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INACTIVATION OF POLYPHENOL OXIDASE AND DEPLETION OF O-DIHYDROXY PHENOL CONTENT DURING THE ENZYMATIC BROWNING REACTION OF FRUIT TISSUES

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(Received: 5 June 1981; accepted: 15 July 1981)

In order to study enzyme inactivation and substrate depletion during the browning reaction of fruit tissues, the changes in these variables were followed in aerated pulps of two apricot and two apple cultivars of different mass-related polyphenol oxidase activities and o-dihydroxy phenol contents. Although enzyme activity decreased from the beginning of aeration, in the majority of samples substrate concentration reached levels near to depletion while polyphenol oxidase activity was still considerable. The results obtained with apricots suggested that not all the o-dihydroxy phenols present in this fruit were substrates of polyphenol oxidase. Enzyme inactivation related to unit decrease in o-dihydroxy phenol content was more marked in the samples of higher initial polyphenol oxidase activities and lower o-dihydroxy phenol contents and vice versa. This means that the reaction inactivation of polyphenol oxidase was inversely related to product formation. Consequently, under the given conditions, enzyme inactivation during the polyphenol oxidase-catalyzed oxidation of o-dihydroxy phenols cannot be caused by quinone binding by the enzyme molecule.

In an earlier study (VÁMOS-VIGYÁZÓ et al., 1977) carried out with fruit homogenates, the ratio of mass-related polyphenol oxidase (PPO) activity and the concentration of its endogenous substrates was found to indicate which of these factors determines the initial browning rate of the product. In fruit of relatively high polyphenol content and low mass-related PPO activity (e.g., the apple cultivars *Jonathan* and *Golden Delicious*) (VÁMOS-VIGYÁZÓ et al., 1980) the latter variable proved to play the primary role; in most of the apricot cultivars studied, where the situation was reversed (GAJZÁGÓ et al., 1979), browning rate was related to polyphenol concentration. It was assumed that in the first case reaction inactivation of the enzyme occurred while substrate was still present (WALKER, 1964; WHITAKER, 1972; PADRÓN et al., 1975). Thus, necessarily, mass-related enzyme activity would be the limiting factor in the browning reaction. In the second case total substrate depletion was assumed to take place prior to enzyme inactivation, thus the reaction would be limited by substrate concentration.

In order to support these considerations by experimental evidence, model experiments were carried out with apple and apricot homogenates of different ratios of mass-related PPO activity and endogenous polyphenol

concentration in which the browning reaction was accelerated by aeration and enzyme activity as well as substrate concentration were determined at intervals during the reaction.

1. Materials and methods

1.1. The fruits

All the fruits were picked in the years 1977 and 1978. The apples of the cultivars *Jonathan* and *Starking* were purchased from the Research Station of the University of Horticulture, Újfehértó, the apricots of the cultivars *Bibor* (Purple) and *Mandula* (Almond) were supplied by courtesy of the Research Station of the Research Institute for Fruits and Ornamental Plants, Cegléd.

1.2. The analytical methods

Enzyme activity was determined in appropriately diluted fruit homogenates by a kinetic spectrophotometric method developed at the Central Food Research Institute (MIHÁLYI & VÁMOS-VIGYÁZÓ, 1976), using chlorogenic acid (Fluka, Switzerland), as substrate. A change in optical density (OD) of 10^{-4} per min was adopted as unit enzyme activity and related to fruit mass (fresh mass basis).

Ortho-dihydroxy phenol (ODP) content was assessed in the methanol extracts of the fruits by a modified version of the method of ALMÁSI and MOLNÁR (1961) based on the Hoepfner reaction. ODP content was expressed as mg chlorogenic acid in 1 g of fruit flesh (fresh mass basis) (VÁMOS-VIGYÁZÓ et al., 1981).

1.3. Aeration of the fruit homogenates

Fruit homogenates were prepared from (7:2), later from (6:1) tap water dilutions of fruit flesh comminuted with a stainless steel knife. Homogenization was carried out in an Atomix blendor equally equipped with stainless steel knives. In the 1977 experiments 3 samples of homogenate of 140 g each were stirred in beakers at 22 ± 2 °C and 180 rpm with vertical stainless steel blades mounted on a vertical stainless steel shaft. In 1978 this equipment was replaced by a 12 cm ∅ sintered glass funnel (G 1), holding 750 g of homogenate. Aeration was performed by an upward flow of air and simultaneous stirring. Aeration and stirring were carried on for several hours at r.t. and samples were taken for the determinations of PPO activity and ODP content before aeration and, subsequently, every 30 and 60 min, resp.

