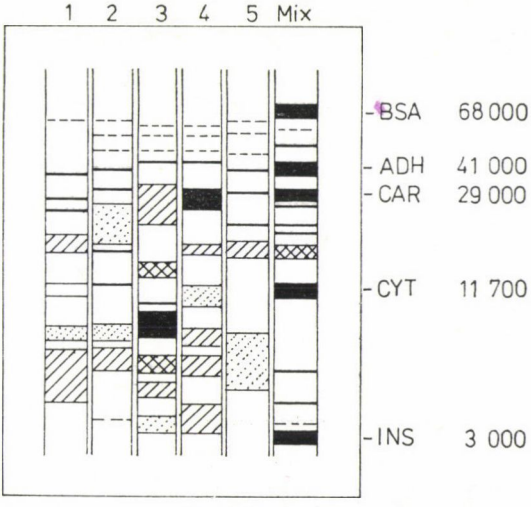

Fig. 2. Schematic diagram of the separation of plasteins by SDS-gel electrophoresis followed by "silver staining". For further explanation see legend to Fig. 1

the zone being the next intensive in the pattern of the papain-plastein exhibited a relatively high molecular weight of about 30 000 Daltons.

The patterns of the plastein, embracing the major substrate zone, indicate that transpeptidation occurs leading to a larger and to a smaller peptide than the original substrate peptide. The relatively intensive zone corresponding to the high molecular weight of 30 000 Daltons in the pattern of the papain-plastein may be ascribed probably to condensation reactions (Fig. 2).

In some separations the pattern of the casein hydrolysate showed a more intensive staining of the zones corresponding to higher molecular weights, e.g. the 15 000 and 30 000 Dalton-zones, and even higher molecular zones were noted. The pattern of the casein hydrolysate also tended to be more diffuse than the plasteins derived from it, indicating a preferential formation, by the plastein reaction, of certain polypeptides with defined molecular weights. Although the staining of these higher molecular zones in the substrate was increased, the higher molecular zones in the plasteins e.g. the 12 000 Dalton-zone in the α-chymotrypsin-plastein and the 30 000 Dalton-zone in the papain-plastein, were still markedly more pronounced.

Since the ratio of SDS to polypeptide was rather low, some experiments with an increased amount of SDS in the sample buffer, namely 5% SDS and 5% β-mercaptoethanol, were conducted. No significant changes in the electrophoretic patterns were noted.

Recently, the development of a "silver stain" rendered the detection of very small amounts of proteins possible, its sensitivity being 10–50 times higher than that of the Coomassie Blue staining. Therefore, the separations were repeated with 1% plastein, denatured with 3% SDS and 5% β-mercaptoethanol (thereby fulfilling the requirements of the SDS to polypeptide ratio of 3 : 1), and the gels were stained with the "silver stain". In Fig. 2 the photograph of this separation is shown.

With the "silver staining" a much higher number of zones is visualised, although the patterns seem to be slightly more diffuse. The main features of the separation, however, are similar to those described for Fig. 1. Again specific patterns for the plasteins synthesized with different enzymes are obtained. The plastein patterns again indicate that both transpeptidation and condensation reactions play a role.

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CONTRIBUTIONS TO THE NUTRITIONAL CHARACTERIZATION OF FENUGREEK (*TRIGONELLA FOENUM-GRAECUM* L. 1753)

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The present paper deals with the amino acid composition and in vitro protein quality of fenugreek (*Trigonella foenum-graecum* L.) seeds. Although the seeds have a high protein content (26.4%), the relatively low (32%) multienzymatic digestibility considerably reduces the protein value of the seeds. The seed protein of fenugreek is rich in lysine (345 mg g^{-1} N), but poor in sulphur containing amino acids (129 mg g^{-1} N) and tryptophan (93 mg g^{-1} N). In comparison to the data for human requirements the protein quality of fenugreek seeds calculated from the amino acid pattern approaches that of soybeans.

Keywords: fenugreek, amino acid composition, in vitro biological value

Due to the increasing protein deficiency all over the world, considerable efforts are being made to discover the nutritional potential of new or neglected sources of protein. The aim even now, but even more so in the future, is to utilise every protein source wherever and however it will have the highest nutritional value. At present, work on the nutritional surveying of unconventional protein sources consists primarily of data collection, since very little is currently known about numerous materials with promising characteristics.

Fenugreek (*Trigonella foenum-graecum* L. 1753) is an ancient Egyptian and East Indian crop, cultivated chiefly for its seeds *(Semina Foeni-graeci)* which have a high mucilage content and are highly perfumed.

It is an annual belonging to the *Leguminosae*, and mature plants reach a height of approx. 50 cm (Fig. 1). It has tri-digitate leaves and yellowy-white papilionaceaous flowers which grow in the axil of the rachis. Greenish-brown seeds, 3–5 mm in size and of various shapes, are found in the 8–15 cm pods (Fig. 2).

The crop is sown in October or November and harvested approximately five months later. The whole plant is harvested and dried in the sun, after which the seeds are separated (e.g. by wind sifting). The dried seeds are marketed.

References to the utilization of fenugreek are found as far back as 1578, when detailed information on the plant is given in the famous Kolozsvár Herbarium compiled by Péter Melius (Melius, 1578). In accordance with the

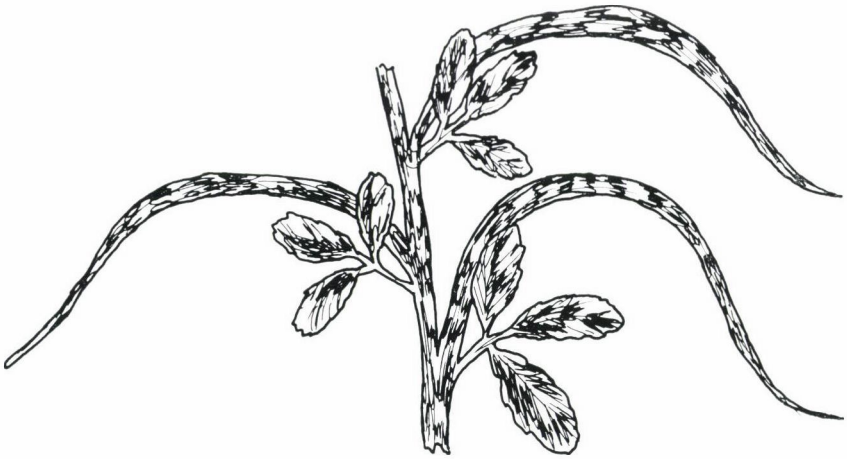

Fig. 1. The fenugreek plant

Fig. 2. Fenugreek seeds. (Magnitude, $n = 1.5$)

mediaeval, intuitive and symbolic view (theory of humoral squares) of what we now know as homeostasis, the Transylvanian Herbarium emphazises the "warming and very drying nature" of fenugreek on the basis of personal experience ("probatum est") and no doubt of antique sources. (According to the Herbarium, similar characteristics are to be found in *Sinapsis alba, Cardamine pratensis, Phoenix dactylifera, Lithospermum officinale, Aloe sp.*, etc.). The medicinal effect of fenugreek is still acknowledged and utilized, mainly for stomach complaints: in Egypt the fenugreek seeds are scalded, and the resulting infusion is drunk sweetened with honey or sugar. The boiled seeds are also eaten. Quite apart from its possible medicinal effect, *Trigonella foenum-graecum* is a popular food. One method of preparation, for instance, is to leave the seeds to germinate for 2–3 days before consumption. Fresh green shoots of fenugreek are also a favourite in Egypt. In many places the grain is completely extracted and the flour used as a supplement in home-baked bread, particularly when using mixed flours based on maize.

Both the fresh green shoots and the seeds of fenugreek are used in feeding cattle.

There is very little literature on fenugreek. Data on the amino acid composition were published by FAO (1970) using the data of JAMALIAN and PELLETT (1968), and general data on the composition by KUZAYLI and coworkers (1966). The effect of germination in connection with the amino acid composition was studied by HUSSEIN and NOUMAN (1969). Lately a review of great value mainly in the field of botany was prepared by MÁTHÉ (1975).

1. Materials and methods

1.1. Materials

Air-dried seeds of fenugreek originating from Egypt were used for the analyses. The representative samples were ground to a fine powder (300μ mesh) in an electric mill (Labor MIM Hungary).

1.2. Chemical analyses

1.2.1. Determination of moisture, nitrogen and fat contents. The determinations of moisture, nitrogen and fat content were carried out using the standard methods currently in force (ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1975).

The crude protein content was obtained by multiplying the value of the nitrogen content by a factor of 6.25.

1.2.2. Determination of the gross amino acid composition

1.2.2.1. Acid hydrolysis. — Samples containing approx. 30 mg protein were measured into heat-resistant test-tubes using an analytical balance. The

test-tubes were placed in a boiling water-bath for 15 min for preliminary hydrolysis with 5 cm^3 6 mol per dm^3 hydrochloric acid containing 1% Na-sulphite. Then a further 5 cm^3 6 mol per dm^3 hydrochloric acid containing 0.5% Na-hydrogen sulphite was added and boiling was continued for another 5 min. After this, nitrogen gas was passed through the samples for three minutes to eliminate the air, then the test-tubes were sealed. The hydrolysis was carried out for 24 hours in a test-tube sand bath thermostat at 110 °C, after which the test-tubes were opened and the contents washed into 25 cm^3 measuring flasks containing 10 cm^3 neutralising solution. The neutralising solution contains NaOH (104 g dm^{-3}), Na-citrate · 2 H_2O (39.2 g dm^{-3}), cc. HCl (33 cm^3 dm^{-3}), thiodiglycol (20 cm^3 dm^{-3}) and 0.5% pentachlorphenol solution (0.1 dm^3 dm^{-3}). The final volume was adjusted using Na-citrate solution with a pH of 2.2. After making up to the mark, the samples were filtered and stored at −18 °C until the analysis was carried out.

1.2.2.2. Separation, determination. — The determination of the amino acid composition was carried out using a Mikrotechna AAA 881 automatic amino acid analyser made in Czechoslovakia, where elution takes place on a single column using four buffer solutions. The ninhydrin colour reaction was used for detection. The reagent was produced by mixing two components. The resulting compound was automatically measured in two flow cuvettes photometrically and recorded at 570 and 440 nm.

The area of the chromatographic peaks measured manually was compared to those of standard amino acids chromatographed under identical conditions.

1.2.2.3. Determination of tryptophan. — The determination of tryptophan, which is destroyed in the course of hydrochloric acid hydrolysis was carried out after alkali hydrolysis using a Contiflo (Labor Instrument Works, Hungary) automatic analyser with the method developed at the Department of Biochemistry and Food Technology, Technical University, Budapest (Örsi, 1984).

1.2.2.3.1. Alkali hydrolysis. — Samples containing approx. 80 mg protein were measured into heat-resistant test-tubes using an analytical balance. Ten cm^3 4 mol per dm^3 sodium hydroxide were added and preliminary hydrolysis was carried out in a 100 °C water-bath for 10 min.

Nitrogen gas was then passed through the samples for 3 min, after which the test-tubes were sealed. The hydrolysis was carried out for 10 hours in a test-tube thermostat at 110 °C. At the end of this period the test-tubes were opened and the contents were washed into 25 cm^3 measuring flasks containing 10 cm^3 4 mol per dm^3 sulphuric acid. One mol per dm^3 sulphuric acid was used to make up to the mark. The samples were analysed immediately after filtration.

1.2.2.3.2. Determination using a Contiflo automatic analyser. – The sample passes from the diluting section into a sulphuric acid–water solution (7 : 3) segmented with air in the determination module, then, after mixing, 0.05% *p*-dimethylamino cinnamic aldehyde reagent dissolved in 20% sulphuric acid is added. The colour reaction takes place at room temperature in a spiral with 40 turns. The coloured product is then measured photometrically at 430 nm. The signals emitted by the photometer are continuously recorded throughout the analysis. Calibration required for evaluation was carried out using standard tryptophan solutions (in the concentration range 0–100 mg per dm^3).

1.2.2.4. Determination of cystine. – In the course of hydrochloric acid hydrolysis sulphur-containing amino acids suffer a greater or lesser degree of damage, so it is advisable to produce stable oxidation forms of these amino acids for analytical purposes.

Samples containing approx. 60 mg protein were measured into heat-resistant test-tubes using an analytical balance. Four cm^3 98% performic acid – 30% hydrogen peroxide (1 : 9), left to stand for 1 hour before use was added and then left to stand in a refrigerator for 20 hours. During this time the sample completely dissolved and oxidation took place. In order to stop the reaction 0.6 cm^3 48% hydrogen bromide were added to the sample, after which the material was evaporated to dryness in a vacuum drying cabinet.

Ten cm^3 6 mol per dm^3 hydrochloric acid were added to the oxidized, evaporated samples, which then underwent preliminary hydrolysis on a 100 °C water-bath for 15 min. Nitrogen gas was then passed through the samples and the test-tubes were sealed. The hydrolysis was carried out for 24 hours in a test-tube thermostat at 110 °C. After hydrolysis the samples were treated as described for the determination of gross amino acid composition.

The amino acid analysis was also carried out in an identical manner, except that in this case the first buffer (pH 3.25) is sufficient for elution.

During the evaluation the chromatographic peak was compared to a standard cysteic acid peak, after which the values were transformed into cystine assuming a 0.93 degree of conversion for the oxidation.

1.2.3. Determination of in vitro digestibility

For the determination of digestibility a modified version of the method given by Hsu and co-workers (1977) was applied: samples containing 200 mg crude protein were suspended in 50 cm^3 distilled water in an ideally mixed, double-walled glass reactor with the temperature stabilized at 37 °C in an ultrathermostat. The pH of the mixture was adjusted to 8.00 with 0.1 *N* NaOH or HCl using a pH meter with a digital display (Reanal, Hungary). After this, 1 cm^3 enzyme solution, made from 10 mg porcine pancreatin

(Gedeon Richter Works, Hungary) and 4 mg porcine trypsin (Merck) and kept in an ice-bath, was pipetted into the system. The drop in pH over a period of ten minutes was recorded. The relative digestibility of the protein in the sample compared to casein was determined by comparison with data obtained in a similar manner using casein (Reanal, Hungary).

1.3. Determination of in vitro protein quality

The in vitro protein quality was determined using the method described by BÉKÉS and co-workers (1982a, 1982b, 1984).

The mathematical form of the expression which models protein quality as a function of amino acid composition data is as follows:

$$\text{In vitro biological value} = 10^{\alpha_0} \prod_{i}^{n} Q_i^{\alpha_i} \tag{1}$$

$$Q_i = \exp(-4.5) \left[\frac{\left[\frac{a_i}{\sum_{i}^{n} a_i} \right] - \left[\frac{a_{i,r}}{\sum_{i}^{n} a_{i,r}} \right]}{\left[\frac{a_{i,r}}{\sum_{i}^{n} a_{i,r}} \right]} \right]^2 \tag{2}$$

where a_i = concentration of the i-th essential amino acid in the sample;
$a_{i,r}$ = requirement for the i-th essential amino acid;
n = number of essential amino acids;
α_0 and α_i = specific weighting factors.

In the present paper two variants of functions (1) and (2) are applied. These differ from each other in the reference composition and in the numerical value of the specific exponents. In the first variant (hereafter: IN VITRO BV(A)) the numerical values of the weighting factors were determined using statistical methods on the basis of data from human in vivo nitrogen balance experiments (BÉKÉS et al., 1984). The method is a further development of that reported by MØRUP and OLESEN (1976), using the same composition for reference, but, due to the greater sensitivity of the mathematical model employed, there is better correlation with in vivo data ($r = 0.986$). The second variant (hereafter: IN VITRO BV(B)) contains no constants determined by statistical methods, and the reference data are the human amino acid requirements taken from the USA standard (NATIONAL RESEARCH COUNCIL, 1974).

Considering the fact that the IN VITRO BV indices cannot, due to the mathematical nature of the matter, be compared linearly (if the index is twice as big, it does not mean that the protein quality is twice as good), indices

referred to as 'protein in vitro nutritional quality' (A) and (B) are given, which can be taken as linear in the above sense. These figures were produced mathematically using the following transformation:

$$\text{protein in vitro nutritional quality} = 100\left[\frac{\text{IN VITRO BV}}{10^{\alpha_0}}\right]^{\frac{1}{n}} \qquad (3)$$

In other words, the geometrical mean of the factors in the product function found in (1) (the individual amino acid increments) represents the average amino acid supply provided by the sample to the consumer.

The reference amino acid data employed are shown in Table 1. Table 2 contains the α weighting factors used in the two cases.

Table 1

Reference amino acid patterns for adult humans
(mg essential amino acids per g total essential amino acids)

Amino acids	A	B
ILE	110	125—
LEU	179	165
LYS	141	124
PHE + TYR	212	160
MET + CYS	89	160
THR	99	86
TRP	30	38
VAL	140	141

A: MØRUP and OLESEN (1976)
B: NATIONAL RESEARCH COUNCIL (1974)

Table 2

Numerical values of the α weighting factors used in the expressions of in vitro protein quality

Factor	A	B
α_0	2.16	2.00
α_{LYS}	0.28	1
α_{TRP}	0.19	1
α_{THR}	3.32	1
$\alpha_{MET+CYS}$	0.67	1
$\alpha_{PHE+TYR}$	0.72	1
α_{ILE}	0.00	1
α_{LEU}	0.00	1
α_{VAL}	0.00	1

2. Results and discussion

The macro composition data for fenugreek seeds are shown in Table 3 measured by the authors listed.

There is considerable deviation between the literary data on protein content; the current results are similar to those reported by KUZAYLI and co-workers (1966). Although fenugreek contains a substantial amount of protein, its nutritional potential is seriously reduced by the high content of

Table 3

The macro composition data for fenugreek seeds

Protein (%)	Fat (%)	Moisture (%)	Reference
26.4 ± 0.2	6.15 ± 0.09	9.0 ± 0.05	Present investigation[a]
30.2			HUSSEIN & NOUMAN (1969)
26.0	6.3	4.2	KUZAYLI et al. (1966)
16.97	7.61	10.30	CASARES LOPEZ et al. (1950)
35.0	4.1 — 8.0		HARBORNE et al. (1971)

[a] On the basis of three analyses

Table 4

Comparison of amino acid composition of fenugreek seeds in mg per g N unit

Amino acid	JAMALIAN and PELLETT (1968)	Present analysis
ASP	622	672
THR	201	226
SER	279	276
GLU	967	883
PRO	301	292
GLY	269	246
ALA	225	212
CYS	100	75
VAL	237	186
MET	82	54
ILE	298	250
LEU	409	361
TYR	191	167
PHE	237	257
LYS	357	345
HIS	144	159
TRP	45	93
ARG	646	524
SAA[a]	182	129
AROM[b]	428	424

[a] MET + CYS
[b] TYR + PHE

Table 5

In vitro biological value and protein nutritional quality data for the seed protein of some legumes

Legumes	IN VITRO BV		PROTEIN IN VITRO NUTRITIONAL QUALITY	
	A	B	A	B
Groundnut meal[a]	56.54	0.657	88.93	53.36
Soybean meal[a]	107.77	4.64	96.40	68.13
Sweet lupin[a]	84.39	1.19	93.49	57.47
Lentils[a]	48.16	0.274	87.16	47.83
Broad bean[a]	24.08	0.322	79.93	48.81
Chickpea[a]	85.85	2.04	93.70	61.48
Fenugreek[a]	119.51	4.58	97.65	68.02
Fenugreek[b]	64.39	2.00	90.39	61.32

[a] Calculated using amino acid composition data from JAMALIAN and PELLETT (1968)
[b] Calculated using data from the present amino acid analysis

bitter and anti-nutritive components: the sapogenine content may be as high as 0.8–2.2% (FAZLI & HARDMAN, 1968). These noxious components do not seem to be localised in any anatomically distinct part of the seed (DEMETER et al., 1983). The effect of the anti-nutritive factors is responsible for the extremely low digestibility of the protein from fenugreek seeds; current analyses show this to be 32 $\pm$ 2% on the basis of four measurements.

The results of the present amino acid analysis and those given in the literature indicate that the amino acid pattern of fenugreek seed protein is characterized by a relatively low quantity of sulphur-containing amino acids and tryptophan, but the amino acid pattern of the protein is otherwise well-balanced and, unlike that of cereals, is particularly rich in lysine (Table 4).

The in vitro protein quality of fenugreek seeds is shown in Table 5 in comparison with other legumes.

It can be seen from the results that the protein quality of fenugreek seeds is approximately equal to that of soybeans, which means that it is higher than that of an "average legume".

On the grounds of the above, it would seem that fenugreek could become a promising nutritional factor as a source of protein for those strata of the Egyptian and Indian population which now have a protein-deficient diet. The main problem is the low digestibility and high content of bitter compounds in the material. Further research will be required in order to overcome these problems.

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COMPARISON BETWEEN THE SENSORIALLY ESTABLISHED AND INSTRUMENTALLY MEASURED COLOUR OF RED WINE

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The correlation between the sensorially established and instrumentally measured colour of red wines, was studied. On the basis of the correlations established we aimed at selecting a colour characteristic, which, while measurable instrumentally is in harmony with the sensory experience, or in other words reflects accurately, linearly the colour as seen by the naked eye. The study was carried out with the must and young wine of increasing colour intensity of Merlot variety from Eger and with a series of white wines of increasing colour intensity using Enocianina grape pigment extract (Transcommerce; Wien) for colouring.

The sensory value of colour of the samples was established by a triangular test and ranking as laid down by the authors.

On the basis of the triangular tests it was found that a difference below 1 between colour intensities could not be established at a probability level of 95% by a panel of 7 judges. The result of the ranking test has shown that all members of the panel ranked the samples according to their increasing colour intensity. On the whole the differences in the colour intensities of the samples were valued by the same difference in scores by all the members.

In order to establish the correlation between the instrumentally measured colour characteristics and the sensory value the generally used colour parameters of the samples were determined: monomer anthocyanin content; tristimulus values; light absorption as measured at 520 nm (value E_{520}); transmission as measured at 520 nm (value T_{520}); sum of light absorption values as measured at 420 and 520 nm (value $E_{420+520}$). The parameters measured were plotted as a function of the averages of the sensory scores.