2. Results

2.1. *Mass-related polyphenol oxidase activity, o-dihydroxy phenol content and the ratio of these two parameters in the fruit homogenates*

Mass-related PPO activity, ODP content and the ratio of the two values (Q) in the fruit homogenates as determined prior to aeration are given in Table 1.

Table 1

Mass-related polyphenol oxidase (PPO) activity, o-dihydroxyphenol (ODP) content and the ratio of the two values (Q) in apple and apricot homogenates

Fruit	Cultivar	Year	PPO activity (kU g^{-1})		ODP content (mg g^{-1})		Q (kU mg^{-1})
			$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	
Apple	Jonathan	1977	1.16	0.09	0.63	0.03	1.84
		1978	0.25	0.03	0.79	0.06	0.32
	Starking	1978	5.14	0.35	0.64	0.03	8.03
Apricot	Mandula	1977	30.0	0.85	0.43	0.03	69.8
		1978	16.2	0.82	0.11	0.007	147
	Bíbor	1977	41.8	1.68	0.12	0.005	348
		1978	14.5	0.14	0.12	0.006	121

Q = PPactivity per ODP content
$\bar{x}$ = mean of parallel determinations
$\pm s$ = standard deviation
n = number of parallel determinations (= 4–5 for PPO and 3 for ODP)

The data of Table 1 are consistent with earlier findings (VÁMOS-VIGYÁZÓ et al., 1977), i.e., mass-related PPO activity was considerably higher, while ODP content was lower in apricot than in apple cultivars. The values of Q varied over a wide range (3 orders of magnitude) and were found to be very different in the samples of a given cultivar picked in different years.

2.2. *Changes in mass-related polyphenol oxidase activity and o-dihydroxy phenol content in apple and apricot homogenates during the enzymatic browning reaction*

PPO activities and ODP contents as determined after different periods of aeration of the fruit pulps are given in Fig. 1.

Both enzyme activity and endogenous substrate concentration decreased during aeration in the pulps of both kinds of fruits.

For the two samples of the apricot cultivar *Bibor* the time course of the decrease in both parameters was very similar, although the mass-related enzyme activity of the 1977 sample was about treble that found in the 1978 one. Changes were most marked during the first 60 min. By that time enzyme activity dropped to 29% and 18% of the initial values in the samples of 1977 and 1978, resp. (i.e., 12.2 ± 0.96 kU g^{-1} and 2.67 ± 0.14 kU g^{-1}). ODP concentration varied accordingly: after 60 min of aeration the residual values were 75% and 53% of the initial ones. Both values represented very low concentrations of substrate: 0.09 mg g^{-1} and 0.06 mg g^{-1}, resp.

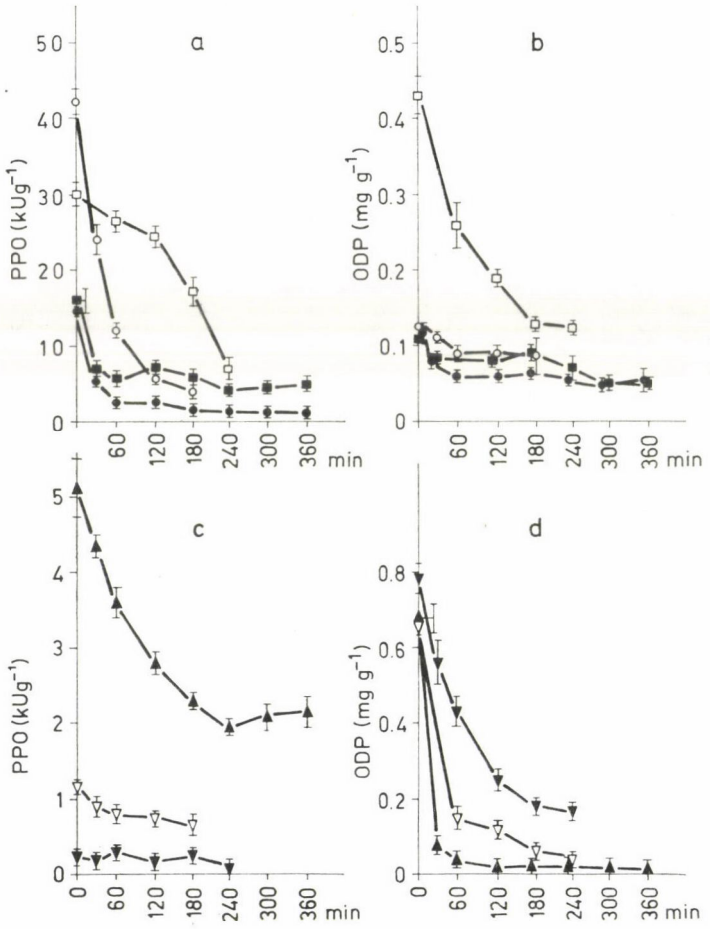

Fig. 1. Changes in polyphenol oxidase (PPO) activity and o-dihydroxy phenol (ODP) content in pulped apricots (a, b) and apples (c, d) during aeration. Cultivars: *Bibor* (circles); *Mandula* (squares); *Starking* (triangles with apex upward); *Jonathan* (triangles with apex downward). Open and solid symbols stand for data obtained with fruit picked in 1977 and 1978, resp. The points of measurements are means of 4–5 (PPO) and 3 (ODP) determinations. The vertical bars represent standard deviations

With the apricot cultivar *Mandula*, the samples picked in two consecutive years behaved differently. For the 1977 sample the decrease in enzyme activity was slower during the first than during the subsequent 120 min. For the substrate concentration, the greatest loss (40%) occurred during the first 60 min and another 30% were lost between the 60th and the 180th min. By the end of the experiment, the residual values of PPO activity and ODP content were 24% and 28%, resp. (7.3 ± 0.85 kU g^{-1} and 0.12 mg g^{-1}). For the sample of 1978, both enzyme activity and substrate concentration showed a quick drop during the first 30 min of the experiment, thereafter changes slowed down, similarly as with the two samples of the cultivar *Bibor*. By the end of aeration (240 min), the residual values were 27% (4.32 ± 0.19 kU g^{-1}) for PPO activity and 67% (0.07 mg g^{-1}) for ODP concentration.

With apples, the time course of the changes was more uniform. For the two *Jonathan* samples picked in 1977 and 1978, resp., residual values of enzyme activity were, after 180 and 240 min, 60 and 54% (0.69 ± 0.12 kU g^{-1} and 0.14 ± 0.04 kU g^{-1}). In the *Starking* sample, residual PPO activity was, after 240 min of aeration, 38% (1.96 ± 0.12 kU g^{-1}). The drop in ODP concentration was most abrupt in the *Starking* pulp: after 30 min, only 5.7% of the original substrate content were present (0.04 mg g^{-1}), these then decreased slowly to about 3% (0.02 mg g^{-1}). The *Jonathan* sample picked in 1977 had a nearly identical initial ODP content as the *Starking* sample and a considerably lower mass-related PPO activity. The decrease in substrate concentration was somewhat slower with the former: after 60 min, about 23% of the initial value were found (0.15 mg g^{-1}) and after 240 min about 6% (0.04 mg g^{-1}). The *Jonathan* sample picked in 1978 had a considerably higher ODP content than the other two apple samples and an exceptionally low mass-related PPO activity. (This was, in fact, the lowest PPO activity ever found in apples in this laboratory.) The decrease in substrate concentration was slower than in the two other apple samples. After 240 min of aeration 22% of the initial ODP content were still present (0.17 mg g^{-1}), while PPO activity was reduced to 54% of the initial value (0.135 kU g^{-1}).

From the aforesaid it is obvious that the decrease in enzyme activity corresponding to a given decrease in substrate concentration was different for the different samples. This can be shown by plotting the values of mass-related PPO activity determined after different times of aeration against the corresponding ODP concentrations (Fig. 2).