Value T_{520} was found to have no linear correlation or correlation tracable to linear with the average values of sensory evaluation. The direct utilization of tristimulus values for the characterisation of the colour of red wine is yet infeasible. A linear correlation was found between the monomer anthocyanin content and log E_{520} values when plotted against the average sensory values. However, the correlation coefficients were low (between 0.6 and 0.7).

On plotting the $E_{420+520}$ values as a function of the average sensory values a homogeneous linear correlation was established with a correlation coefficient above 0.9. On the basis of the experiments the $\log E_{420+520}$ value (colour intensity according to Sudraud) in the 1–27 colour intensity range showed a linear correlation with the colour sensation of the naked eye. On the basis of the data obtained in the present experiments the introduction of parameter 23 log $E_{420+520}$, termed logarithmic colour intensity, and marked P is suggested. This parameter lends itself to routine measurement, is simple to calculate and reflects accurately, linearly the colour intensity as established by the naked eye.

Keywords: colour of red wine, tristimulus method

The most striking characteristic of red wines is their colour. Therefore, in grading red wines and in evaluation of their manufacturing technologies with view to improving and checking their quality it is important to establish

their colour objectively and in harmony with the colour sensation. In the knowledge of the authors so far no effort was made to find a correlation between the instrumentally measured colour parameters and the colour as established by the naked eye.

The anthocyanins, as the main colour components of red wine grapes and red wine were widely investigated by authors abroad and in Hungary. The quality and quantity of anthocyanin pigments (GOMBKÖTŐ, 1963; 1964; 1965), changes during ripening, fermentation and aging (LITTLE, 1977; MARTINIERE et al., 1973; NAGEL & WULF, 1979; ÁSVÁNY & DONKÓ, 1981), were studied. The red wine pigments were found to undergo changes during fermentation and aging, inasmuch the concentration of the anthocyanin monomers decreases (ÁSVÁNY & DONKÓ, 1981; KAMPIS, 1980) while that of polymerized anthocyanins increases (KAMPIS, 1980).

In order to throw light on the pigments and the mechanism of their changes and to be able to stabilize the colour of red wines it is inevitable to have an objective instrumental method for colour measurement.

Several methods are applied to characterize the colour of red wines. The method of measuring the total anthocyanin content is widely used (AUBERT, 1970; ÁSVÁNY, 1973; SOMERS & EVANS, 1979). Similarly widely used is the measurement of colour intensity (value $E_{420+520}$) and hue (value E_{420}/E_{520}) according to SUDRAUD (1958) and PALLOTTA (1982).

Lately the value E_{520} is frequently used in the related literature to characterize the colour of red wines (SOMERS & EVANS, 1974; 1977) and for the comparison of musts and wines made by different red wine vinification technologies (TARJÁN & URBÁN, 1979). An essay was made also to use the transmission value as measured at 520 nm for the characterization of the colour of red wine (TÖRÖK, 1980).

The truly objective determination of colour is possible by measuring and calculating the tristimulus value as standardized by C.I.E. (Commission Internationale de l'Eclairage). This method is seldom used in wine analysis, although it was endeavoured to use it for the elucidation of the correlation between the qualitative and quantitative analysis or anthocyanins and their tristimulus values (BALLINGER & MANESS, 1974; LITTLE, 1977). In related Hungarian literature a single paper was published discussing the characterization of red wine colour with tristimulus values (MOLNÁR & LUKÁCS, 1978).

Since for the characterization of the colour of red wines only an objective, instrumental method in harmony with sensory values, can be used, the chief aim of the present study was to correlate the colour characteristics instrumentally measured with the visual sensation, to establish the correlation between the sensitivity of the eye and that of the instrument and to study the possible saturation of the subjective colour sensation.

1. Materials and methods

1.1. Materials

1.1.1. Red wines of increasing colour intensity. Merlot grape variety grown at the Research Station in Eger of the Research Institute for Viticulture and Enology in 1981 was used for the experiments. Fifty kg grapes were, after crushing and stemming, fermented in glass jars without sulfiting. During fermentation 2 dm^3 samples were taken in every 24 h from the must. The samples were pressed and those containing unfermented sugar were preserved with bromoacetic acid–ethylene glycol ester (Pandurol). With the advance of fermentation the pigment concentration and colour intensity of the samples increased.

1.1.2. Model red wine sample series. A series of samples of increasing colour intensity was prepared from white wine and a grape pigment extract named Enocianina (Transcommerce, Wien). The colour intensity of the identical white wine samples was increased by the addition of the appropriate amount of Enocianina from OD-1 to OD-12 (OD = optical density) according to SUDRAUD (1958). The series was complemented by two samples: OD-14 and OD-27 colour intensity. This series of samples was prepared to check the capability of the panel members to distinguish between samples differing by one unit of colour intensity. The possible saturation of subjective colour sensation in higher intensity ranges was also investigated.

1.2. Methods

1.2.1. Instrumental measurement of colour characteristics. The colour characteristics instrumentally measured were determined by a Spectromom 195 (Hungarian Optical Works, Budapest) spectrophotometer.

1.2.1.1. Measurement of the anthocyanin content. – The total monomer anthocyanin content of the wine or must was determined by the method of AUBERT (1970). The measurement was carried out in a solution of pH 1 at 550 nm. The anthocyanin content was expressed as malvidin-3-monoglucoside in mg dm^{-3} unit.

1.2.1.2. Determination of colour intensity according to Sudraud. – Colour intensity according to Sudraud is the sum of the extinction values as measured at the two characteristic wavelength (420 and 520 nm) taken in the visible wavelength range of the spectrum of red wine and calculated for 1 cm optical path. Colour intensity is measured at the original pH of the wine, thus only the light absorption visible in this pH range is measured. The colour intensity value of Sudraud is therefore $E_{420}+E_{520}$, $E_{420+520}$, resp., in general use. (Sudraud's hue value [$E_{420/520}$] was not investigated because it is not unanimously valued and in the related literature it is rarely referred to.)

1.2.1.3. Determination of the E_{520} *values.* – The optical density of the red must or wine was measured at 520 nm and calculated for an optical path of 1 cm.

1.2.1.4. Determination of the T_{520} *value.* – The transmission of red must or wine was measured on a 0.2 cm optical path at 520 nm.

1.2.1.5. Determination of tristimulus values. – Measurements were carried out on a Momcolor (Hungarian Optical Works, Budapest) instrument in a cuvette of 0.5 cm width adjusted to distilled water. Values X_1, X_2, Y and Z recorded on the instrument were used to calculate colour values x, y, z as standardized in the C.I.E. system.

1.2.2. Sensory colour evaluation. Parallel to the instrumental measurements the colour of the samples was established also by sensory test. The triangular test and a ranking system with a scale from 0 to 30 points, set up by the authors, was used by a panel of 7 members.

1.2.2.1. Triangular test. – Panelists received 3 standardized tasting glasses each containing a red wine sample and they had to say which of the samples differed from the other two. As regards colour intensity the order of the three samples was random. The results were evaluated by mathematical statistical methods at the 95% probability level. The difference between sam-

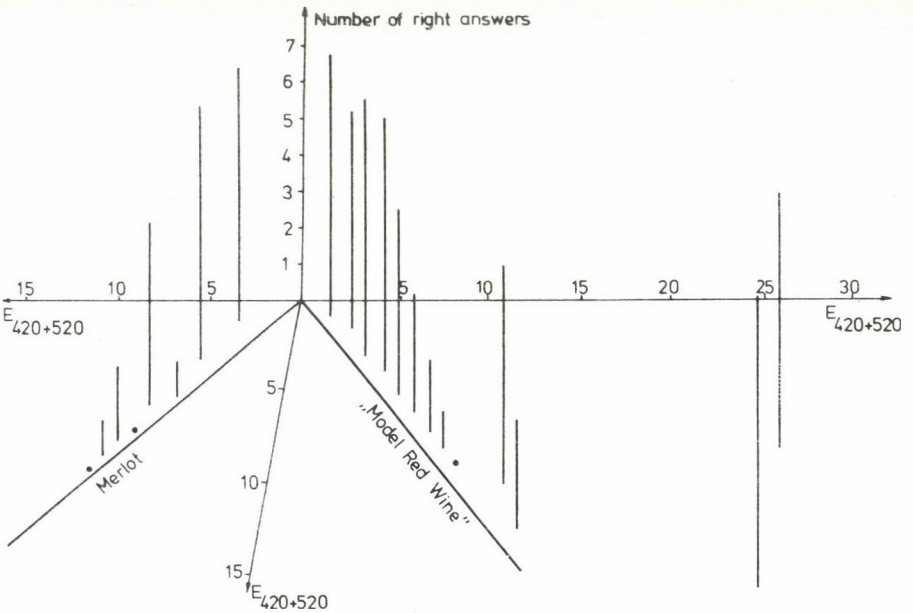

Fig. 1. Results of the triangular tests in the Merlot and red wine series. Number of panel members: 7. Data related to Merlot wine are on the left side, those belonging to the model series on the right side. The colour intensity values according to Sudraud are shown on the base plane. The standard deviations are summarized in Tables 2 and 3

ples was considered significant at the 95% probability level when 5 of the judgements was correct (AMERINE et al., 1965).

1.2.2.2. Ranking test. – The samples were put in test-tubes of identical size and width and ordered randomly. Panel members had to rank the samples according their colour intensities. Samples of different colour intensity were scored on the scale between 0 to 30, use of the full scale being obligatory. Selection of the scoring range was arbitrary. The scale of 0 to 30 seemed to enable realistic differentiation between samples.

2. Results

2.1. Results of the sensory evaluation

2.1.1. Results of the triangular test. Results are summarized in Fig. 1. Each sample was characterized by its colour intensity according to SUDRAUD (1958). The colour intensities are shown on the base plane. A greater difference in colour intensity is represented by greater difference from the bisector.

Table 1

Results of the ranking test

	No. of sample	Scores of panelists							Result		$E_{420+520}$-values	
		1	2	3	4	5	6	7	$\bar{x}$	±s	$\bar{x}$	±s
Model red wine	1	0	0	0	0	0	0	0	0	Ø	0.120	0.002
	2	3	3	3	3	3	2	3	2.86	0.38	1.005	0.014
	3	5	7	7	7	6	6	6	6.29	0.76	1.865	0.039
	4	13	10	11	11	13	10	10	11.14	1.35	3.110	0.059
	5	16	13	13	13	16	12	12	13.57	1.72	3.764	0.068
	6	18	16	16	16	18	15	15	16.29	1.25	5.150	0.118
	7	20	17	17	20	20	17	17	18.29	1.60	6.110	0.153
	8	23	19	18	23	21	19	19	20.29	1.89	7.150	0.136
	9	24	20	20	24	21	20	20	21.29	2.06	8.175	0.131
	10	25	22	21	25	22	21	21	22.43	1.81	9.035	0.208
	11	26	23	22	26	23	22	22	23.43	0.90	9.925	0.188
	12	27	26	26	27	24	24	24	25.43	1.40	12.775	0.268
	13	28	27	28	28	25	25	27	26.86	1.95	14.00	0.294
	14	30	30	30	30	30	30	30	30.00	Ø	27.253	0.953
Merlot series	1	0	0	0	0	0	0	0	0	Ø	1.175	0.020
	2	11	8	11	10	9	10	10	9.85	1.07	3.355	0.070
	3	18	18	20	19	18	18	19	18.56	0.79	5.108	0.087
	4	23	19	22	19	18	20	22	20.42	1.90	5.824	0.116
	5	27	26	27	26	25	25	27	26.14	0.90	7.565	0.136
	6	27	25	27	26	25	25	27	26.00	1.00	7.625	0.175
	7	29	27	29	29	28	28	29	28.43	0.79	8.478	0.127
	8	29	28	30	30	29	29	29	29.14	0.69	9.083	0.200
	9	30	30	30	30	30	30	30	30.00	Ø	9.356	0.178

Column heights represent the number of correct judgements in the triangular test. Dots represent the tests where none of the answers was correct.

2.1.2. Results of the ranking test. In both sample series all the samples were placed by all the panel members in the correct order, in the order of increasing colour intensity. The scores of the panelists, the average and the standard deviations of the scores of all the 7 panel members are summed up in Table 1.

2.2. Analysis of the correlation between instrumental characteristics and sensory evaluation

In order to be able to analyze the correlation the instrumentally measured characteristics were plotted as a function of the average value of scores obtained in the ranking test. The colour characteristics instrumentally measured are presented in Tables 2 and 3.

Table 2

Instrumentally measured colour characteristics of the Merlot series

No. of sample	$E_{420+520}$		E_{520}		T_{520}		Anthocyanin (mg dm^{-3})	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
1	1.175	0.020	0.530	0.010	78.0	1.72	21	0.48
2	3.355	0.070	1.830	0.036	47.3	1.65	106	1.59
3	5.108	0.087	2.650	0.060	28.6	0.54	165	3.79
4	5.824	0.116	3.746	0.060	23.6	0.53	261	5.48
5	7.565	0.136	4.367	0.109	13.3	0.38	262	6.03
6	7.625	0.175	4.900	0.102	12.5	0.44	311	5.91
7	8.478	0.127	5.225	0.089	8.1	0.41	320	6.72
8	9.083	0.200	6.321	0.120	6.0	0.41	440	7.92
9	9.356	0.178	6.634	0.159	5.5	0.49	409	11.45

Table 3

Instrumentally measured colour characteristics of the Model red wine

No. of sample	$E_{420+520}$		E_{520}		T_{520}		Anthocyanin (mg dm^{-3})	
	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s	$\bar{x}$	±s
1	0.120	0.002	0.030	Ø	98.5	5.91	Ø	Ø
2	1.005	0.014	0.555	0.007	77.5	3.25	20	0.40
3	1.865	0.039	1.065	0.019	61.5	3.26	36	0.76
4	3.110	0.059	1.913	0.034	41.5	1.16	63	1.57
5	3.764	0.068	1.954	0.036	35.5	1.06	54	0.86
6	5.150	0.118	2.745	0.052	23.5	0.59	87	1.48
7	6.110	0.153	4.162	0.079	18.0	0.47	85	2.04
8	7.150	0.136	4.418	0.093	13.0	0.66	161	3.86
9	8.175	0.131	4.698	0.099	9.0	0.61	139	2.92
10	9.035	0.208	5.483	0.104	7.0	0.56	158	3.00
11	9.925	0.188	5.854	0.105	5.0	0.40	240	4.08
12	12.775	0.268	8.484	0.161	2.0	0.12	221	4.20
13	14.000	0.294	8.723	0.166	0.5	0.06	270	4.86
14	27.253	0.953	17.252	0.690	Ø	Ø	267	11.91

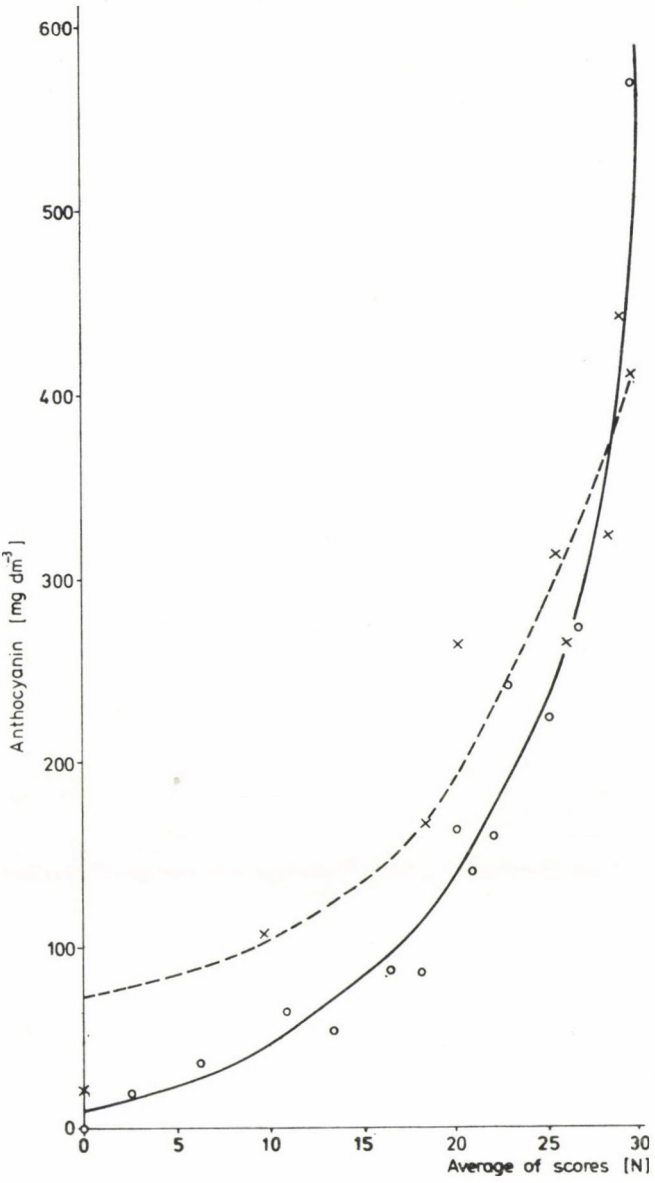

Fig. 2. Relationship between the monomer anthocyanin content (mg dm^{-3}) and the colour as seen by the naked eye (average value of the scores given in the ranking test). Points marked ×; dashed line: Merlot series; equation of the curve: $y = 47.0 \times 10^{0.032x}$; $n = 9$, where y is the anthocyanin content and x the average score in the sensory evaluation. Points marked ○; solid line: model red wine series; equation of the curve $y = 14.0 \times 10^{0.051x}$; $n = 14$. Points represent average of three measurements; standard deviations are given in Tables 2 and 3

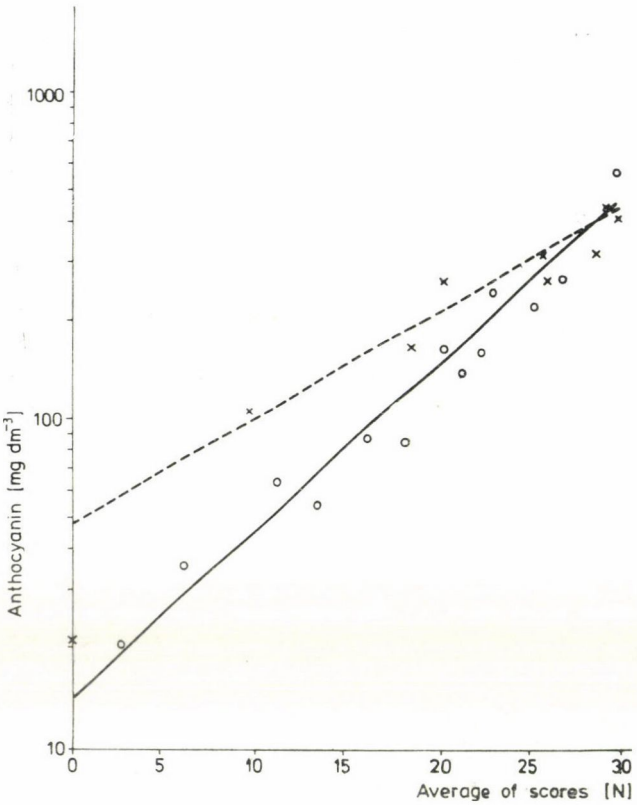

Fig. 3. Relationship between the logarithm of the monomer anthocyanin content and the colour as seen by the naked eye (average values of scores given in the ranking test). Points marked ×; dashed line: Merlot wine series; equation of the straight line: $\log y = 1.672 + 0.032x$; $r = 0.64$; $n = 9$ (y stands for the anthocyanin content, x for the average score in the ranking test). Points marked ○; solid line: model red wine series; equation of the straight line: $\log y = 1.146 + 0.051x$; $r = 0.69$; $n = 14$. Points are the averages of three measurements. Standard deviations are given in Tables 2 and 3

2.2.1. Correlation between the anthocyanin content and the colour as seen by the naked eye. According to the results of the two series of experiments the correlation between the monomer anthocyanin content and the visual sensation is of exponential character (Fig. 2). When the logarithm of the anthocyanin content is plotted against the average score obtained in the sensory test and fitted to linear regression straight line is obtained (Fig. 3). The correlation coefficient of both lines is, however, below 0.7.

2.2.2. Correlation between tristimulus values and the visual colour sensation. Plotting of the tristimulus value as a function of the average scores of sensory judgement is not possible, because this would require the graphic representation of a four-variable function. As it can be seen from the tristimulus values presented in Tables 4 and 5, values x and Y show a monotonic change, while

y value changes only slightly with the increase of colour intensity. In both series of experiments the x values show a monotonic increase with increasing colour intensity, while Y values show a monotonic decrease.

2.2.3. Correlation between the T_{520} *values and the visual colour sensation.* In both the Merlot wine and the model red wine series the results show a correlation of monotonically decreasing character between the transmission values as measured at 520 nm and the average scores of panel members (Fig. 4). On investigating the logarithm of the T_{520} values as a function of the average scores (Fig. 5) it is seen that the correlation is non-linear.