With the apple cultivars (curves 5 to 7) in which substrate concentrations were relatively higher, a considerable decrease in ODP concentration corresponded to a very slight decrease in PPO activity, while with the apricot cultivars (curves 1 to 4) in which substrate concentration relative to enzyme activity was low, the situation was reversed. In other words, less enzyme was inactivated in the samples in which substrate transformation, i.e., product formation

was more pronounced. The slopes of the curves which represent the decrease in enzyme activity corresponding to unit decrease in substrate concentration are the steeper the higher the Q values. With the samples of medium Q values (apple cultivar *Starking* and apricot cultivar *Mandula*, 1977) the curves were

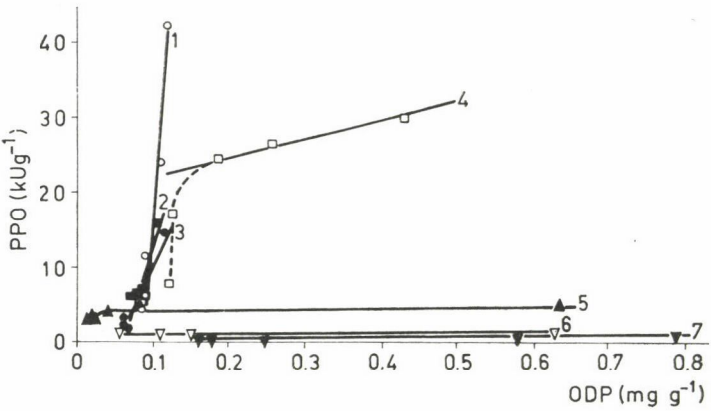

Fig. 2. Relationships between mass-related polyphenol oxidase (PPO) activity and o-dihydroxy phenol (ODP) concentration in pulped apricot and apple cultivars after different periods of aeration. Apricot cultivars: *Bibor* (circles; curves 1 and 3); *Mandula* (squares; curves 2 and 4). Apple cultivars: *Starking* (triangles with apex upward, curve 5); *Jonathan* (triangles with apex downward, curves 6 and 7). Open and solid symbols stand for data obtained with fruit picked in 1977 and 1978, resp. Regression equations of the linear parts of the curves:

1. PPO = −86.80 + 1040.45 ODP; $r^2 = 0.9672^{**}$; $n = 5$
2. PPO = −20.88 + 341.43 ODP; $r^2 = 0.9605^{*}$; $n = 4$
3. PPO = −12.06 + 225.21 ODP; $r^2 = 0.9655^{**}$; $n = 5$
4. PPO = 20.42 + 22.45 ODP; $r^2 = 0.9970^{*}$; $n = 3$
5. —
6. PPO = 0.64 + 0.818 ODP; $r^2 = 0.9979^{*}$; $n = 4$
7. PPO = 0.11 + 0.180 ODP; $r^2 = 0.9851^{*}$; $n = 4$

* and **: correlations are significant at the levels of probability of 95% and 99%, resp.

not linear over the whole range of measurements: the slopes of the curves became steeper after a certain decrease in substrate concentration, i.e., at the point where Q reached the values of 120 and 132, resp.

3. Conclusions

During the enzymatic browning reaction of apricot and apple pulp a definite dependence of the course of substrate transformation and enzyme inactivation on the initial ratio of PPO activity and ODP content was observed. Enzyme inactivation was found to be less marked, while substrate depletion was quicker in pulps of relatively higher ODP contents. These findings are contrary to expectations and not consistent either with the theory according

to which reaction inactivation of PPO is due to the binding of quinones near or at the active site of the enzyme (WHITAKER, 1972). The results equally upset the authors' working hypothesis.

The inactivation of a purified PPO preparation from apples catalyzing the transformation of an o-diphenol under the conditions of substrate saturation has been proven by WALKER (1964). Similar results have been obtained with purified banana PPO by PADRÓN and co-workers (1975).

However, with fruit pulps, the situation is different in many respects. The substrate present in the tissues is, among others, heterogeneous, being composed of various o-diphenols (NÁDUDVARI-MÁRKUS & VÁMOS-VIGYÁZÓ, 1980) towards which the affinity of the enzyme is different. In apple samples of the cultivars *Jonathan* and *Starking* the concentration of endogenous substrates was found insufficient to saturate the enzyme (VÁMOS-VIGYÁZÓ & GAJZÁGÓ, 1978). Accordingly, the results presented showed the o-diphenols in the 1977 sample of *Jonathan* and in the sample of *Starking* to be practically depleted while residual enzyme activities were still considerable (0.69 kU g^{-1} and 2.14 kU g^{-1}, resp.). In the *Jonathan* sample 1978 of exceptionally low mass-related PPO activity the substrate was not consumed during the 240 min of the experiment. However, its concentration dropped to 22% of the initial, while 54% of the initial enzyme activity were still present.

In 3 out of the 4 apricot samples investigated, the initial ODP content amounted only to 14–17% of the concentration present in apples, while mass-related PPO activity was 2.7- to 167-fold. In spite of that, substrate transformation came to a standstill at higher concentrations (0.5–0.9 mg g^{-1}) than in the samples of *Jonathan* 1977 and *Starking*. These findings suggest that perhaps not all the o-diphenols present in apricots are substrates of PPO. Investigations into the separation and identification of these compounds have been considered. In the 1977 sample of the cultivar *Mandula* neither substrate depletion nor enzyme inactivation was completely achieved during the 240 min of aeration.

Summarizing these considerations, it can be said that out of the 7 samples investigated, in 5 cases the depletion of the endogenous substrates stopped the browning reaction in the fruit. However, this occurred only after several hours of aeration and cannot be related to the initial rate of browning.

Enzyme inactivation being a consequence of quinone binding by the enzyme molecule may be ruled out under the given experimental conditions. Contradictions found in the literature clearly show that the conditions of the enzymatically catalyzed polyphenol oxidation strongly influence the mechanism of the reaction. For example, MATHEIS and BELITZ (1977) as well as RIVAS and WHITAKER (1973) both found the reaction to occur according to an ordered sequential mechanism. The former authors carried out their experiments with the enzyme in potato tissue and found O_2 to be the first substrate bound, while

the latter performed them with a purified fraction of pear PPO and reported the phenolic substrate to be bound first. Moreover, kinetic studies revealed the mechanism of the enzyme (ordered sequential and ping-pong, resp.) to depend on the nature of the phenolic substrate (RIVAS & WHITAKER, 1973).

A possible explanation of the phenomena observed is that enzyme inactivation might be caused by oxidative denaturation. Oxygen excess was higher in the pulps of lower concentrations of phenols as in these less oxygen was consumed for quinone formation. Such an assumption would be consistent with findings, however, it lacks supporting evidence.

The results did not answer the question why the initial rate of enzymatic browning depends on the ratio of PPO activity and ODP concentration in the way described in the introduction. Nevertheless, the findings that there might be conditions in which the browning reaction is stopped by factors other than product inactivation of the enzyme, seemed to us worth publishing.

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POSSIBILITIES FOR CONTROL OF PECTOLYTIC ENZYME FORMATION BY *ASPERGILLUS NIGER* IN CONTINUOUS CULTURE

Kornélia ZETELAKI-HORVÁTH

(Received: 18 June 1981; accepted: 15 July 1981)

In this work, the effect of various parameters (dilution rate, pH, carbon source concentration, oxygen transfer rate) was studied on the pectolytic enzyme formation of *Aspergillus niger* in continuous culture, with the main aim to increase the formation of pectin lyase (PL) at the expense of pectin esterase (PE).

The increase of the dilution rate from 0.03 h^{-1} to 0.08 h^{-1}, resulted in a very highly significant decrease in the specific PL activity of the culture filtrate (from 1.93 to 1.34 μmol min^{-1} cm^{-3}) while PE activity increased (from 1.56 to 1.93 mval min^{-1} cm^{-3}).

The increase in the *CCS* of the diluting medium from 2 to 3% resulted in a very highly significant increase in the specific PL activity of the culture (from 0.45 to 1.18 μmol min^{-1} cm^{-3}) while PE activity showed no remarkable change.

According to our results, at an OTR of 34 mmol l^{-1} h^{-1}, the specific PE activity proved to be the highest and decreased significantly with the increase in OTR from 34 to 140 mmol l^{-1} h^{-1}. The formation of PL was the highest at OTR = 140, very highly significantly higher, than at an OTR value of 34.