2.2.4. Correlation between the E_{520} *values and the colour as seen by the naked eye.* The results of measurements in red wine and the model series of

Table 4

The tristimulus values of the Merlot series

No. of sample	x		y		Y	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
1	0.3480	0.0052	0.3296	0.0046	50.82	0.56
2	0.4397	0.0061	0.3223	0.0051	21.46	0.26
3	0.5057	0.0076	0.3101	0.0049	11.32	0.15
4	0.5323	0.0085	0.3074	0.0046	9.46	0.10
5	0.5864	0.0111	0.2923	0.0040	6.24	0.07
6	0.5933	0.0083	0.2915	0.0046	6.81	0.09
7	0.6127	0.0122	0.2864	0.0049	6.11	0.08
8	0.6227	0.0099	0.2806	0.0045	5.20	0.07
9	0.6303	0.0113	0.2814	0.0045	5.01	0.05

Table 5

The tristimulus values of the Model red wine

No. of sample	x		y		Y	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
1	0.2731	0.0013	0.3073	0.0015	70.92	0.71
2	0.3401	0.0017	0.3382	0.0017	28.95	0.28
3	0.3836	0.0019	0.3298	0.0014	26.13	0.30
4	0.4273	0.0022	0.3286	0.0015	24.89	0.26
5	0.4715	0.0025	0.3187	0.0016	17.62	0.19
6	0.5094	0.0020	0.2981	0.0013	11.24	0.13
7	0.5438	0.0026	0.2935	0.0015	9.31	0.11
8	0.5622	0.0027	0.2930	0.0015	6.57	0.10
9	0.6027	0.0030	0.2893	0.0014	6.32	0.09
10	0.6228	0.0031	0.2837	0.0017	5.19	0.07
11	0.6475	0.0030	0.2809	0.0011	4.38	0.07
12	0.6761	0 0034	0.3810	0.0014	3.82	0.06
13	0.6993	0.0032	0.2784	0.0016	2.08	0.04
14	0.7180	0.0036	0.2703	0.0013	1.23	0.03

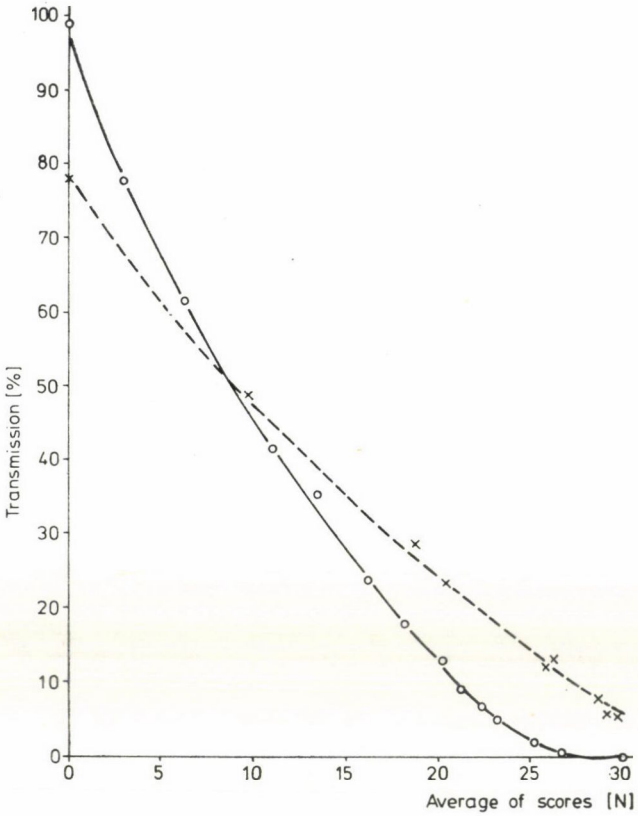

Fig. 4. Relationship between values T_{520} and the colour as seen by the naked eye (average of scores given in the ranking test). Points marked ×; dashed line: Merlot red wine series. Points marked ○; solid line: model red wine series. Points are the averages of three parallel measurements. Standard deviations are contained in Tables 2 and 3

pigmented white wine show that the correlation between the extinction values as measured at 520 nm and the average colour values as established by the panel members is of monotonically increasing character (Fig. 6). When plotting the logarithm of E_{520} values as a function of the average of sensory values the correlation was linear (Fig. 7). Results were evaluated by mathematical statistical methods. The lines were fitted by linear regression and the correlation coefficients were in both cases below 0.7.

2.2.5. Correlation between values $E_{420+520}$ *and the colour as seen by the naked eye.* The results of both experimental series show that the correlation between the values as measured and as seen by the naked eye is of monotonically increasing character (Fig. 8). On plotting the logarithm of value $E_{420+520}$ as a function of the average score of the visible colour (colour intensity according to SUDRAUD, 1958) a homogeneous linear correlation was ob-

tained (Fig. 9). Equation of the straight line on the basis of measurements in the model red wine

$$N = 23 \times \log E_{420+520}$$

where N = the average score,
$E_{420+520}$ = colour intensity according to Sudraud.

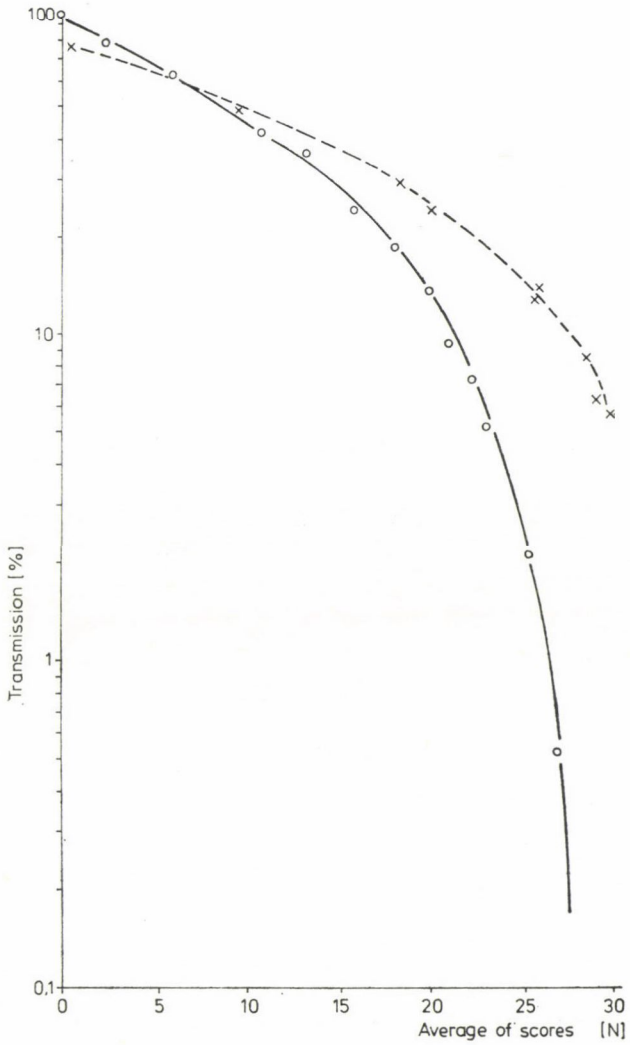

Fig. 5. Relationship between the logarithms of values T_{520} and the colour as seen by the naked eye (average of scores given in the ranking test). Points marked x; dashed line: Merlot red wine series. Points marked o; solid line: model red wine series. Points are the averages of 3 parallel measurements. Standard deviations are shown in Tables 2 and 3

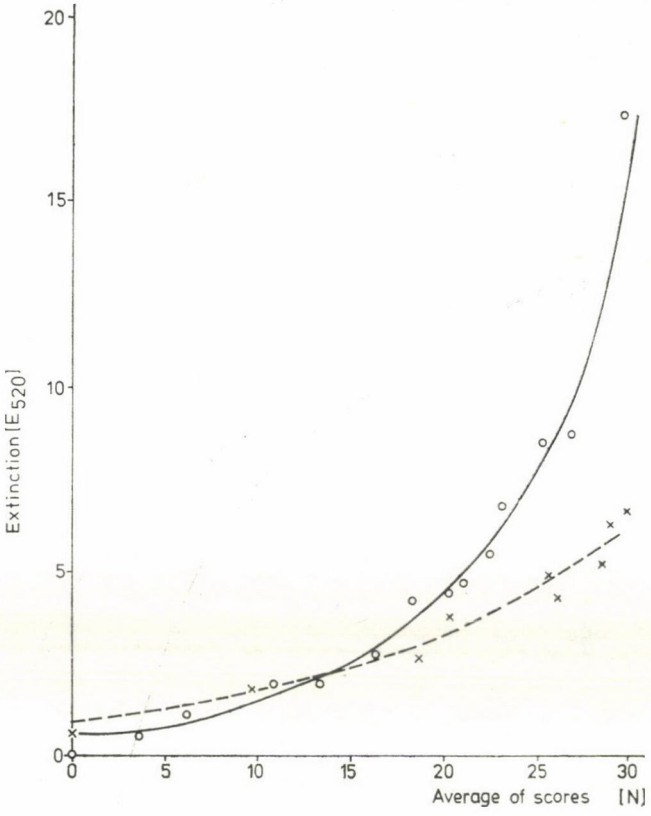

Fig. 6. Relationship between values E_{520} and the colour as seen by the naked eye (average of scores given in the ranking test). Points marked ×; dashed line: Merlot series; equation of the curve: $y = 0.916 \times 10^{0.023x}$; $n = 9$, where $y = E_{520}$; $x =$ average score in the ranking test. Points marked ○; unbroken line: model red wine series; equation of the curve: $y = 0.624 \times 10^{0.043x}$; $n = 14$. Points are the averages of three parallel measurements. Standard deviations are shown in Tables 2 and 3

Since the scores given the sample of lightest and darkest colour deviate from the straight line because of the lower (0) and upper (30) limitation of the scores, they were not taken into account in fitting the straight line by linear regression. The results show that the logarithm of Sudraud's colour intensity values (SUDRAUD, 1958) and the visible colour values are correlated linearly in the 1–27 colour intensity range.

3. Conclusions

In the course of the sensory evaluation of colour it was proven by the triangular tests that differences of colour intensity below 1 cannot be significantly distinguished at a probability level of P = 95% (Fig. 1). In the colour

intensity range of 1–5 panelists could differentiate at the 95% significance level between the three samples of the test differing only by 1 colour intensity value. In the range of 6 to 14, however, the resolving power of the human eye is lower, thus, panelists could not distinguish at the 95% significance level between samples differing only by 1 colour intensity value. The model red wine series permitted of checking the possible saturation of the subjective colour sensation. It was found that the subjective colour sensation does not become saturated and panelists were capable of distinguishing between samples in the 8 to 14 colour intensity range at the probability level

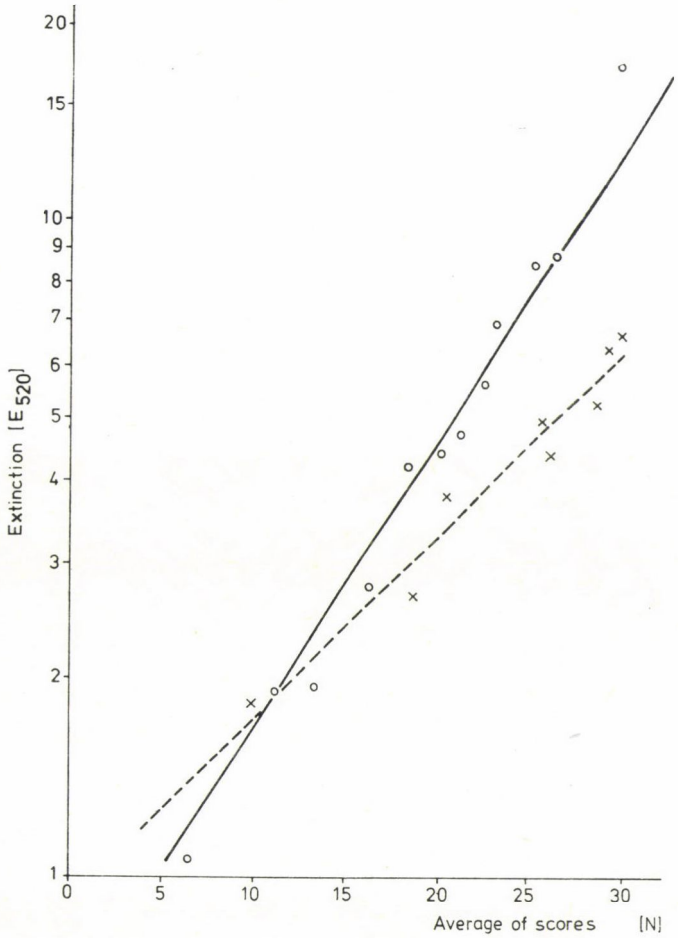

Fig. 7. Correlation between the logarithms of value E_{520} and the colour as seen by the naked eye (average of scores given in the ranking test). Points marked x; dashed line: Merlot series; equation of the straight line: $\log y = -0.038 + 0.028x$; $r = 0.65$; $n = 9$. Points marked o; unbroken line; model red wine series: equation of the straight line: $\log y = -0.205 + 0.043x$; $r = 0.69$; $n = 14$ ($y = E_{520}$; $x =$ average of scores in the ranking test). Points represent averages of three parallel measurements. Standard deviations are shown in Tables 2 and 3

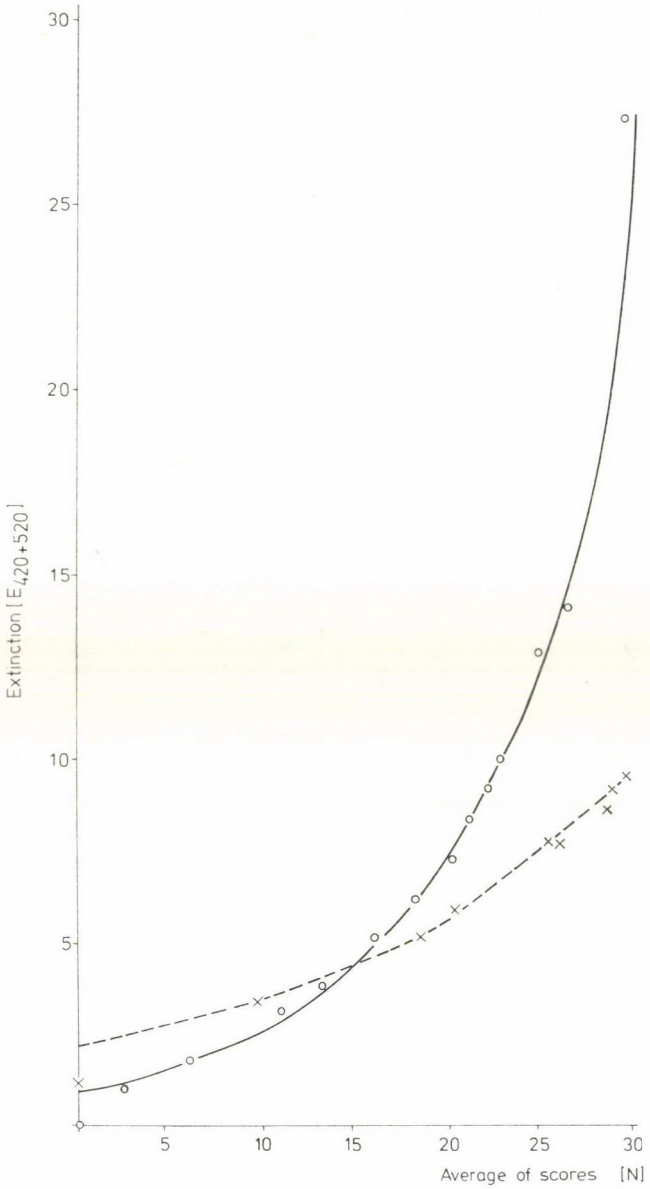

Fig. 8. Correlation between values $E_{420+520}$ (colour density according to Sudraud) and the colour as seen by the naked eye (average scores given in the ranking test). Points marked ×; dashed line: Merlot series; equation of the curve: $y = 2.07 \times 10^{0.0217x}$; $n = 9$. Points marked ○; unbroken line; model red wine series: equation of the curve $y = 1.00 \times 10^{0.0349x}$; $n = 14$ ($y = E_{420+520}$; x = average of scores given in the ranking test). Points represent averages of three parallel measurements. Standard deviations are shown in Tables 2 and 3

of 95%. Similarly significant difference could be established between samples of 8 and 13 or 14 and 27 colour intensity, respectively.

On plotting the monomer anthocyanin content as a function of the average sensory scores a linear correlation was obtained, however, because of the correlation coefficient below 0.7, the monomer anthocyanin content is not suitable to characterize safely the colour of red wine. The reason for the

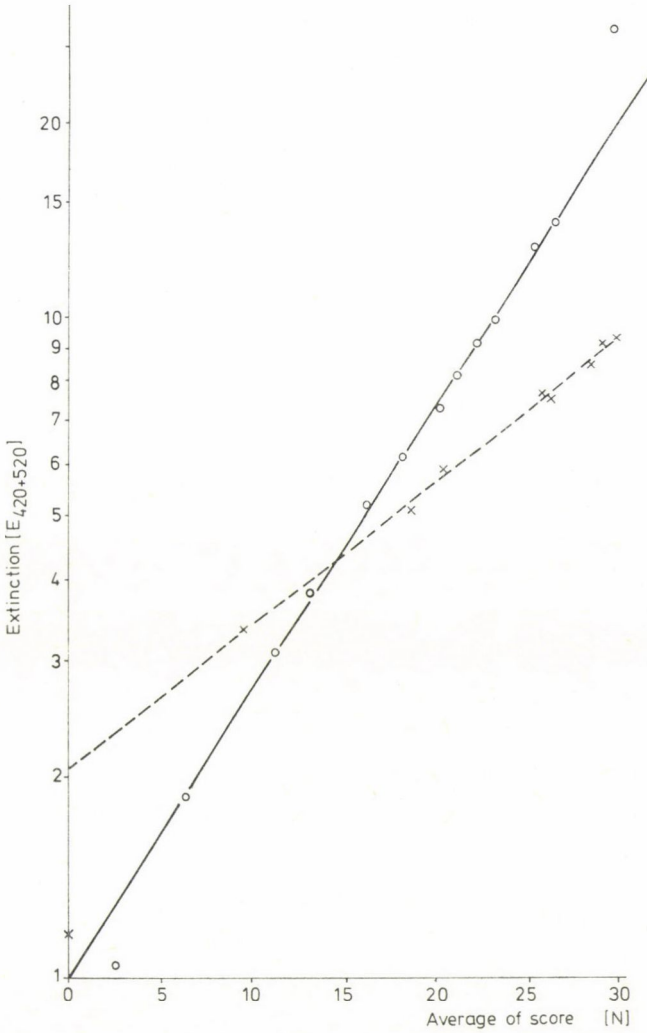

Fig. 9. Correlation between the logarithms of values $E_{420+520}$ and the colour as seen by the naked eye (average of scores given in the ranking test). Points marked x; dashed line: Merlot series; equation of the straight line: $\log y = 0.316 + 0.127x$; $r = 0.96$; $n = 9$. Points marked o; unbroken line; model red wine series: equation of the straight line: $\log y = 0.04348x$; $r = 0.98$; $n = 14$ ($y = E_{420+520}$; x = average of scores given in the ranking test). Points represent averages of three parallel measurements. Standard deviations are shown in Tables 2 and 3

weak correlation can be explained by the fact that the measurement of the monomer anthocyanin content is carried out at pH = 1. At this pH all monomer anthocyanins are in coloured form, while at the pH of wine (3.0–3.8) about 80% of the total anthocyanin is present in colourless form. As an effect of the free sulfurous acid present in wines a part of the anthocyanins is reversibly discoloured (KAMPIS & ÁSVÁNY, 1979). The colour of red wines is highly affected further by the polymerized and co-pigmented anthocyanins (SOMERS & EVANS, 1974; 1977; 1979; KAMPIS, 1980). These factors affecting colour cannot be taken into account in the determination of the monomer anthocyanin content.

In order to be able to use the tristimulus values for the characterization of red wine colour a great number of further measurements, their statistical mathematical evaluation is required.

According to the results obtained the correlation between the T_{520} values and visible colour of red wines is not linear neither can it be traced back to linearity. To characterize the colour of red wine it is expedient to choose an instrumental parameter reflecting linear correlation or one traceable to linearity to colour visible to the naked eye. That is why the T_{520} values are not considered suitable to characterize the colour of red wine safely and in harmony with sensory experiences.

A linear correlation exists between the logarithm of the E_{520} values and the average scores obtained by sensory evaluation. The correlation coefficients of the straight lines fitted by linear regression are, however, lower than 0.7 and therefore the E_{520} values are not suitable either to reliably reflect the colour of red wines as seen by the naked eye.

A linear correlation was found between the logarithm of the $E_{420+520}$ values (Sudraud's colour intensity value; SUDRAUD, 1958) and the average scores obtained by sensory evaluation. The correlation can be considered close because the correlation coefficients of both straight lines fitted by linear regression were above 0.9. On the basis of these results it is suggested to use for characterizing the colour of red wines the relationship

$$N = 23 \times \log (E_{420+520})$$

as obtained for the data of the model red wine series. The parameter (P) = $= 23 \times \log (E_{420+520})$ termed "logarithmic colour intensity" is suitable for routine measurement, can be simply calculated from the colour intensity values according to Sudraud and reflects truly the colour intensity as seen by the naked eye. Identical differences as measured on the logarithmic scale of colour intensity can be considered identical on the sensory scale, too. Theoretically the colour characteristic thus defined may have negative value, however, rose, "Siller" and red wines are placed on the N (log colour intensity) scale in the

0–20 range. Since the scale is still linear at $N = 27$, it can be used for the characterization – free of distortion – of the colour of all red wines.

The parameter can be used also for the evaluation of red wine processing technologies from the aspect of colour. It seems also suitable for the establishment of optimum doses of colouring wines and grape pigment preparations and to esteem and check the increase in visible colour intensity as obtained by these products.

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INVESTIGATIONS OF DIFFERENT EQUATIONS PREDICTING MOISTURE, FAT AND PROTEIN CONTENT OF RAW MEAT BY NIR-TECHNIQUE

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To estimate the moisture, fat and protein content of raw meat equations of varied form and different variables were constructed using the basic spectrum and first and second derivative variables. Accuracy of the equations was checked by measurement of control samples and comparing the standard errors of prediction (SEP), the standard errors according to Demming (DSEP) and the mean differences (BIAS).

It was established that the accuracy of the optimal predicting equations of different form and of different variables, derived from identical calibration sample population, is similar. Equivalent accuracy can be achieved also by equations of identical form and identical number of variables when the variables are measured at different wavelengths. Correlation existing between the components of natural meat samples is reflected also in the similarity of the correlation spectra and leads to the selection of similar 'characteristic' wavelengths for the prediction of different components. If the calibration and control sample groups were formed by dividing one sample population or the control samples were collected at large time intervals, the SEP values were similar, while the BIAS increased manifold. The equations of highest accuracy showed DSEP values of about 0.8 mass % for the moisture content, 0.7 mass % for the fat content and 0.6 mass % for the protein content in unknown meat samples. (Percentage values as given in the text, in tables and figures indicate always mass percentages.)

Keywords: NIR-technique, meat composition

The available literature on non-destructive methods for measuring meat composition by near infrared spectrophotometry has been reviewed in an earlier paper (NÁDAI, 1983). Recently ARNETH (1982), MARTENS and co-workers (1980, 1981) and KRUGGEL and co-workers (1981) applied for similar purposes homogeneous linear predicting equations using instruments with interference filters of fix wavelengths. LANZA (1983) described analyses by a scanning instrument using predicting equations of second derivative ratios.

The aim of the present study was to construct and determine the accuracy of predicting equations of different form and of different number of variables for the control of the moisture, fat and protein content in raw meat.

1. Materials and methods

1.1. Meat samples

Meat samples were randomly selected at the slaughterhouse from meats originating from different parts of the animal body and of different quality. The samples were then ground and homogenized on a Moulinette chopper-blender. The composition and the diffuse reflexion spectra of the samples, were determined.

Two hundred samples of beef and 31 samples of pork were analyzed in about 3 months' time. One year later 63 samples of beef and 20 samples of pork were analyzed.

1.2. Laboratory analysis of composition

The moisture, fat and protein contents of the meat samples were determined by standard reference methods.

To determine the moisture content HUNGARIAN STANDARD (1980) method was applied. The sample was dried in an oven of 105 °C to constant mass. The standard deviation of the method was found to be about 0.25 mass %.

Fat content in samples of first grade (with a fat content below 7%) was determined by Soxhlet's method. With samples of higher fat content Gerber butirometer was used in accord with HUNGARIAN STANDARD (1979). The standard deviations were found to be 0.16 and 0.26 mass %, respectively.

Protein was determined by Kjeldahl's method as described in HUNGARIAN STANDARD (1978). The standard deviation was found to be about 0.35 mass %.

All three components of each sample were investigated in two parallel tests and the average values of the results were used in data processing. When the difference between parallel moisture or fat content determinations exceeded 0.5% the determinations were repeated. For the protein content determinations were repeated above a difference of 1%.

1.3. Spectrum measurements

The diffuse reflexion spectra of the meat samples were measured with Neotec 6450 type Research Composition Analyzer (RCA) according to the method as described in an earlier paper (NÁDAI, 1983). The instrument stored the $V(\lambda)$ basic spectra on floppy disk in the wavelength range of 1100–2500 nm in steps of 2 nm. The average of three parallel spectrum measurements was used for data processing.

1.4. Data processing

Different types of predicting equations were constructed from the data of the RCA and from the results of the reference methods by multiple regression technique. The C composition characters of the raw meat are given by these equations as the multivariable F functions, taken at different wavelengths (λ_1, $\lambda_2 \ldots \lambda_n$), of the 'basic spectrum' $V(\lambda)$ or its G transformation [e.g. first derivative $V'(\lambda)$ or second derivative $V''(\lambda)$ by wavelength]. The general form is as follows:

$$C = F\{G[V(\lambda_1, \lambda_2 \ldots \lambda_n)]\}$$

where n is the number of optical data in the predicting equation.

The first derivative spectrum is replaced by the computer program as follows:

$$G_1 \equiv V'(\lambda) \approx V(\lambda + \Delta\lambda) - V(\lambda)$$

The second derivative spectrum is approximated by the following function:

$$G_2 \equiv V''(\lambda) \approx V(\lambda - \Delta\lambda) + V(\lambda + \Delta\lambda) - 2\,V(\lambda)$$

In the course of the 'calibration procedure' the n number of optical data was increased step by step. In this way predicting equations of different forms can be constructed. A chosen new $G[V(\lambda_k)]$ optical date can be used as e.g.:

– a new term variable of a homogeneous linear equation, or

– a numerator or denominator of a term in an equation containing a sum of optical data fractions, or

– members in terms of optical data differences, etc. (We should differenciate between the n number of optical data and p number of term variables in the predicting equations which is equal to the number of the regression coefficients.)

Optimizing the predicting equations the wavelengths can be modified cyclically as long as the r multiple correlation coefficient increases. Increasing the p number of terms as well as optimizing the wavelengths, the SEC (standard error of calibration) decreases and tends towards a minimal limit value as it was reviewed e.g. by NÁDAI and KÖRMENDY (1982). They suggested the calculation by Demming regression of the prediction error (DSEP) to characterize the accuracy of the predicting equation. This, accounting for the incertainty of the reference methods of composition analysis, gives a realistic value for the error derived from the NIR-technique. The optimal number of variables of the predicting equation is indicated by the minimum of the DSEP value. Further factors of accuracy: mean of differences (BIAS) between reference and predicted data and the standard error of prediction (SEP) as it is used in related literature, were also calculated and are given in this paper.

Table 1

Wavelength and accuracy data obtained for

Calibration												
No.	Type	p	λ_1 (nm)	λ_2 (nm)	λ_3 (nm)	λ_4 (nm)	λ_5 (nm)	λ_6 (nm)	SEC (%)	*B*1 SEP (%)	*B*1 DSEP (%)	*B*1 BIAS (%)
1	1	1	2070						1.59			
2	1	2	2070	2306					1.23			
3	1	3	2070	2306	2336				1.04			
4	1	4	2070	2306	2336	1634			0.89			
5	1	5	2070	2306	2336	1634	1696		0.75			
6	1	6	2070	2306	2336	1634	1696	1852	0.73			
7	1	1	2066						1.68	1.52	1.45	−0.21
8	1	2	2066	2306					1.25	1.27	1.18	−0.12
9	1	3	2066	2306	2328				1.02	1.09	1.03	−0.05
10	1	4	2066	2306	2328	1640			0.94	1.01	0.96	0.04
11	1	5	2066	2306	2328	1640	1726		0.75	0.87	0.84	0.07
12	1	6	2066	2306	2328	1640	1726	1816	0.75	0.85	0.81	0.09
13	1	4	1776	1742	1930	1366			0.70			
14	1	4	2324	2310	1720	1846			0.77			
15	2	1	1772						1.06			
16	2	2	1772	1354					0.80			
17	2	3	1772	1354	2352				0.73			
18	2	4	1772	1354	2352	2006			0.70			
19	2	5	1772	1354	2352	2006	1736		0.68			
20	3	1	1716	1756					0,96			
21	3	2	1716	1756	1384	1270			0.78			
22	3	3	1716	1756	1384	1270	2002	1998	0.74			
23	4	2	1738	1390	1902	2154			0.79			
24	5	2	1744	1280	1690				0.81			

2. Results and conclusions

Two hundred samples of beef were randomly divided into two groups of 100 each (*B*1 and *B*2) and for both groups predicting equations, as homogeneous linear functions of the basic spectrum, were constructed for the moisture content:

$$C = K_0 + \sum_{i=1}^{p} K_i \, V(\lambda_i) \tag{1}$$

where p is the number of the terms in the predicting equations.

First, without iterative optimization of the wavelengths, the number of variables was increased up to 6. As it can be seen in rows 1 to 12 of Table 1, the SEC value characterizing the fitting of the predicting equations to calibration data, decreases with increasing number of variables, similarly for both calibration groups towards limit values of 0.73 and 0.75 mass %, respectively. The wavelengths selected at the maxima of the correlation spectra were found to be nearly identical with only a slight difference of a few nanometers.

moisture predicting equations

Control											
B2			*B3*			*P1*			*P2*		
SEP (%)	DSEP (%)	BIAS (%)	SEP (%)	DSEP (%)	BIAS (%)	SEP (%)	DSEP (%)	BIAS (%)	SEP (%)	DSEP (%)	BIAS (%)
1.69	1.69	0.15	2.41	2.23	2.61	2.10	1.50	0.42	3.81	2.57	0.47
1.24	1.25	−0.14	1.94	1.91	−1.61	1.79	1.35	−0.09	3.20	2.55	−2.72
1.03	1.08	0.08	1.71	1.44	0.84	1.67	1.36	−0.10	3.16	1.93	−0.96
0.93	0.99	0.03	1.35	1.14	−0.07	1.34	1.09	−0.03	2.74	1.70	−1.51
0.87	0.84	−0.04	1.02	0.96	0.46	1.13	0.95	0.28	2.23	1.91	0.14
0.84	0.81	−0.05	0.98	0.91	0.74	1.16	0.99	0.21	2.04	1.73	0.32
			2.41	2.22	2.55	2.11	1.49	0.32	3.84	2.57	0.39
			1.90	1.88	−1.67	1.87	1.36	−0.19	3.25	2.54	−2.81
			1.67	1.37	0.14	1.61	1.28	−0.09	3.05	1.79	−1.50
			1.44	1.28	−0.86	1.40	1.07	0.00	2.81	1.76	−2.13
			1.00	0.89	0.82	1.25	0.95	0.03	2.16	1.63	−0.06
			1.02	0.89	0.85	1.28	0.93	−0.02	2.06	1.52	−0.15
0.81	0.78	−0.03	1.14	1.09	0.76	1.22	0.88	−0.54	1.87	1.65	−0.55
0.80	0.80	−0.05	1.26	1.08	1.64	1.46	1.17	−0.15	1.79	1.53	0.36
1.11	1.09	−0.12	1.12	1.09	0.92	1.36	1.15	−0.41	1.53	1.43	0.38
0.85	0.82	−0.09	0.96	0.94	0.28	1.44	1.16	−0.52	1.59	1.53	−1.04
0.83	0.79	−0.02	1.06	0.99	1.77	1.46	1.15	−0.56	1.72	1.59	−0.06
0.90	0.87	−0.01	1.21	1.16	1.65	1.58	1.22	−0.93	1.82	1.64	−0.42
0.93	0.89	−0.01	1.22	1.16	1.13	1.62	1.24	−0.98	1.92	1.68	−0.64
1.03	0.99	−0.12	1.06	1.04	0.90	1.61	1.52	0.20	1.93	1.70	0.89
0.91	0.87	−0.09	1.18	1.12	0.34	1.60	1.41	−0.08	2.01	1.79	−0.43
0.98	0.93	−0.09	1.37	1.14	−0.20	1.78	1.53	−0.39	2.26	1.84	−0.93
0.78	0.75	0.01	0.88	0.85	0.26	0.96	0.92	0.07	1.73	1.66	0.30
0.84	0.32	−0.05	0.90	0.87	0.66	0.99	0.90	0.24	1.86	1.78	0.49

To check the equations thus obtained they were applied to control sample groups. First the equations obtained from sample group *B*1 were checked with sample group *B*2 and vice versa. Then they were applied to sample group *P*1 (pork), measured at the same time as the samples above, then to new beef samples *B*3 and pork sample group *P*2, investigated a year later. Columns marked 'control' in Table 1 contain the results.

The next step undertaken was the cyclic optimization of the wavelength with data of sample group *B*1 with a four-variable equation similar to (1). Optimization was carried out in two different orders. Results are contained in rows 13 and 14 of Table 1. By comparing the four-variable linear predicting equations hitherto constructed (Table 1, rows 4, 10, 13 and 14) it can be seen that depending on the course of optimization they stabilized at wavelengths differing from one another and from the wavelengths of calculation without optimization. The DSEP values of the optimized equations cannot be considered better than those of non-optimized equations.

For sample group *B*1 similar homogeneous linear predicting equations were constructed as multi-variable functions of the first derivative of the basic

Table 2

Wavelength and accuracy data obtained for fat

Calibration									
No.	Type	p	λ_1 (nm)	λ_2 (nm)	λ_3 (nm)	λ_4 (nm)	λ_5 (nm)	λ_6 (nm)	SEC (%)
1	1	1	2068						2.29
2	1	2	2068	2304					1.55
3	1	3	2068	2304	2330				1.27
4	1	4	2068	2304	2330	1300			1.15
5	1	5	2068	2304	2330	1300	1718		0.89
6	1	6	2068	2304	2330	1300	1718	2156	0.82
7	1	1	2076						1.08
8	1	2	2076	2310					1.00
9	1	3	2076	2310	1898				0.98
10	1	4	2076	2310	1898	1730			0.95
11	1	5	2076	2310	1898	1730	1176		0.96
12	1	6	2076	2310	1898	1730	1176	1186	0.95
13	3	1	1838	1758					0.98
14	3	2	1838	1758	1550	1300			0.89
15	3	3	1838	1758	1550	1300	2270	2120	0.86
16	4	2	1698	1380	2206	2366			0.66
17	5	2	1750	1320	2190	1884			0.68
18	2	1	1990						0.95
19	2	2	1990	2212					0.92
20	2	3	1990	2212	1486				0.90
21	3	1	2038	1926					0.94
22	3	2	2038	1926	1800	1570			0.90
23	3	3	2038	1926	1800	1570	1700	1600	0.89
24	4	2	1710	1314	2366	1750			0.86
25	4	2	1486	1850	2168	2204			0.83
26	5	2	2078	1916	1770				0.89

spectrum at different wavelengths:

$$C = K_0 + \sum_{i=1}^{p} K_i \, V'(\lambda_i) \tag{2}$$

The number of variables was increased up to 5. Parameters of the equations are contained in rows 15 to 19 of Table 1.

It can be seen that, as it was expected, calibrations carried out with beef can be applied to the measurement of unknown pork samples with less accuracy than to beef samples. With control samples measured at a substantially later time the prediction error was slightly greater than for control samples taken from the same sample population as the calibration samples, while the BIAS increased manifold. The prediction error data with identical number of variables of very different wavelengths are practically equivalent (i.e. many equivalent variable combinations can be used with the same measuring instrument, thus, significantly optimal 'characteristic wavelengths' cannot be selected by this method). When the number of variables is low the equation with variables of first derivative is more accurate than the equation constructed

and protein predicting equations

Control											
	B2			*B3*			*P1*			*P2*	
SEP (%)	DSEP (%)	BIAS (%)	SEP (%)	DSEP (%)	BIAS (%)	SEP (%)	DSEP (%)	BIAS (%)	SEP (%)	DSEP (%)	BIAS (%)
2.66	2.55	−0.24	2.93	2.67	2.86	2.97	1.75	0.24	5.32	3.35	0.59
1.68	1.66	−0.09	2.36	2.25	4.47	2.39	1.56	1.28	4.29	3.49	6.15
1.51	1.47	−0.01	1.95	1.55	0.75	1.95	1.33	1.33	4.02	2.27	3.40
1.45	1.37	0.12	1.63	1.32	2.08	1.64	0.99	1.24	3.64	2.04	4.34
1.07	1.00	0.04	1.48	1.19	0.13	1.64	1.15	1.13	3.01	2.04	1.79
0.97	0.89	0.06	1.66	1.15	0.76	1.64	0.98	0.91	2.85	1.95	1.08
1.18	0.90	0.06	0.93	0.68	0.63	1.64	0.61	−0.52	1.61	0.53	−0.08
1.03	0.83	0.05	0.79	0.72	2.37	1.59	0.69	−0.72	1.34	0.64	−1.41
1.03	0.82	0.03	0.77	0.69	2.41	1.51	0.68	−0.58	1.21	0.65	−1.40
1.02	0.81	0.04	0.75	0.61	1.78	1.47	0.66	−0.54	1.29	0.66	−0.89
0.98	0.77	0.06	0.83	0.60	0.96	1.45	0.62	−0.23	1.42	0.68	−0.07
0.98	0.77	0.06	0.98	0.63	0.63	1.53	0.65	−0.28	1.63	0.82	0.03
1.19	1.16	0.15	1.91	1.63	0.75	2.26	1.70	1.84	2.25	2.17	0.77
1.16	1.11	0.12	1.55	1.34	0.65	2.32	1.60	1.58	2.53	2.09	2.29
1.13	1.01	0.12	1.36	1.28	0.89	2.33	1.51	1.63	2.27	2.06	1.06
0.90	0.87	−0.04	1.07	0.99	0.31	1.2	0.89	0.57	4.42	4.29	0.02
0.75	0.73	−0.03	1.04	0.92	0.22	1.5	0.74	1.04	2.06	1.98	0.88
1.17	0.92	0.12	0.97	0.70	0.72	1.6	0.72	−0.37	1.49	0.50	−0.25
1.05	0.84	0.15	0.85	0.77	1.44	1.5	0.64	−0.38	1.34	0.57	−0.65
1.05	0.81	0.10	0.86	0.80	1.60	1.5	0.55	−0.24	1.23	0.57	−0.36
1.17	0.91	0.12	0.96	0.69	0.66	1.58	0.70	−0.34	1.51	0.50	−0.21
1.06	0.82	0.09	0.76	0.62	−0.89	1.49	0.64	−0.41	1.20	0.54	−0.10
1.07	0.83	0.11	0.81	0.69	0.97	1.64	0.75	−0.41	1.39	0.52	−0.40
1.06	0.89	0.17	0.76	0.53	1.15	1.58	0.68	−0.60	1.38	0.57	−0.44
1.03	0.94	0.15	1.33	0.64	0.19	2.03	1.35	−0.12	1.73	0.59	0.79
1.05	0.83	0.13	0.84	0.68	1.33	1.82	0.61	−0.61	1.42	0.62	−0.83

from the variables of the basic spectrum, however, with derivative variables the accuracy changes only slightly as a function of the number of variables. It appears, that the optimum (minimum) value of the prediction error, in the case of homogeneous linear predicting equations, is practically independent of the starting point being the basic spectrum or first derivative spectrum.

In order to investigate whether some transformation or another equation form could bring about a significant improvement of accuracy further predicting equations with other variables and of different form were constructed and tested. A predicting equation constructed as linear function of differences between basic spectrum values as measured at optimized wavelengths:

$$C = K_0 + \sum_{i=1}^{p} K_i \left[V(\lambda_{1i}) - V(\lambda_{2i}) \right] \tag{3}$$

With this type of equation it was observed that while the calibration error was reduced, the prediction error practically did not change or slightly increased for both beef and pork as a function of the number of variables. (See

rows 20 to 22 in Table 1.) No explanation was found for this observation. The minimum prediction error of this type of equation practically corresponds, too, to that of former equations. The linear functions of the quotients of the first and second derivatives, were also tested:

$$C = K_0 + \sum_{i=1}^{p} K_i \frac{V'(\lambda_{1i})}{V'(\lambda_{2i})} \tag{4}$$

$$C = K_0 + \sum_{i=1}^{p} K_i \frac{V''(\lambda_{1i})}{V''(\lambda_{2i})} \tag{5}$$

The wavelength values of both equations were optimized by cyclic iteration. The optima of the $\Delta\lambda$ values of derivative transformations were also established and were found for the first derivative 40 nm and for the second derivative 65 nm. With the equation of the second derivative we stopped at three wavelengths because the introduction of a fourth wavelength did not improve the SEC value.

The characteristics of these equations are contained in rows 23 and 24 of Table 1. As it can be seen from the data, the fractional function with the first derivative, represented by row 23, appears to be the 'best' one. However, to test the stability in time of the accuracy characters and the order according to quality among the predicting equations, the analysis of further sample groups seems expedient.

Hereafter, predicting equations to establish the fat content were sought for and the number of variables was varied in type (1) equations from 1 up to 6. Data are shown in rows 1 to 6 of Table 2. It was found striking that, at least for the first few variables, the best correlating 'characteristic' wavelengths were found to come close to those obtained for moisture content. Then similar calibration was carried out for the protein content and again the first few wavelengths were found to be near those of the predicting equations for moisture content (rows 7 to 12 in Table 2).

Not only the 'characteristic wavelengths' but the correlation spectra, too, were found very similar for all three components. In Fig. 1 the correlation spectra for selecting the first wavelength, are shown. Their values are different while their course is similar (because of the difference in the relative accuracy of the reference methods used to determine the 3 components related to their range of change). In the similarity of the correlation spectra the correlation existing between the components of natural meats is reflected. To illustrate this the composition of the calibration sample population is shown in a triangle diagram in Fig. 2.

In the diagram the composition point of the three-component sample is determined by two composition data, presuming that the sum of the three components is 100%. Since all the three components of the meat samples

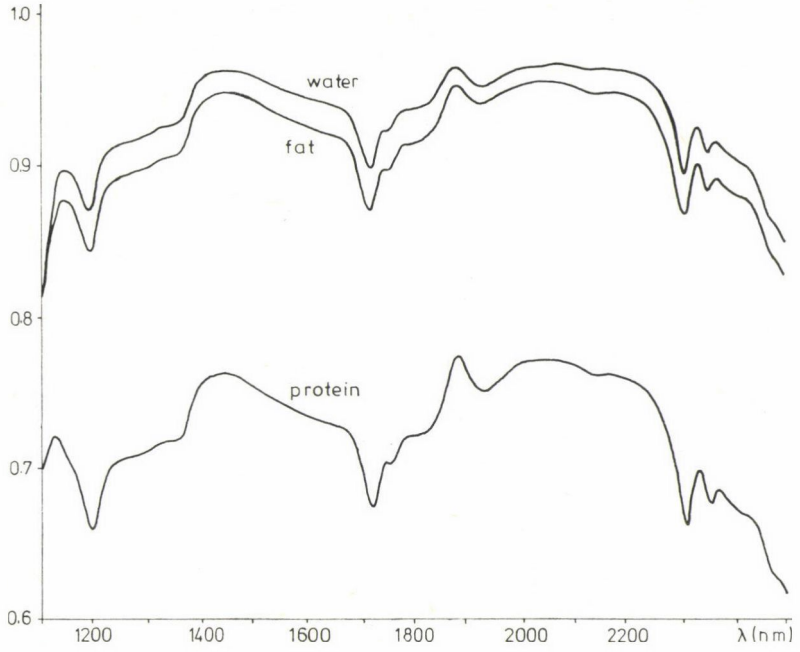

Fig. 1. Correlation spectra related to the first wavelength of type (1) predicting equations for the three components of beef

were measured, by plotting 3 data of each sample by pairs, three points were obtained in the diagram for each sample. The length of the sides of the triangles determined by the three points is proportional to the difference of the sum of the three data from 100%. The position of the triangle depends on the sign of difference. If the peak of the triangle stands upwards the sum is higher than 100%, if it is directed downwards the sum is below 100%. It can also be seen in the diagram that the higher the moisture content of the sample the greater the sum of data as obtained by reference methods for the three components. An explanation for this can be that the loss in dry matter content occurring during drying, erroneously accounted for by gravimetry as moisture content, is proportional to the moisture content. It seems to be advisable, after the thorough study of this phenomenon, to modify the basic method by correcting the value of moisture content.