In the course of this work, apple juice clarifying and macerating effects o the pectolytic enzyme complex of *Aspergillus niger* as well as the activity of endof PG were measured, too.

Pectin lyases (PL) split pectic substrates by transelimination without water uptake. These are generally synthesized by fungi.

Pectin lyases are produced in microbial fermentation together with other pectolytic enzymes. PL is never synthesized alone, though its individual effect is remarkable without the presence of pectinesterase (PE) as purified PL showed a good clarifying and macerating effect, too (ISHII, 1976; ISHII & YOKOTSUKA, 1971a; b).

In the present work, cultural conditions were attempted to be determined, by the use of which the formation of PL and PE components of the pectolytic enzyme complex of *Aspergillus niger* can be controlled (dilution rate, pH, carbon source concentration and oxygen transfer rate).

1. Materials and methods

An *Aspergillus niger* strain (obtained after mutagenic treatment with NaN_3) was used as the microorganism.

Cultivation was carried out in an automatic fermenter (Biostat, B. Braun, Melsungen, GFR). An extract of dried sugar beet cossettes was used as carbon

source. The values of the dry matter content of the extract are referred to later as concentration of carbon source (CCS). The correlation of the dry matter (*DM*) content (measured by a refractometer) and the carbohydrate content (measured by the method of HERBERT et al., 1971) of the extract, can be

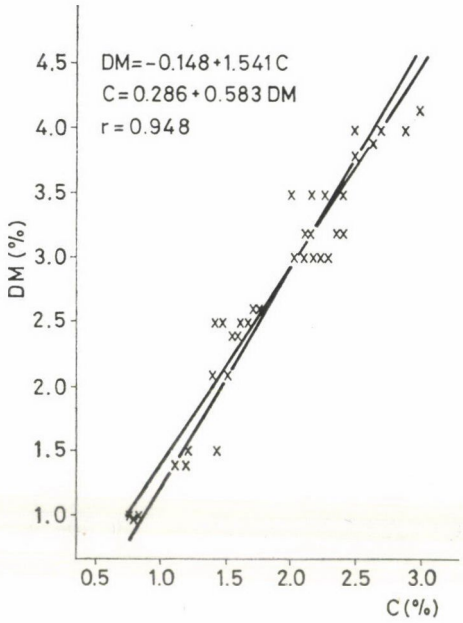


Fig. 1. Correlation between the dry matter content (DM) (measured refractometrically) and the carbohydrate content (C) (measured by the phenol method) of the extract of sugar beet cossettes

characterized by the equation given in Fig. 1. CCS of the medium in the batch culture was 4%, while in the case of the diluting medium it was 2%.

A 24-h vegetative culture was used as the inoculum, in the ratio of 500 cm^3 to 4.5 l culture medium.

Changing over to continuous cultivation started in the 30-h culture. In the course of continuous cultivation one parameter was altered, while all the others were kept constant. Under each set of fixed conditions, the system was maintained for five to six residence times.

Samples were collected automatically every four h by circulating the culture in a tube, coupling the fermentor with the sample collector placed in a refrigerator.

Mycelium was determined from the culture by filtration and its quantity was determined after drying in an oven at 105 °C.

PL activity was determined by measuring the absorption of unsaturated galacturonic acids (the reaction product of PL) by a spectrophotometer at

235 nm. The method of ALBERSHEIM and co-workers (1960) was modified by ZETELAKI-HORVÁTH (1982). Pectinesterase activity was measured according to KERTESZ (1955) and ZETELAKI-HORVÁTH (1981), resp.

Apple juice clarifying activity (a-PG) as well as the *endo-PG* (e-PG) *activity* were measured by a viscosimetric method using fresh apple juice (variety *Jonathan* with a specific viscosity adjusted to 1.0) and Na-polypectate (Serva Entwicklungslabor, Heidelberg, GFR) as substrates, and an *Ostwald* type viscometer (ZETELAKI & VAS, 1972).

Macerating activity was determined by an instrumental method (developed directly for this purpose, ZETELAKI-HORVÁTH, 1974), using potato tissue (of standard variety *(Rózsa)* and size) as the substrate.