As it can be seen in Fig. 2, the composition points of the calibration samples are grouped more or less along a line the two ends of which represent the composition of pure muscle tissue and pure adipose tissue, respectively. In nature every raw meat sample is a mixture of different proportions of these two components. The predicting equations indicate the place of an unknown sample along this line. This indication occurs not necessarily on the basis of parameters (e.g. wavelengths) selectively characterizing any pure component

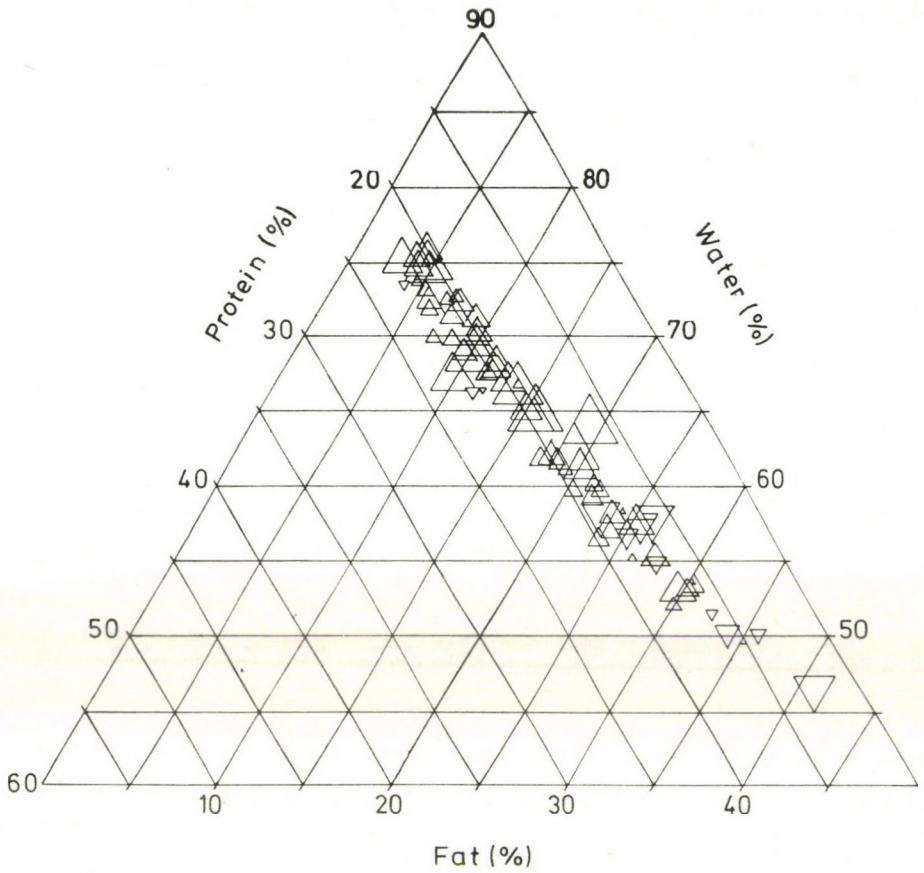

Fig. 2. Composition of $B1$ beef sample group

thus, it cannot be expected that the predicting equations determined for natural meats shall be laws of nature reflecting causal correlation with the moisture, fat and protein content. From this it can be concluded that the predicting equations are not necessarily valid for meat samples outside the adipose tissue–muscle tissue line, e.g. by adding pure water or protein additives, etc. Clarification of the problem is being studied.

It should be noted, however, that every physical parameter measurable with sufficient accuracy is suitable for the determination of composition in natural meat samples for which, their 'equipotential' lines in the triangle diagram, intersect the composition line of natural meats at an approximately perpendicular angle. Measuring instruments based on densitometry, X-ray absorption, visible light reflectivity and other principles, work in this way.

Predicting equations of other types were constructed also for fat and protein contents. Related data are listed in Table 2, for fat rows 13 to 17,

for protein rows 18 to 26 represent such equations. No explanation was found for the SEP values of sample group *B*3 being lower than the SEC values for protein. To investigate this and to carry out further study of the predicting equations for practical use measurements of other control sample groups are intended.

*

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STUDY INTO DETERMINING DIETARY FIBER OF WHEAT BRAN BY NIR-TECHNIQUE

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A preliminary study reported that we explored the opportunity for determining dietary fiber as well as its components by a quick, non-destructive, near-infrared, spectrophotometric method in wheat bran. Fifty-seven wheat bran samples were prepared with known composition. An enzymatic method was used to determine dietary fiber and its components. Starch and protein content were also determined because the wheat bran contains a large quantity of these two constituents.

The chemical analysis was carried out in Hungary at the Faculty of Advanced Paramedical Training at the Institute for Postgraduate Medical Education, Department of Dietetics, Budapest. For the spectral analysis, the computerized spectrophotometer, built in the Instrumentation Research Laboratory, BARC, USDA, USA, was used. Near-infrared reflectance factor (R) spectra were recorded and transformed to log (1/R). The second derivatives of log (1/R) spectra were correlated with compositional data. A linear regression technique was used to determine the optimum wavelengths and other parameters for predicting each constituent. The correlation coefficients were above 0.95 for each dietary fiber component, with the highest correlation coefficient for cellulose, $r = 0.989$. The standard error of calibration for dietary fiber was 0.26 mass percent; for water-insoluble dietary fiber 1.12 mass percent; and for water-soluble dietary fiber 0.60 mass percent. For the other dietary fiber components, the standard error of calibration was lowest for the water-insoluble pectin 0.027 mass percent and highest for the water-insoluble hemicellulose 1.00 mass percent.

In the calculation of content of each component, the influence of the other components was also tested by determining the correlation coefficients between the constituents before and after NIR analysis. The correlation coefficients between the constituents determined by chemical analysis seem to be the limit for correlation coefficients of predicted contents.

The sums of dietary fiber components were determined directly using a ratio of second derivatives of log (1/R) spectra and indirectly by addition of components where each component was determined in a direct way. Determining the dietary fiber content by addition of components is preferred.

Because in these samples some constituents occur in a natural form and as added pure components, the wavelength for best calibration must be suitable for both types of constituents.

Keywords: Dietary fiber, NIR-technique, analysis of wheat bran

According to the epidemiological appraisals nutrition deficiency in dietary fiber acts as a risk-factor in the development of a number of diseases peculiar to civilized communities. The most frequent diseases correlated to

the insufficiency of dietary fiber consumption are constipation, diverticulosis, colon cancer, and irritable colon syndrome. Connection has been indicated between nutritional deficiency in dietary fiber and hyperlipoproteinaemia, diabetes mellitus, atherosclerosis, and the development of ischaemic heart diseases as well. The INSTITUTE OF FOOD TECHNOLOGISTS (1979) divide the physiological effect of dietary fiber into three categories:

- Definite value of dietary fiber is its beneficial effect on constipation due to the increase of the water content of the feces.
- Probable value of dietary fiber is the preventing and treating effect on diverticulosis.
- Possible value of dietary fiber is the serum-cholesterol reducing effect and the role in the prevention of some diseases; i.e., ischaemic heart disease, colon cancer, diabetes mellitus, hemorrhoids, obesity, etc.

The significant physiological effect of dietary fiber rests upon the physico-chemical properties of the components. VISEK (1978) discussed the effect of cellulose in reducing ammonia. Decrease of the serum-carbamide level after carboxymethyl-cellulose consumption was observed by RIGÓ and co-workers (1977) in their clinical experiments. CUMMINGS and co-workers (1978) found close correlation between the pentose content of hemicelluloses and the fecal weight and transit time. KAY and TRUSWELL (1977) found decrease in the serum-cholesterol level after the consumption of pectin. The protective effect of lignin against bacterial digestion was also confirmed by HARTLEY (1978).

To determine the dietary fiber in the food and its components separately is highly desirable with rapid and accurate methods because of the importance of each component. The favorable nutritional effects of the components of dietary fiber can be summarized as follows:

Physico-chemical properties of cellulose: water-holding capacity, saturating effect, reduction of intraluminal pressure in the colon, reduction of ammonia in the colon, reduction of serum-carbamide, reduction of the activity of the digestive enzymes, and hindering of absorption.

Physico-chemical properties of hemicelluloses: water-holding capacity, increasing fecal weight, decreasing transit time, reduction of intraluminal pressure, cation-exchange capacity, hindering of absorption, and increasing the motility of the bowels.

Physico-chemical properties of pectins: water-holding capacity, gel formation effect, binding of bile acids, reduction of serum-cholesterol, binding of toxic materials, hindering carbohydrate absorption, and increasing steroids in feces.

Physico-chemical properties of lignin: binding steroids, antioxidant effect, and protective effect against bacterial decomposition of cellulose.

The analysis of dietary fiber is a problematic question even now. The reason for this is the idea of dietary fiber itself as it was defined by TROWELL

(1972) and TROWELL and co-workers (1976) resp., as the sum of the polysaccharides and lignin which cannot be decomposed by the human digestive enzymes. Dietary fiber consists of various different compounds which change and create different ratios in the different materials. They are mainly water-insoluble, with some which can be dissolved in water; e.g., the arabinoxylans in cereals; some acidic polysaccharides, mucilages and gums; some reserve polysaccharides such as guaran; water-soluble pectic substances, etc.

The methods used for the analysis of dietary fiber are based upon very different ideas. Direct carbohydrate determination is very exact but rather complicated, time consuming, and needs a large amount of samples (SOUTHGATE 1969; 1981; ENGLYST 1981). The ADF (Acid Detergent Fiber) and NDF (Neutral Detergent Fiber) method of VAN SOEST (1967) is more rapid, but the disadvantage of it is the lack of the determination of the water-soluble components, and if these are to be determined, the usefulness of this method would be lost (THEANDER, 1981). The method of HELLENDOORN and co-workers (1975), based on the enzymatic decomposition of starch and protein, completed with the determination of the water-soluble components of dietary fiber, seems to be suitable for a routine analysis. Such methods are described by SCHWEIZER and WÜRSCH (1979), ASP and JOHANSSON (1981) and FURDA (1981). Dietary fiber analysis by NIR-technique is described by NORRIS and co-workers (1976) and BAKER and co-workers (1979). In the latter studies, the ADF and NDF method of VAN SOEST (1967) was used as a reference method.

A rather simple method for determining the components of the water-insoluble dietary fiber is published by ELCHAZLY and THOMAS (1976). The separation of the components is carried out on the basis of their resistance to the 5% by volume H_2SO_4 and 72% by weight H_2SO_4, respectively. In this test, the fraction which can be decomposed by the 5% H_2SO_4, is called "crude hemicellulose", that which is decomposed only by the 72% H_2SO_4 is called "crude cellulose", and the remnant is called "crude lignin and minerals". Lignin can be removed from the latter by ashing at 800 °C. The disadvantage of this method is the lack of the determination of the pectic substances.

In this investigation of dietary fiber of wheat bran by NIR-technique, the enzymatic method was modified to include the determination of the water-soluble dietary fiber. The components of the water-insoluble dietary fiber, together with the pectins, were also determined, and the total dietary fiber was calculated by adding up the water-soluble and water-insoluble components.

The water-insoluble dietary fiber contains a rather large amount of undigested protein according to the investigations of SAUNDERS (1980). To confirm this, the protein content of both the samples and their water-insoluble dietary fiber, which remained after the enzymatic decomposition, were determined as well.

Because of the high starch content of cereals, we determined the starch content of our samples.

Here we report a new NIR spectrophotometric technique which can be used for accurately and rapidly determining dietary fiber and its components non-destructively in wheat bran.

1. Materials and methods

1.1. Preparation and chemical analysis of the wheat bran samples

Fifty-seven bran samples of different composition, made up in an artificial way, were used for the calibration. Wheat bran purchased from trade was chosen as a basic material, and its composition was altered in five directions by mixing with different materials in predetermined ratios. These materials were:

- whole wheat flour (from trade),
- special bran with high dietary fiber content (prepared from the basic bran by washing out of starch and some other water-soluble components with lukewarm water and drying at 50 °C),
- water-insoluble dietary fiber (prepared from the basic bran),

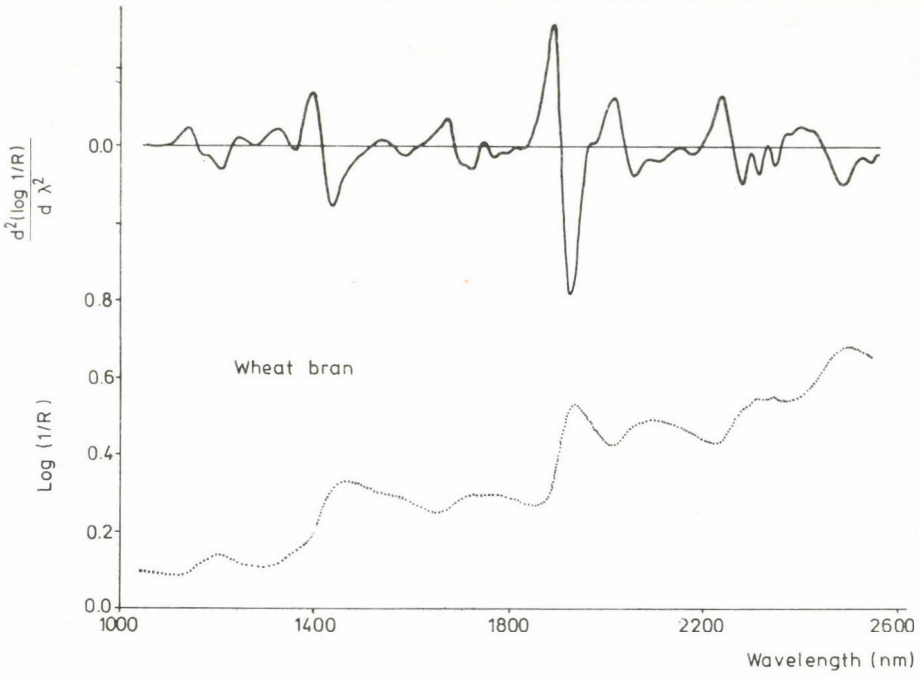

Fig. 1. The log (1/R) spectrum and the second derivative curve of wheat bran

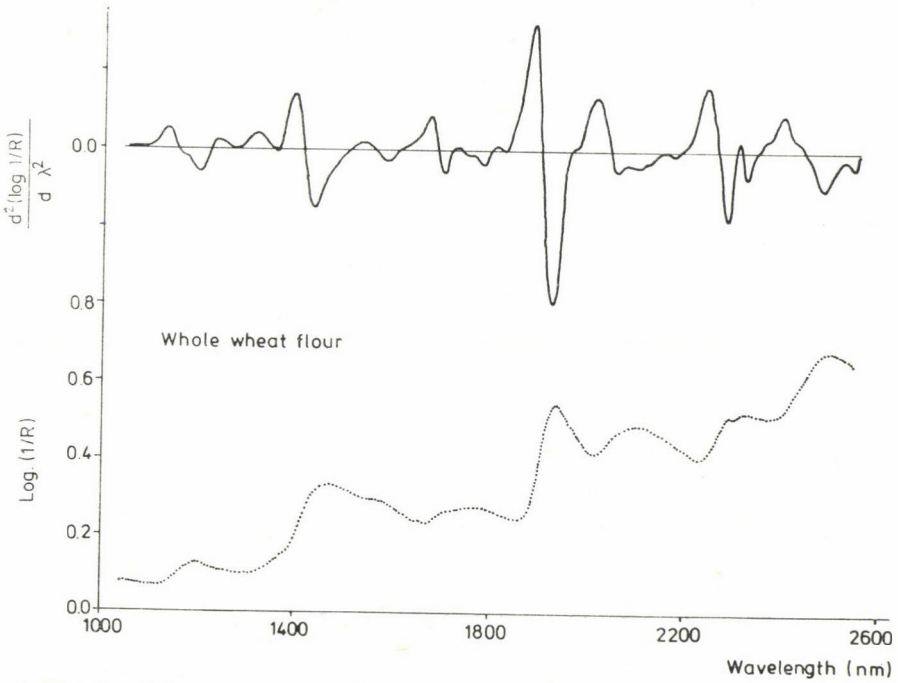

Fig. 2. The log (1/R) spectrum and the second derivative curve of whole wheat flour

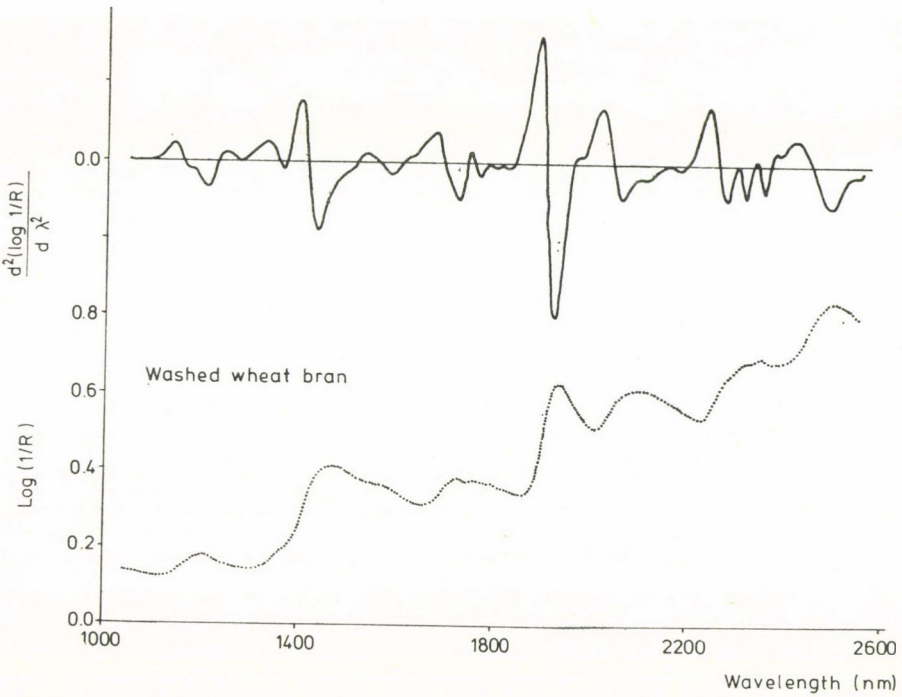

Fig. 3. The log (1/R) spectrum and the second derivative curve of washed wheat bran

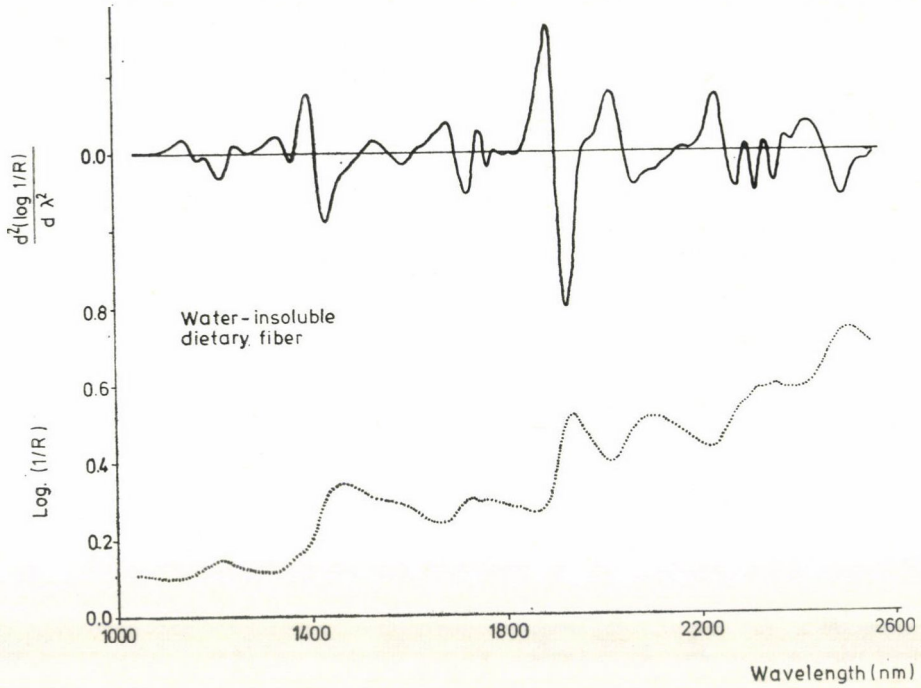

Fig. 4. The log (1/R) spectrum and the second derivative curve of water insoluble dietary fiber

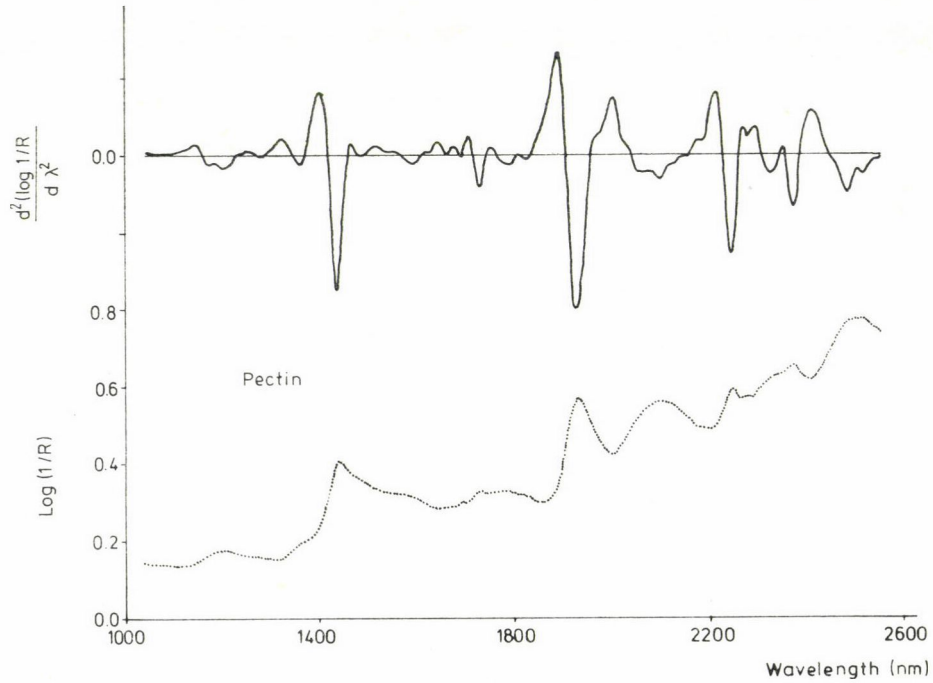

Fig. 5. The log (1/R) spectrum and the second derivative curve of pectin

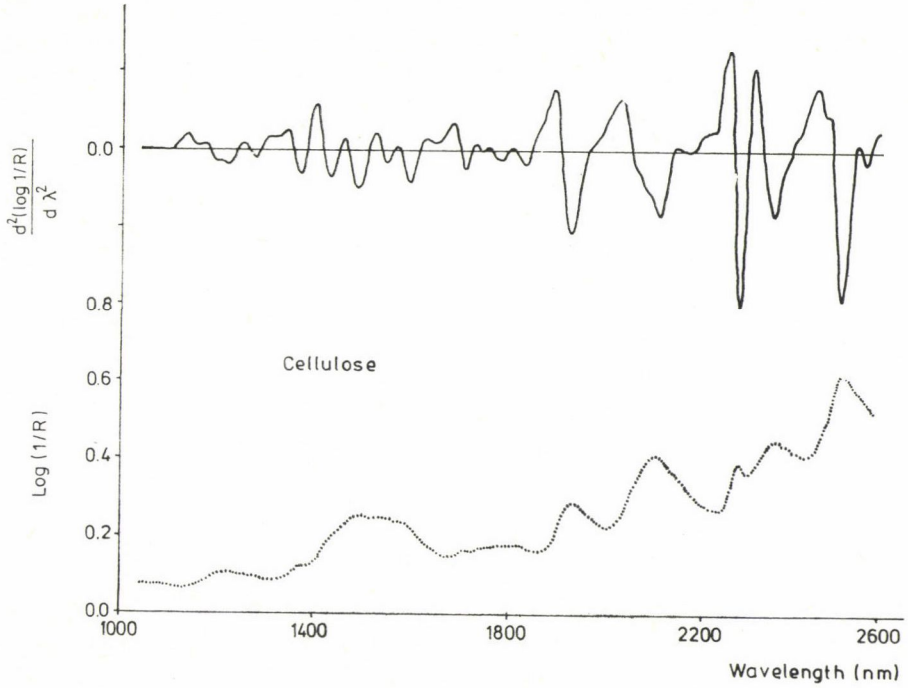

Fig. 6. The log (1/R) spectrum and the second derivative curve of cellulose

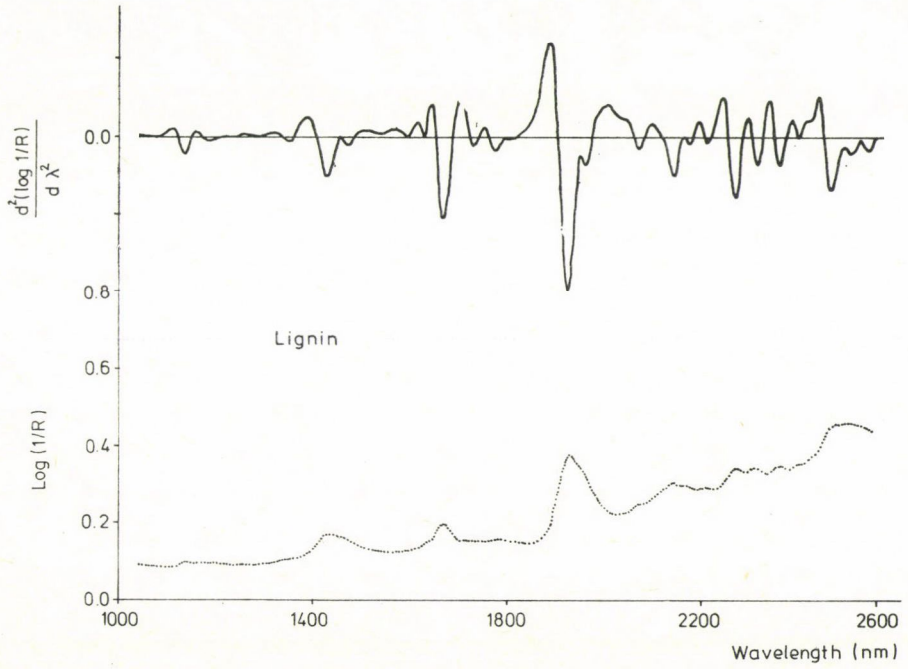

Fig. 7. The log (1/R) spectrum and the second derivative curve of lignin

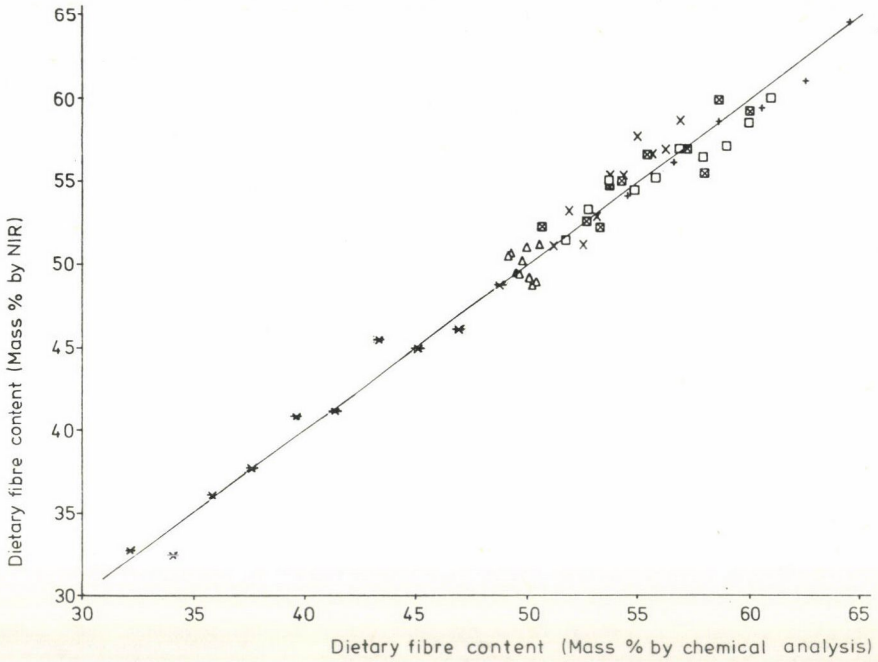

Fig. 8. Relationship between water-insoluble dietary fiber content determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths. *: Samples with whole wheat flour; □: samples with washed-wheat bran; +: samples with water-insoluble dietary fiber; △: samples with pectin; ×: samples with cellulose; ⊠: samples with lignin

- pectin (water-soluble citrus pectin),
- cellulose powder,
- lignin powder.

Altogether 57 samples were made in six groups in the following way:

1 – bran–whole wheat flour mixtures: from 0 mass % to 50 mass %, increasing amount of whole-wheat flour was added to the decreasing amount of bran to make it up to 100 mass %, in equipartition in ten steps;

2 – bran–special bran mixtures: from 0 mass % to 50 mass %, increasing amount of special bran was added to the decreasing amount of bran to make it up to 100 mass %, in equipartition in ten steps;

3 – bran–water insoluble dietary fiber preparation mixtures: water-insoluble dietary fiber content of bran was increased by adding water-insoluble dietary fiber preparation, from the original 50.7 mass % to 64.5 mass %, in equipartition in seven steps;

4 – bran–pectin mixtures: pectin content of bran was increased from the original 3.0 mass % to 5.9 mass % by adding pectin powder, in equipartition in ten steps;

5 – bran–cellulose mixtures: cellulose content of bran was increased from the original 10.7 mass % to 21.9 mass % by adding cellulose powder, in equipartition in ten steps;

6 – bran–lignin mixtures: lignin content of bran was increased from the original 6.2 mass % to 14.8 mass % by adding lignin powder, in ten steps.

All the components were chemically analyzed, and the composition of the samples was calculated knowing the analytical data and mixing ratios.

Dietary fiber is given as the sum of water-insoluble and water-soluble dietary fiber. Water-insoluble dietary fiber was determined by a modified enzymatic method, water-soluble dietary fiber was precipitated by ethanol and weighed (SCHWEIZER & WÜRSCH, 1979; HORVÁTH-MOSONYI, 1983).

The components of the water-insoluble dietary fiber were determined according to the method of ELCHAZLY and THOMAS (1976), with slight modifications, as it is described by HORVÁTH-MOSONYI and co-workers (1983). Minerals in the water-insoluble dietary fiber were not determined, because

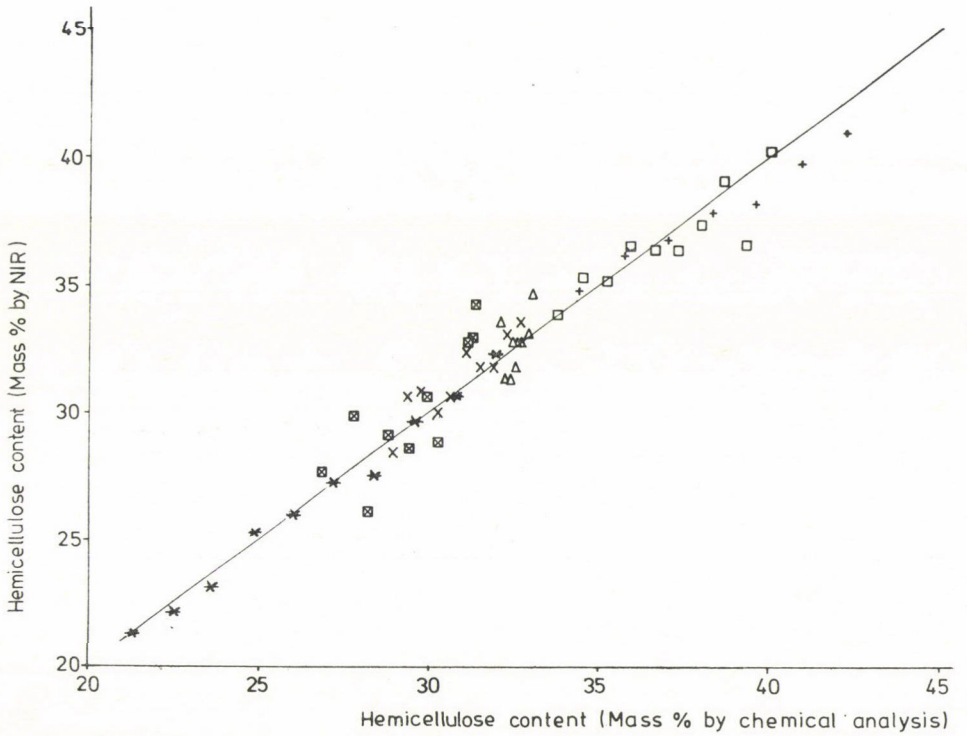

Fig. 9. Relationship between hemicellulose content in water-insoluble dietary fiber determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths. *: Samples with whole wheat flour; □: samples with washed wheat bran; +: samples with water-insoluble dietary fiber; △: samples with pectin; ×: samples with cellulose; ⊠: samples with lignin

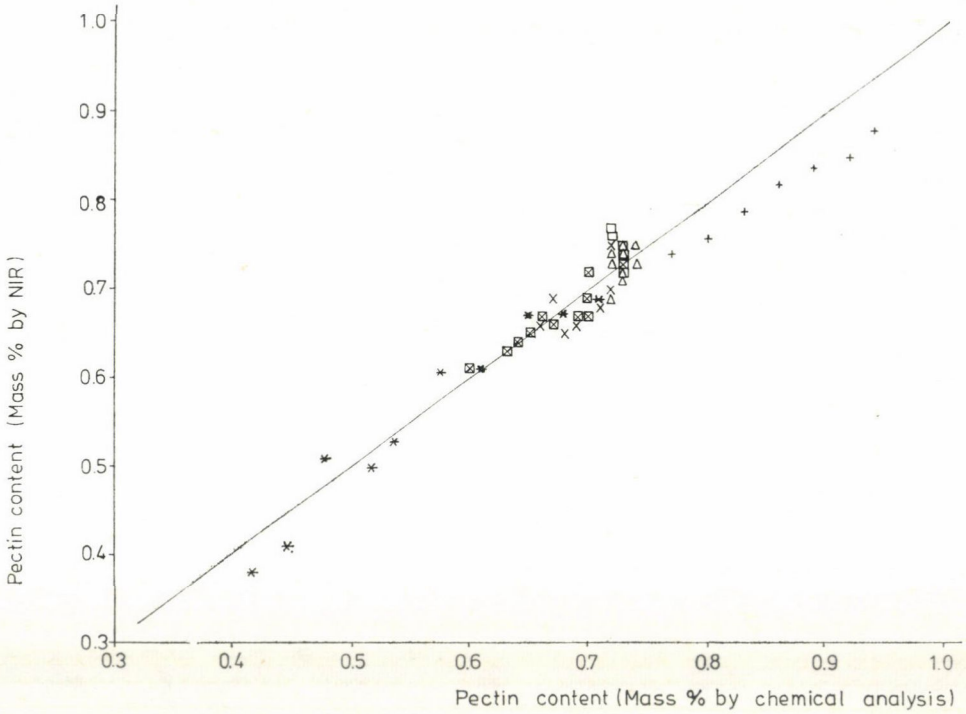

Fig. 10. Relationship between pectin content in water-insoluble dietary fiber determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths. *: Samples with whole wheat flour; □: samples with washed-wheat bran; +: samples with water-insoluble dietary fiber; △: samples with pectin; ×: samples with cellulose; ⊠: samples with lignin

in the course of our preliminary study, their amount was found to be smaller than the error of the used analytical methods. So it burdens the amount of "crude lignin" resistant to the decomposition effect of the 72% by weight H_2SO_4.

The determination of the pectic substances was carried out by carbazole reaction after dissolving the water-soluble pectins, pectats and pectinats by oxalate solution and protopectin by NaOH solution (ROUSE & ATKINS, 1955; MCCOMB & MCCREADY, 1952; HORVÁTH-MOSONYI et al., 1981).

Nitrogen content was estimated by an automatic Kjel-Foss 162-10 instrument and not expressed as protein because the protein/nitrogen ratios were different and unknown.

Starch content was determined by the method of Ewers according to the HUNGARIAN STANDARD (1953).

Dry matter content was estimated by drying the samples at 105 ± 2 °C until constant weight was reached.

In the method of HORVÁTH-MOSONYI and co-workers (1983), pectins have no "place" among the components of dietary fiber. They are distributed

among the different fractions. Some are dissolved by water or decomposed by the 5% H_2SO_4, together with the water-insoluble hemicelluloses, or perhaps resisting the decomposing effect of the 5% H_2SO_4, some pectins remain in the "crude cellulose" fraction. For this study we consider that the water-soluble pectins have been dissolved and precipitated by ethanol, together with the other water-soluble components, and they were weighed as the part of water-soluble dietary fiber; whereas the water-insoluble pectic substances occurred mainly in the "crude hemicellulose" fraction of the water-insoluble dietary fiber. We performed our calculations aiming to get the composition of the samples according to the suppositions mentioned above.

1.2. The NIR spectrophotometer used

The reflectance spectra of all samples were recorded with a multipurpose computerized spectrophotometer using a Cary Model 14 prism-grating mono-

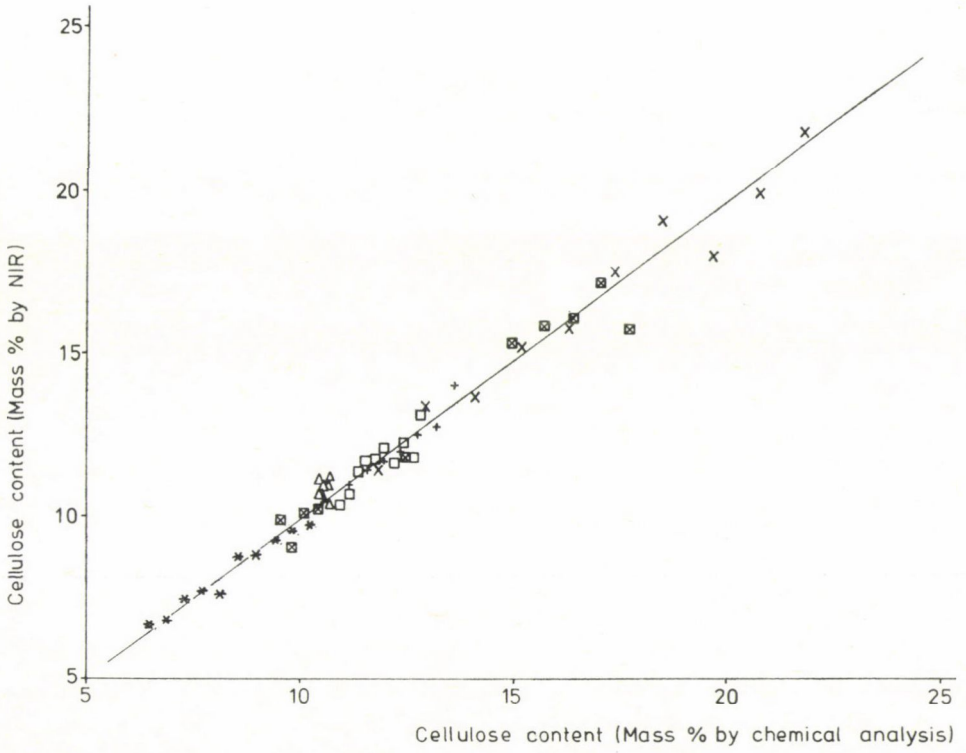

Fig. 11. Relationship between cellulose content in water-insoluble dietary fiber determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths. *: Samples with whole wheat flour; □: samples with washed-wheat bran; +: samples with water-insoluble dietary fiber; △: samples with pectin; ×: samples with cellulose; ⊠: samples with lignin

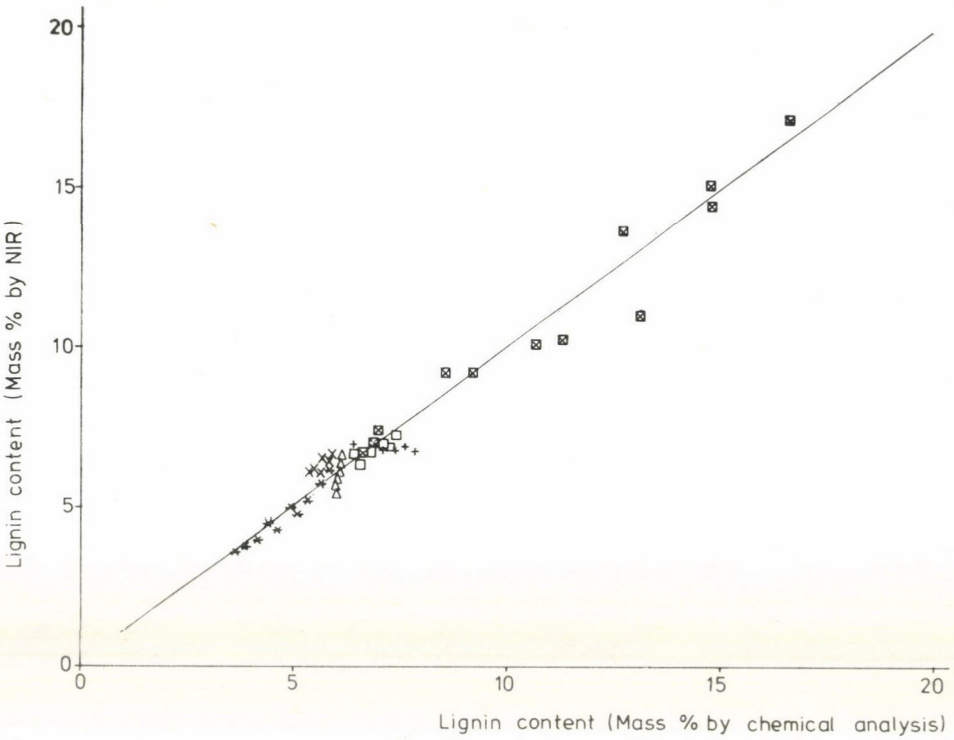

Fig. 12. Relationship between lignin content in water-insoluble dietary fiber determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths. *: Samples with whole wheat flour; □: samples with washed-wheat bran; +: samples with water-insoluble dietary fiber; △: samples with pectin; ×: samples with cellulose; ⊠: samples with lignin

chromator built at the Instrumentation Research Laboratory, BARC, USDA, Beltsville. The instrument is operated in a single-beam mode, with slits at 2 mm, giving an effective bandpass of 7 nm. Ceramic material was used as a reference standard. A new spectrum of reference standard was recorded once each hour to minimize the influence from long-term drift. The measuring geometry was 0°/45°. The wavelength range from 1000 to 2638.4 nm was scanned with a speed of 10 nm per s. Measuring a reflectance spectrum required about 164 s. Reflectance (R) data measured with four lead sulfide detectors were collected every 0.2 nm, with 256 readings per point. We obtained 8192 reflectance points for the whole wavelength range. For data processing, the 8192-point spectral curves were smoothed by a running average of 21 points and shrunk to 1024-point curves by using the average of each 8 points.

Compressed curves for all samples were transformed to log (1/R) because this function gives a linear correlation with the concentration of a given

measured component. Thereafter, the data were recorded on magnetic tape for further processing on a Hewlett–Packard 1000F computer. After recording measured chemical data, the second derivative of the (1/R) was calculated. The studies of NORRIS and co-workers (1976, 1982) and KAFFKA and co-workers (1982 a, b, c) indicated that using second-derivative form of log (1/R) gives better results than log (1/R). The second-derivative calculation was incorporated into the computer program for the linear regression analysis. A regression analysis was made to determine the optimum wavelengths, the constants and coefficients for the regression equation, as well as standard errors of calibration and correlation coefficients.

The definition of standard error of calibration used in the regression analysis is

$$\sqrt{\frac{\Sigma(Q_s - Q_c)^2}{n - p - 1}}$$

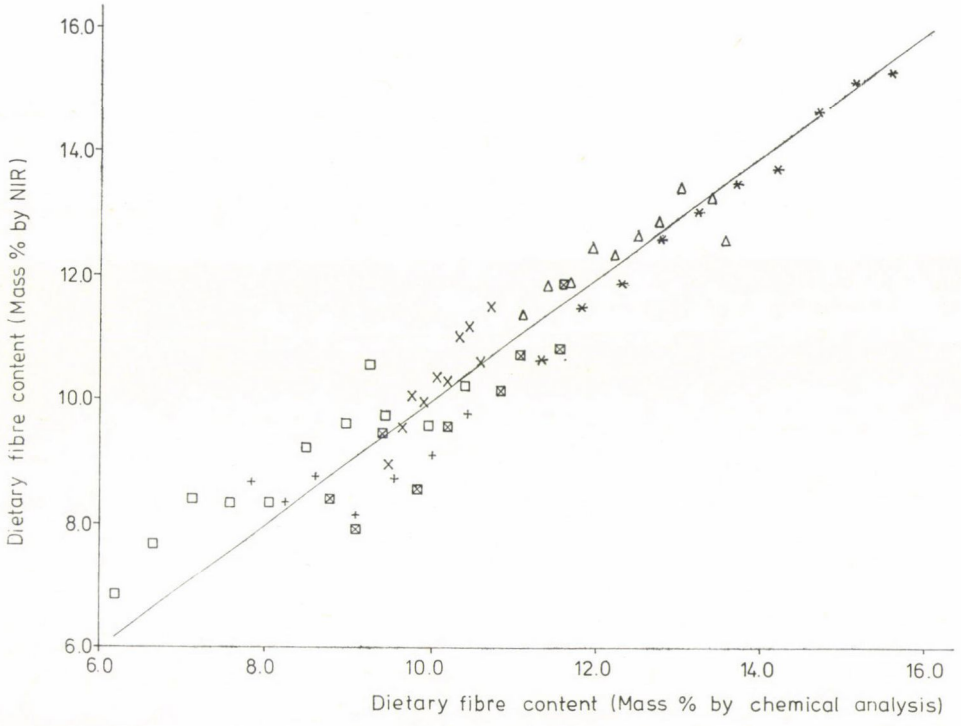

Fig. 13. Relationship between water-soluble dietary fiber content determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths. *: Samples with whole wheat flour; □: samples with washed-wheat bran; +: samples with water-insoluble dietary fiber; △: samples with pectin; ×: samples with cellulose; ⊠: samples with lignin

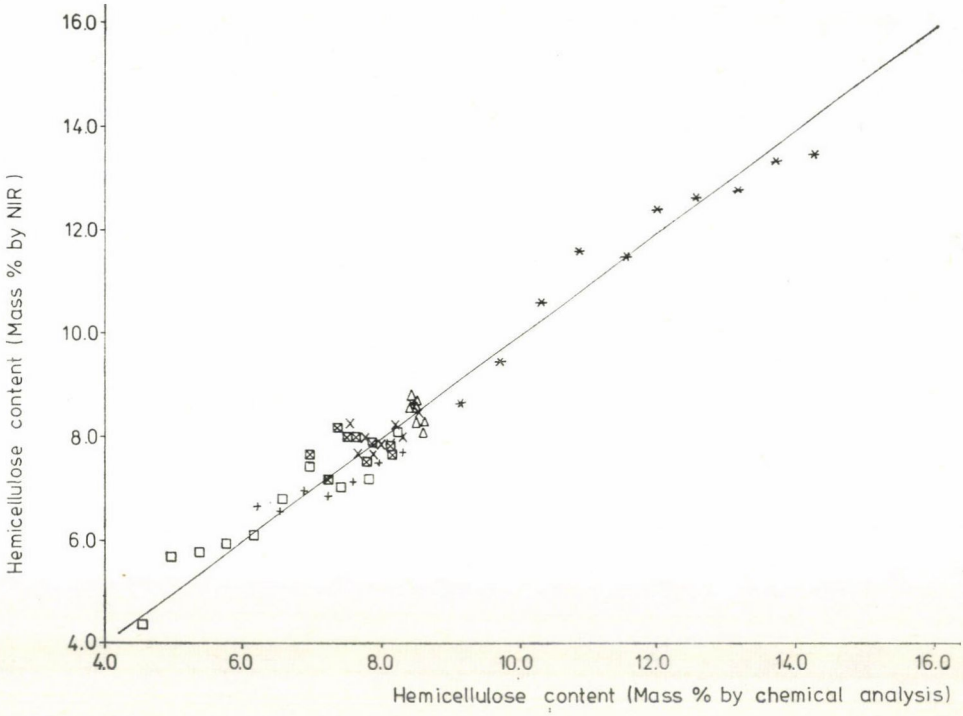

Fig. 14. Relationship between hemicellulose content in water-soluble dietary fiber determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths. *: Samples with whole wheat flour; □: samples with washed wheat bran; +: samples with water-insoluble dietary fiber; △: samples with pectin; ×: samples with cellulose; ⊠: samples with lignin

where Q_s = the component concentration determined by chemical analysis as standard in mass percent;

Q_c = the component concentration calculated by the regression equation in mass percent;

n = the number of samples;

p = the number of independent variables.

The reflectance measurements described were carried out in an air-conditioned laboratory at a temperature of 22 °C.

2. Results

The mean, maximum and minimum values of the compositional data determined by chemical analysis of the six groups of 57 wheat-bran samples are summarized in Table 1.

The occurrence of components in the 57 wheat bran samples are not independent from each other. The correlation coefficients between the compositional data determined by chemical analysis can be seen in Table 2.

The correlation between the components of dietary fiber is important because a high correlation makes it difficult to find the optimum conditions for correlating the spectral data to each component. The hemicellulose and pectin content of wheat bran had a high correlation, 0.897. The two hemicellulose contents are also highly correlated, $r = 0.791$.

The particle size distribution of the wheat bran used for the 57 samples is presented in Table 3.

The log (1/R) spectra and the second derivative curves of wheat bran whole wheat flour, washed wheat bran, water-insoluble dietary fiber, pectin, cellulose, and lignin are shown in Figs. 1–7.

The greatest differences between samples can be seen in the range of 1600–1840 nm and above 2040 nm.

The optimum wavelengths for a second derivative log (1/R) ratio calibration for each constituent was determined by an iterative linear regression procedure. Initial tests used the average log (1/R) spectra of three replicas from each sample. Predicted results from the individual scans showed sample

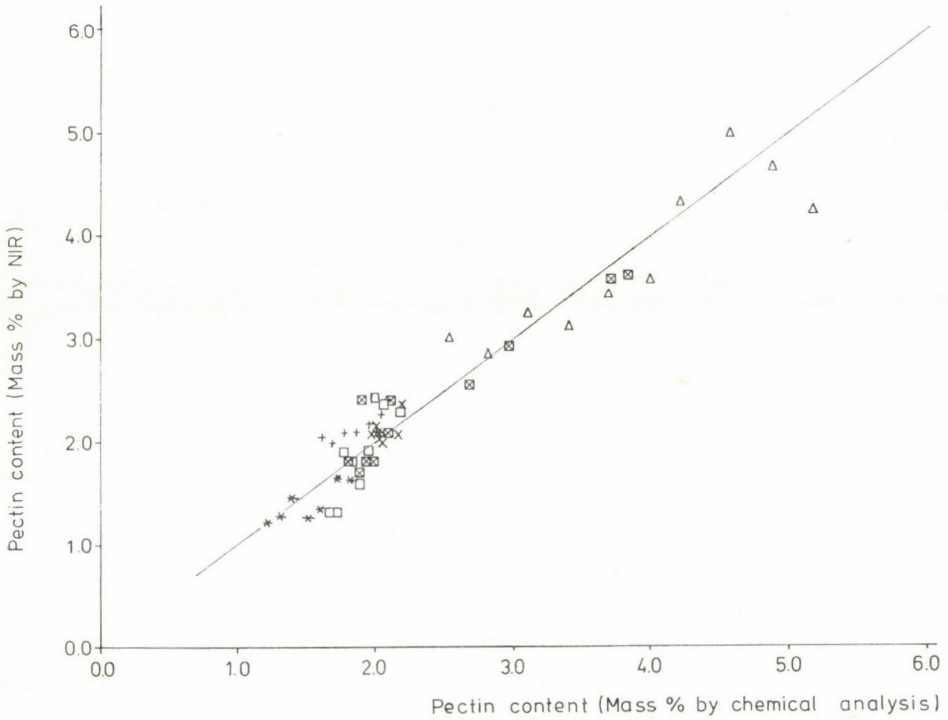

Fig. 15. Relationship between pectin content in water-soluble dietary fiber determined by chemical analysis and predicted value from linear regression ratio of the second derivatives of the log (1/R) curves at two wavelengths. *: Samples with whole wheat flour; □: samples with washed wheat bran; +: samples with water-insoluble dietary fiber; △: samples with pectin; ×: samples with cellulose; ⊠: samples with lignin

Table 1

The composition of the six groups of the 57 bran samples in mass %, analyzed by chemical methods

Group No.	Number of value	Value	WIDF	Components of WIDF				WSDF	Components of WSDF		DF	Nitrogen	Starch
				hemicellulose	pectin	cellulose	lignin		hemicellulose	pectin			
1	10	mean	40.4	26.7	0.56	8.4	4.8	13.5	11.8	1.7	53.9	2.53	24.2
		max.	48.8	32.0	0.71	10.3	5.9	15.6	14.4	2.1	60.2	2.56	33.3
		min.	31.9	21.3	0.42	6.5	3.7	11.4	9.2	1.2	47.6	2.48	14.7
2	10	mean	56.3	37.0	0.73	11.9	6.9	8.3	6.4	1.9	64.7	2.52	10.4
		max.	61.0	40.0	0.74	12.9	7.4	10.4	8.2	2.2	67.2	2.56	12.2
		min.	51.8	33.9	0.72	10.9	6.2	6.2	4.5	1.7	62.2	2.47	8.7
3	7	mean	58.6	38.3	0.85	12.4	7.1	9.1	7.3	1.9	67.8	2.34	10.6
		max.	64.5	42.2	0.94	13.7	7.9	10.5	8.3	2.1	72.4	2.52	12.1
		min.	54.7	34.5	0.77	11.1	6.4	7.8	6.2	1.6	63.1	2.18	9.1
4	10	mean	49.9	32.6	0.73	10.5	6.1	12.4	8.5	3.8	62.2	2.53	12.4
		max.	50.6	33.1	0.74	10.7	6.2	13.6	8.6	5.2	62.8	2.57	12.6
		min.	49.2	32.2	0.72	10.4	6.0	11.2	8.4	2.5	61.7	2.50	12.2
5	10	mean	54.1	30.9	0.69	16.9	5.8	10.1	8.0	2.1	64.2	2.40	11.7
		max.	56.9	32.8	0.73	21.9	6.1	10.7	8.5	2.2	66.4	2.54	12.4
		min.	51.3	29.0	0.65	11.8	5.4	9.5	7.6	2.0	62.1	2.25	11.0
6	10	mean	55.4	29.6	0.66	13.4	11.9	10.2	7.8	2.5	65.6	2.29	11.2
		max.	56.0	31.4	0.70	16.4	14.8	11.6	8.9	3.8	68.8	2.43	11.9
		min.	50.7	26.9	0.63	9.5	8.6	8.8	7.0	1.8	61.5	2.09	10.2

WIDF = water-insoluble dietary fiber
WSDF = water-soluble dietary fiber
DF = dietary fiber

inhomogeneity to represent a significant error for two of the sample sets. Five additional spectral scans were made on these samples, and final results were determined by averaging eight spectra for these two sample sets.

The parameters of the best calibration equations are summarized in Table 4. The gaps for numerators and denominators are the distances among the three wavelengths used to determine the second derivative. The best results for each component of wheat bran are represented in Figs. 8–18. In the Figures, the samples of the six groups of mixtures are plotted with different plotting symbols for each group.

Table 2

The correlation coefficients between the compositional data determined by chemical analysis

Components	WIDF	Components of WIDF				WSDF	Components of WSDF		DF	Nitrogen	Starch
		hemi-cellulose	pectin	cellulose	lignin		hemi-cellulose	pectin			
No.	1	2	3	4	5	6	7	8	9	10	11
1	1.000	0.732	0.741	0.653	0.506	−0.915	−0.956	−0.018	0.986	−0.551	−0.926
2	0.732	1.000	0.897	0.127	0.023	−0.731	−0.791	0.046	0.697	0.029	−0.719
3	0.741	0.897	1.000	0.221	0.096	−0.595	−0.720	0.204	0.764	−0.107	−0.777
4	0.653	0.127	0.221	1.000	0.207	−0.592	−0.540	−0.184	0.646	−0.638	−0.571
5	0.506	0.023	0.096	0.207	1.000	−0.370	−0.436	−0.101	0.537	−0.662	−0.441
6	−0.915	−0.731	−0.595	−0.592	−0.370	1.000	0.903	0.332	−0.834	0.409	0.782
7	−0.956	−0.791	−0.720	−0.540	−0.436	0.903	1.000	−0.106	−0.931	−0.340	0.949
8	−0.018	0.046	0.204	−0.184	0.101	0.332	−0.106	1.000	0.112	0.201	−0.274
9	0.986	0.697	0.764	0.646	0.537	−0.834	−0.931	0.112	1.000	−0.582	−0.940
10	−0.551	0.029	0.107	−0.638	−0.662	0.409	0.340	0.201	−0.582	1.000	0.322
11	−0.926	−0.719	−0.777	−0.571	−0.441	0.782	0.949	−0.274	−0.940	0.322	1.000

WIDF = water-insoluble dietary fiber
WSDF = water-soluble dietary fiber
DF = dietary fiber

Table 3

The particle size distribution of the wheat bran

Particle size (mm)	Content (mass %)
0.16	10.0
0.16—0.25	64.4
0.25—0.40	16.6
0.40—0.63	6.7
0.63—1.25	1.0
1.25	1.3

Because the components of dietary fiber were separately determined by NIR-technique, it is possible to determine the water-soluble, water-insoluble, and total dietary fiber by addition of their components. These results appear in Figs. 19–21.

It is possible that the highest correlation for a component occurs at a wavelength where other components also have a high correlation. The result

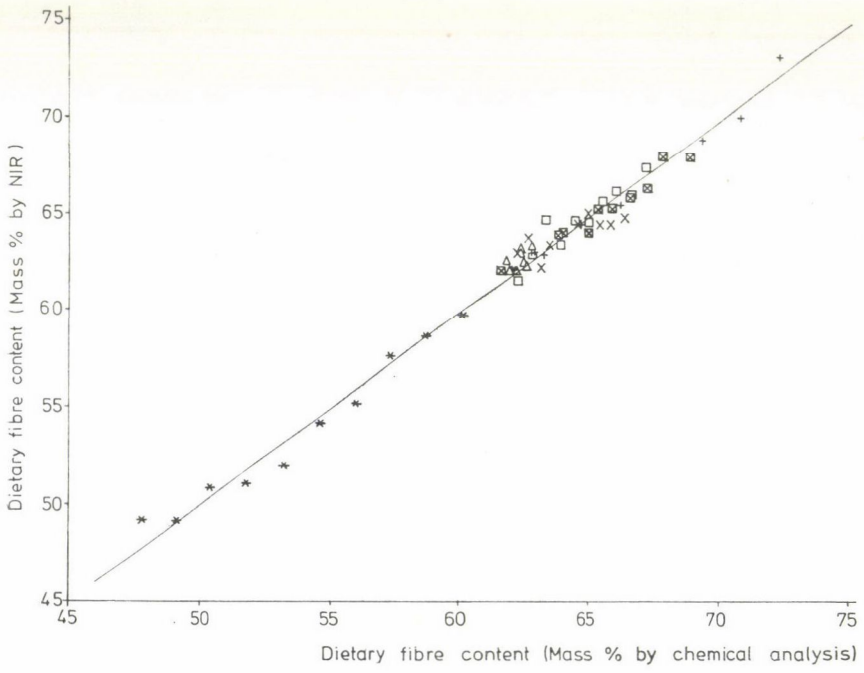

Fig. 16. Relationship between dietary fiber content determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths. *: Samples with whole wheat flour; □: samples with washed wheat bran; +: samples with water-insoluble dietary fiber; △: samples with pectin; ×: samples with cellulose; ⊠: samples with lignin

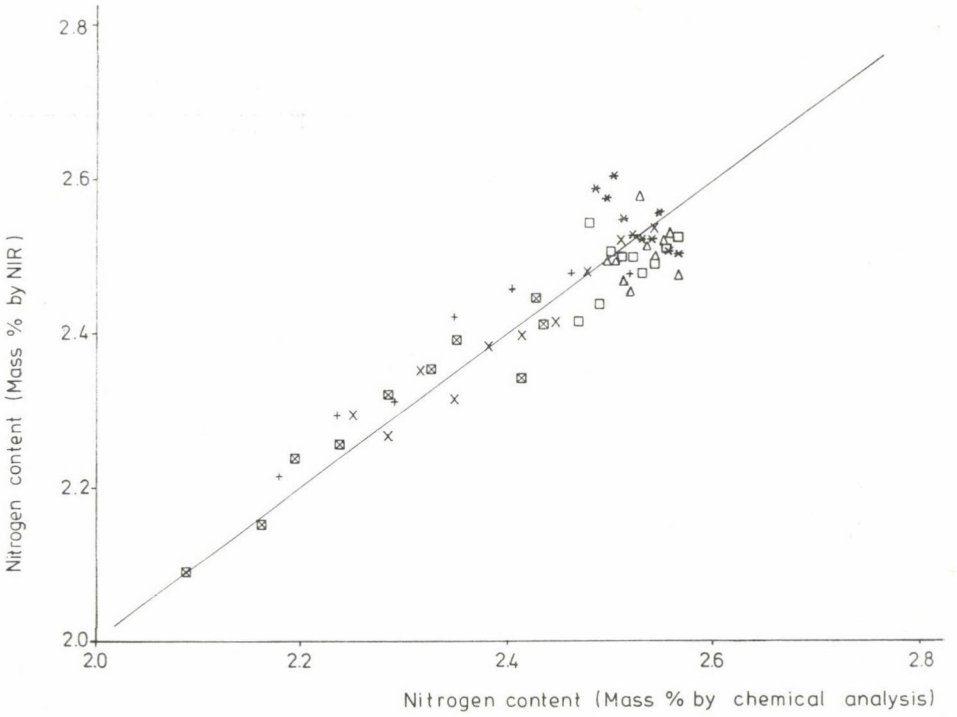

Fig. 17. Relationship between nitrogen content determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths. *: Samples with whole wheat flour; □: samples with washed wheat bran; +: samples with water-insoluble dietary fiber; △: samples with pectin; ×: samples with cellulose; ⊠: samples with lignin

of this occurrence is that such a wavelength is not suitable to differentiate one component from the others.

To test the results, the equation used for calculation of each component (see in Table 4) was used to predict the other 10 components. The correlation coefficients between the compositional data determined by chemical analysis and the predicted data are summarized in Table 5.

Several different wavelengths can usually be used as a numerator wavelength for each constituent. The three wavelengths giving the highest correlations were tested with the above correlation procedure to be certain that an optimum choice was not overlooked. For the spectral analysis, the numerator wavelength should be chosen to give the best relationship to the component being measured and having the least interference from other components. Some components, such as dietary fiber, consist of the sum of several individual components. In this case, the total component can be determined by a direct correlation to the reflectance data or indirectly by summing up the results of predictions from each of the individual component calibrations.

Table 4

Summary of linear regression analyses relating data from chemical analyses and values of the

Equation form: $Q_i = K_{0i} +$

Component	WIDF	Components of WIDF			
		hemicellulose	pectin	cellulose	lignin
Equation No.	I.	II.	III.	IV.	V.
λ_1 (nm)	1748.8	1712	1740.8	2342	2265.6
λ_2 (nm)	1340.8	1312	2438.4	1400	2329.6
K_0	93.123	−6.0366	0.44077	−8.1654	−4.6125
K_1	111.39	−19.305	0.74958	−75.856	9.2931
Gap at λ_1	35.2	30.4	22.4	38.4	40.40
Gap at λ_2	36.8	24	22.4	35.2	32.32
SEC (mass%)	1.117	0.995	0.027	0.491	0.534
r	0.986	−0.975	0.960	−0.989	0.980

WIDF = water-insoluble dietary fiber
WSDF = waer-soluble dietary fiber
DF = dietary fiber

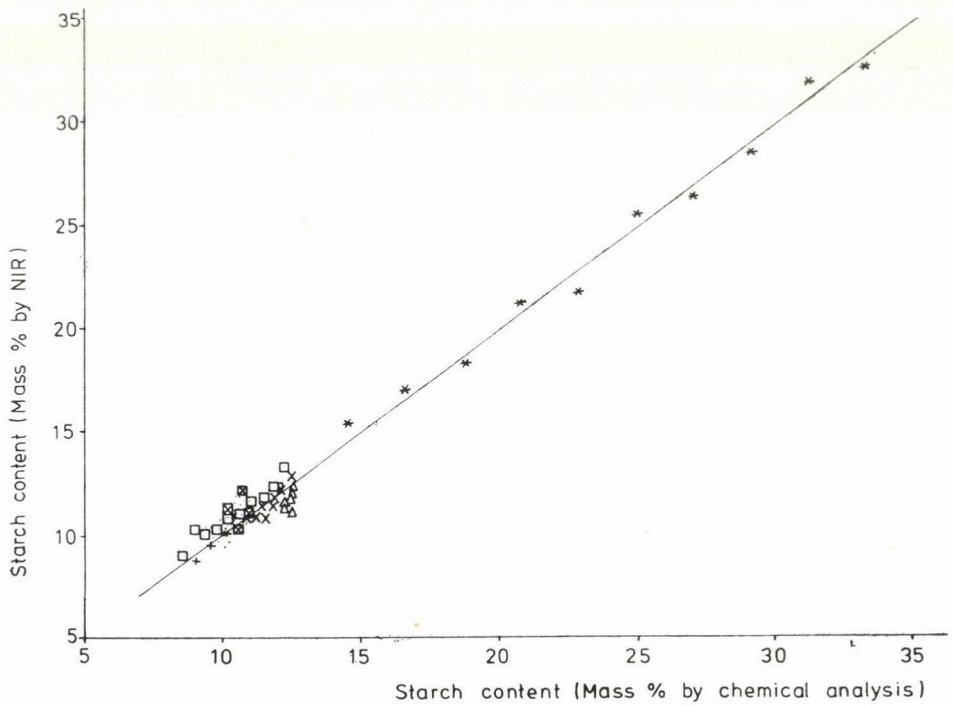

Fig. 18. Relationship between starch content determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths. *: Samples with whole wheat flour; □: samples with washed wheat bran; +: samples with water-insoluble dietary fiber; △: samples with pectin; ×: samples with cellulose; ⊠: samples with lignin

second derivative of log (1/R) curves at two characteristic wavelengths for 57 wheat bran samples

$+ K_{1i}(V''\lambda_{1i}/V''\lambda_{2i})$

WSDF	Components of WSDF		DF	Nitrogen	Starch
	hemicellulose	pectin			
VI.	VII.	VIII.	IX.	X.	XI.
1217.6	1422.4	2200	1745.6	1184	2284.8
1393.6	1784	1222.4	1243.2	2468.8	1710.4
41.412	23.041	4.4443	55.859	0.4422	1.0361
129.30	3.0142	18.62	38.721	6.8207	10.172
30.4	25.6	20.8	25.6	27.2	22.4
36.8	30.4	40	41.6	38.4	22.4
0.601	0.387	0.261	0.687	0.044	0.580
0.959	−0.981	−0.957	0.991	0.933	0.995

SEC = standard error of calibration
r = correlation coefficient

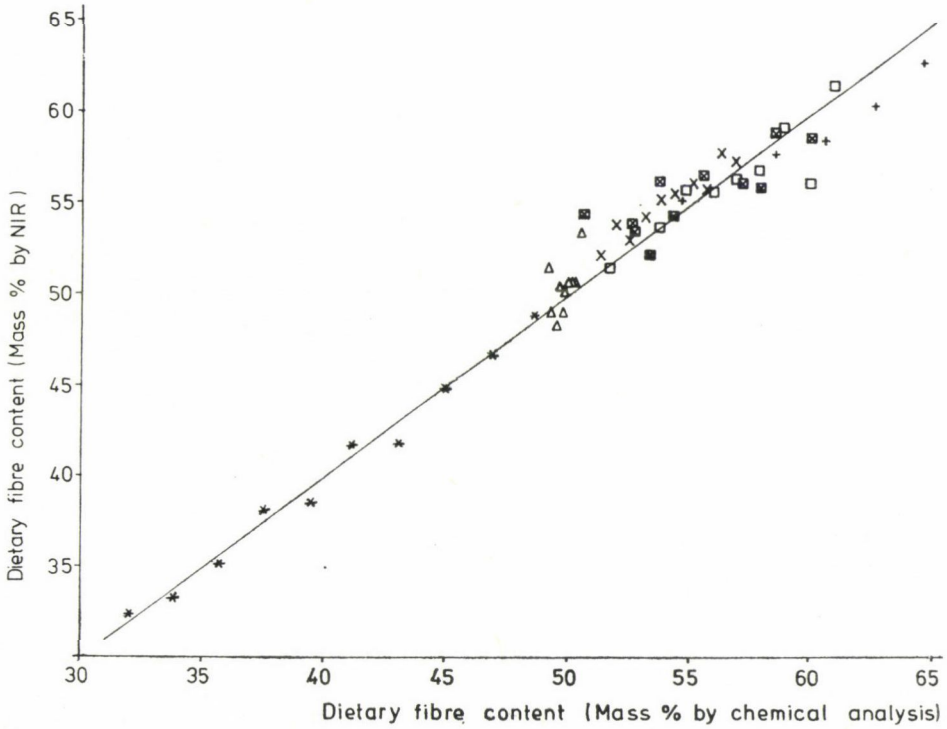

Fig. 19. Relationship between water-insoluble dietary fiber content determined by chemical analysis, and predicted value of sum of components from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths. *: Samples with whole wheat flour; □: samples with washed wheat bran; +: samples with water-insoluble dietary fiber; △: samples with pectin; ×: samples with cellulose; ⊠: samples with lignin

The results were very similar. The correlation coefficients approached the corresponding correlation coefficient determined between the chemical data.

The correlation coefficients between the compositional data determined by chemical analysis given in Table 2 and between the compositional data determined by NIR analysis given in Table 5 should be compared. One way to compare these two groups of correlation coefficients is to create the difference of absolute values of corresponding coefficients. These differences are summarized in Table 6.

3. Conclusions

It can be seen in Table 1 that the dietary fiber components of wheat bran samples have very different ranges. For example, water-insoluble pectin content has the smallest range, 0.42%–0.94%; and water-insoluble hemicellulose content has the largest range, 21.3%–42.2%. Total dietary fiber

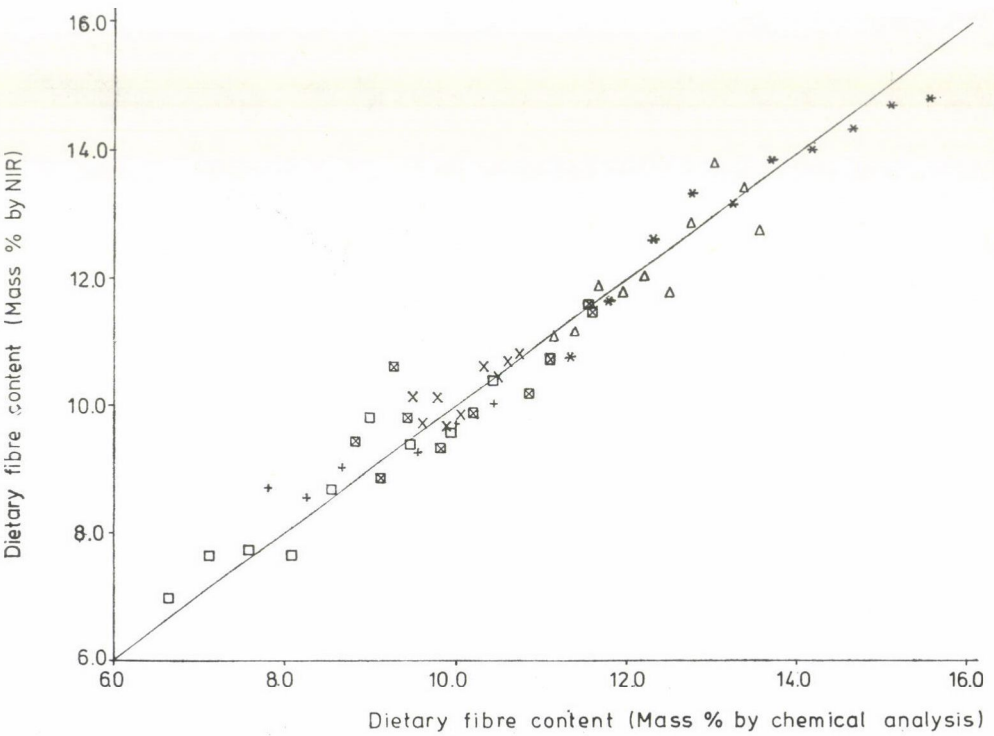

Fig. 20. Relationship between water-soluble dietary fiber content determined by chemical analysis, and predicted value of sum of components from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths. *: Samples with whole wheat flour; □: samples with washed wheat bran; +: samples with water-insoluble dietary fiber; △: samples with pectin; ×: samples with cellulose; ⊠: samples with lignin

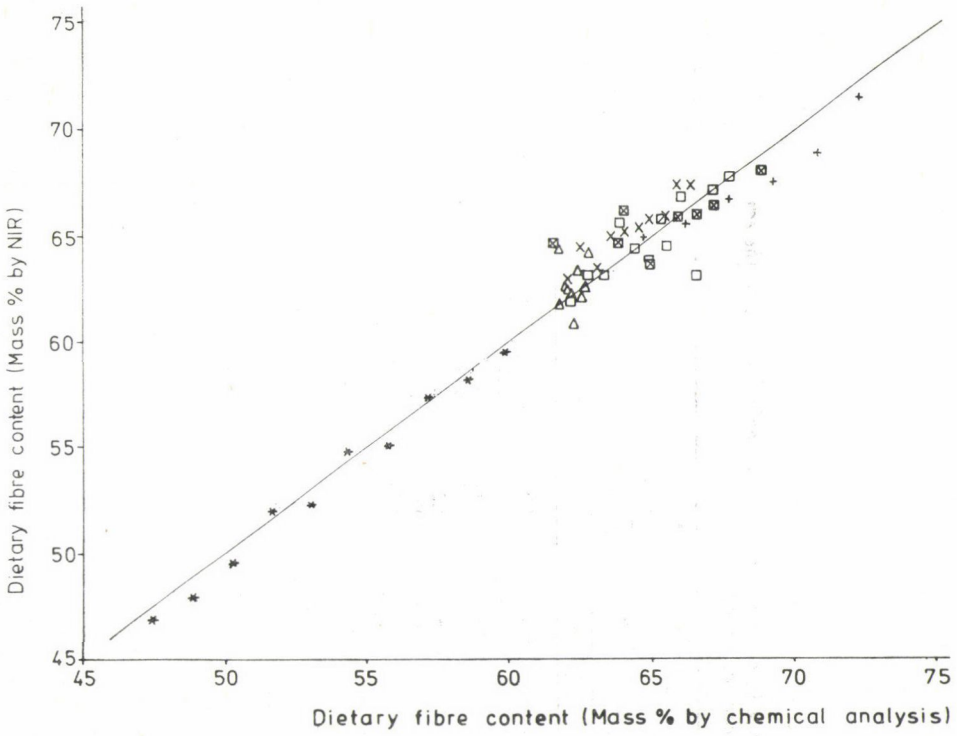

Fig. 21. Relationship between dietary fiber content determined by chemical analysis and predicted value of sum of components from linear regression of the ratio of the second derivatives of the log (1/R) curves of two wavelengths. *: Samples with whole wheat flour; □: samples with washed wheat bran; +: samples with water-insoluble dietary fiber; △: samples with pectin; ×: samples with cellulose; ⊠: samples with lignin

content, 47.6%–72.4%, and starch content, 8.7%–83.3%, also have large ranges. Although the ranges of the components are very different, the NIR calibrations for each were carried out with success. Table 4 shows that the correlation coefficients of each dietary fiber component is above 0.956. The highest correlation coefficient was found for starch, 0.995.

The correlation coefficients given in Table 2 show that the components are not independent from each other. This situation is based on the natural relationship between some components. Namely, the dietary fiber content is the sum of water-soluble and water-insoluble dietary fiber; the sum of water-soluble components gives the water-soluble dietary fiber content; and the sum of water-insoluble components gives the water-insoluble dietary fiber content. The high correlation between water-insoluble hemicellulose and pectin occurred because the mixtures used in this study did not affect the natural ratio of these two components. The high correlation, $r = 0.986$, between water-insoluble dietary fiber and total dietary fiber, makes it difficult to find optical

Table 5

The correlation coefficients between the compositional data determined by chemical analysis and calculated using the equations given in Table 4 for each component

Equation No.	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.
Components	WIDF	Components of WIDF				WSDF	Components of WSDF		DF	Nitrogen	Starch
		hemi-cellulose	pectin	cellulose	lignin		hemi-cellulose	pectin			
No.	1	2	3	4	5	6	7	8	9	10	11
1	0.987	0.723	0.769	−0.644	0.486	−0.901	0.937	−0.049	0.976	−0.661	−0.911
2	0.694	−0.975	0.923	−0.141	−0.012	−0.672	0.810	−0.117	0.724	−0.068	−0.707
3	0.722	−0.898	0.960	−0.241	0.075	−0.587	0.749	−0.314	0.788	−0.218	−0.786
4	0.701	−0.168	0.223	−0.989	0.231	−0.619	0.513	0.159	0.607	−0.720	−0.577
5	0.477	−0.007	0.122	−0.175	0.980	−0.401	0.391	−0.119	0.515	−0.677	−0.415
6	−0.889	0.729	−0.656	0.569	−0.353	0.959	−0.893	−0.278	−0.823	0.508	0.747
7	−0.939	0.791	−0.798	0.534	−0.424	0.861	−0.981	0.144	−0.932	0.482	0.930
8	0.004	−0.050	0.235	0.133	0.113	0.328	0.088	−0.957	0.140	0.121	0.312
9	0.979	−0.693	0.777	−0.643	0.517	−0.833	0.909	−0.180	0.991	−0.691	−0.934
10	−0.561	−0.047	−0.036	0.609	−0.626	0.470	−0.290	−0.172	−0.537	0.933	0.319
11	−0.926	0.741	−0.831	0.578	−0.448	0.769	−0.941	0.333	−0.944	0.497	0.995

WIDF = water-insoluble dietary fiber
WSDF = water-soluble dietary fiber
DF = dietary fiber

Table 6

Summary of differences between the absolute values of correlation coefficients, calculated between the compositional data determined by chemical analysis given in Table 2 and calculated between the compositional data using the equations for each component given in Table 5

Components	WIDF	Components of WIDF				WSDF	Components of WSDF		DF	Nitrogen	Starch
		hemi-cellulose	pectin	cellulose	lignin		hemi-cellulose	pectin			
No.	1	2	3	4	5	6	7	8	9	10	11
1	0.013	0.009	−0.028	0.007	0.020	0.014	0.019	−0.031	0.010	−0.110	0.015
2	0.038	0.025	−0.026	−0.014	0.011	0.059	−0.019	−0.071	−0.027	−0.039	0.012
3	0.019	0.006	0.040	−0.020	0.021	0.008	−0.029	−0.110	−0.024	−0.111	−0.009
4	−0.048	0.041	−0.002	0.011	−0.024	−0.027	0.027	0.025	0.039	−0.082	−0.006
5	0.029	0.016	−0.026	0.032	0.012	−0.031	0.046	−0.018	0.022	−0.015	0.026
6	0.026	0.002	−0.061	0.023	0.017	0.041	0.010	0.054	0.011	−0.099	0.035
7	0.017	0.000	−0.078	0.006	0.012	0.042	0.019	−0.038	−0.001	−0.142	0.019
8	0.014	−0.004	−0.031	0.051	−0.012	0.004	0.018	0.043	−0.028	0.080	−0.038
9	0.007	0.004	−0.013	0.003	0.020	0.001	0.022	−0.068	0.009	−0.109	0.006
10	−0.010	−0.018	0.071	0.029	0.036	−0.061	0.050	0.029	0.045	0.067	0.003
11	0.000	−0.022	−0.054	−0.007	−0.007	0.073	0.008	−0.059	−0.004	−0.175	0.005

WIDF = water-insoluble dietary fiber
WSDF = water-soluble dietary fiber
DF = dietary fiber

data to provide a distinction between these components. This is reflected in the choice of numerator wavelengths for these two components. The two wavelengths, 1748.8 and 1745.6 nm, respectively, are essentially the same; and they do not provide an independent calibration for the separate components.

For the spectral analysis, the numerator wavelength should be chosen to give the best relationship to the component being measured and having the least interference from other components. Some components, such as dietary fiber, consist of the sum of several individual components. In this case, the total component can be determined by a direct correlation to the reflectance data or indirectly by summing the results of predictions from each of the individual component calibrations. Both ways of calibration were used for dietary fiber and its water-soluble and insoluble part. The results of direct calibration are presented in Figs. 8, 13, and 16. The results of summation of components can be seen in Figs. 19, 20, and 21.

Comparing the two groups of results proved that both ways of calibration can be used. In the addition of components, the error can be higher because the individual errors of predicting the components are also added. This situation was found as dietary fiber was summarized from its six components. In spite of this, for water-soluble and insoluble part of dietary fiber, the addition produced the better calibration with less error.

Independently of this, results of calibration by addition seem to be more suitable, because using a common wavelength instead of characteristic wavelengths of each component must have more physical uncertainty.

The best calibration equations for each component were used also for predicting all other components. The calculations were repeated for the three best numerator wavelengths with similar results.

The correlation coefficient between the chemical data shown in Table 2 gives the limits for NIR analysis because the optical data were correlated to data determined by chemical analysis. Therefore, the correlation coefficients, determined by NIR analysis given in Table 5, must be compared with the correlation coefficients given in Table 2.

Comparing these two tables, if a correlation between two components for the NIR results are significantly higher than the respective correlations for the chemical data, then the NIR calibration is influenced by a component different from that being measured. If this occurs, the wavelength selected for calibration is likely to be that of a foreign absorption band. In this study, no significant differences were observed between the correlation coefficients for the chemical data and those of the NIR data (Table 6).

Some constituents, such as cellulose and lignin, are present in two forms in the samples with one form being the natural constituent and the other an added constituent. The NIR calibration should fit both forms of the con-

stituents. The data plotted in Fig. 11 for cellulose and Fig. 12 for lignin show that both forms of the constituents are on the same calibration line. In Group 1 (*), the cellulose and lignin are from the natural product; in Group 5 (x), the cellulose is an added constituent; and in Group 6 (⊠), the lignin is an added constituent. Therefore, in this groups, cellulose and lignin are presented in both forms.

The calibration results of this study have not been tested against unknown samples, but the high correlation coefficients indicate the possibility to predict the dietary fiber and its components non-destructively by NIR-techniques.

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BOOK REVIEW

Plant proteins for human food

C. E. BODWELL and L. PETIT (Eds), J. GUEGUEN (coordinator)
Martinus Nijhoff/Dr. W. Junk Publishers, The Hague, 1983; 471 pages

The volume contains the proceedings of a European Congress, held in Nantes, 5—7 Octobre, 1981 and organised by the Institut National de la Recherche Agronomique of France. The material was published also in Qualitas Plantarum, Plant Foods for Human Nutrition, Vol. 32, Nos. 3/4.

G. FAUCONNEAU discusses the importance of plant proteins in the world's protein supplies. Protein consumption is between 50—110 g per day per capita but half of the world population consumes 50 g protein or less, the 80% of which is of plant origin. The average protein intake in developed countries is over 90 g and the half of it is of animal origin: this population represents one fourth of humanity. These facts suggest that the increase of the production of plant proteins alone is not sufficient to satisfy the protein demand, at the same time the improvement of animal protein sources is necessary, too.

J. MOSSÉ and J. BAUDET deal with the crude protein content and amino acid composition of different seeds (cereals, oil-seeds, pea and bean species, etc.). Both undergo alterations in connection with phenotypical variation and biochemical mutations. The production of a variant with more favourable amino acid composition may be performed by genetic transfer. D. BOULTER analysed the proteins of peas (Pisum) and fababeans (Vicia). The storage proteins may be placed in two major groups (legumin and vicilin). L. M. MONTI and S. GRILLO emphasize the antinutritive factors of seeds of legume species, the presence of which is genetically controlled.

As soybean cannot be cultivated everywhere, other legume species were studied for detecting new protein sources. J. GUEGUEN presented the extraction methods of proteins from *Pisum sativum*, *Vicia faba* and *Lupinus albus* as well as the characteristics of the final products. The isolates and concentrates, produced by the above mentioned processes may replace the functions of soyabean.

Sunflower and rapeseed flour cannot be applied directly in food because of their unfavourable components (polyphenolic pigments, hulls, glucosinolates, etc.). Using extraction processes, however, rather useful products may be obtained. The general use of these processes is to be expected in the near future (L. TRANCHIRO et al.).

The application of leaf-protein concentrates in animal feeding has been worked out but preparing them for human consumption is yet in the experimental stage (R. FIORENTINI, C. GALOPPINI).

The technological, nutritional and agronomical aspects of protein extraction from tobacco leaves are presented by P. FANTOZZI and A. SENSIDONI. The extraction of protein diminishes the toxicity of the tobacco leaf and at the same time the leaf may be used for the production of smoking material. The extracted protein's in vitro and in vivo digestibility is better than that of any protein examined. The use of high planting densities and nitrogen fertilization results in an about 1800 kg per hectare yield of protein from tobacco.

A. M. HERMANSSON dealt with the function of proteins in food microstructure: gel formation as the base of waterbinding and texture characteristics. The functional properties of soya change depending on processing methods. The example of gluten shows that other types of components may also cause drastic alterations in the ingredient function of proteins during processing. The same subject is elaborated in the work of D. J. WRIGHT, P. J. LILLFORD, J. ADLER et al., and in the work of C. GIDDEY, too. They deal with the physico-chemical properties of plant proteins and with the correlations between plant proteins and chemical structure (amino acid composition, secondary and tertiary structure), with the difficulty of defining the properties which determine the functional availability, with the texturation of mixed plant and animal proteins.

In the extraction of plant proteins and then during the preparation and storage, such physical and chemical phenomena can occur which may modify the nutritive quality or may produce certain physiological effects. Chlorinated solvents and alkaline treatment must not be applied, oxidative agents are to be manipulated carefully, conditions causing Maillard reaction have to be controlled (P. A. FINOT).

R. FERRANDO summarizes the antinutritive factors present in European plant protein sources: toxic amino acids, anti-enzymes, hemagglutinins, hormonal and anti-hormonal actions, chelate formation, anti-vitamins and flatulence factors.

GY. BIRÓ

ANNOUNCEMENT

INTERNATIONAL SYMPOSIUM ON FOOD IRRADIATION PROCESSING WASHINGTON, D.C. 4—8 MARCH 1985

ORGANIZED BY:	FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO, ROME, ITALY) AND INTERNATIONAL ATOMIC ENERGY AGENCY (IAEA, VIENNA, AUSTRIA)
SCIENTIFIC SECRETARY:	J. G. von Kooij Joint FAO/IAEA Division of Isotope and Radiation Applications of Atomic Energy for Food and Agricultural Development. IAEA, Vienna International Centre, P.O. Box 100, A-1400 Vienna, Austria.
MAJOR TOPICS:	Technological Feasibility of Food Irradiation Economic Feasibility Institutional Issues of Food Irradiation Acceptance
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REGISTRATION FEE:	NO REGISTRATION FEE IS CHARGED TO PARTICIPANTS.

RECENTLY ACCEPTED PAPERS

Effect of refined hydrogenated karanja oil on lipid metabolism in adult male albino rats. A comparative study
MANDAL, B., GHOSH MAJUMDAR, S. & MAITY, C. R.
Heat conservation of soft drinks prepared by enzymes
SZILÁGYI-TÓTH E., REICHART, O. & ŽETELAKI-HORVÁTH, K.
IVth Conference on Enzymology, Budapest, 1983

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

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

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

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

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

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

References. The reference list (Literature) should be in alphabetical order as follows:
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Books: Names and initials of all the authors; the year of publication in parentheses; colon; title of the book; publishing firm, place of publication; inclusive page numbers.

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CONTENTS

Index: 26.039