2. Results

2.1. Effects of dilution rate

It was found in our previous work that pectolytic enzyme formation of an *Aspergillus* strain was highest at low dilution rates (ZETELAKI-HORVÁTH, 1980). The effects of dilution rates were therefore tested in a rather narrow range (from 0.03 to 0.08 h^{-1}) (Fig. 2).

Cultivation started by batch fermentation. The speed of agitation in the batch culture was 400 rpm while in the continuous culture it usually was 800 rpm. An aeration rate of $1.0\,l^{-1}\,min^{-1}$, and a temperature of 30 °C was used in both the batch and continuous culture.

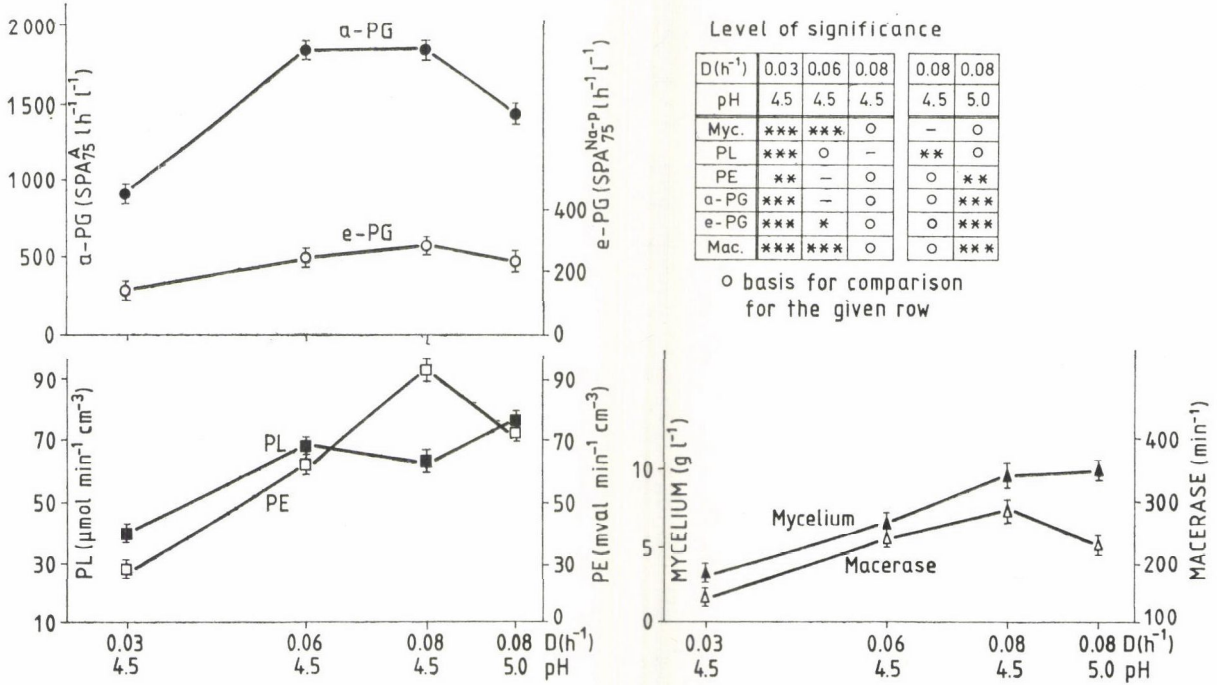
The increase in the dilution rate (from 0.03 to 0.08 g^{-1}) resulted in no significant change in the mycelium formation of the culture.

PL concentration of the culture filtrate was highest at the dilution rate of 0.03 h^{-1} and decreased very highly significantly at a dilution rate of 0.08 h^{-1}.

PE activity changed in the opposite direction than that of PL, it increased significantly with the increase of the dilution rate. PE activity of the culture diluted at a rate of 0.08 h^{-1} was highly significantly higher than that measured at 0.06 h^{-1}.

The e-PG and the apple juice clarifying activities of the culture changed proportionately with PL activity, they decreased significantly and very highly significantly with the increase in the dilution rate from 0.03 to 0.06 and 0.08 h^{-1}, resp.

When a pH of 5 was maintained in the culture instead of 4.5 at $D = 0.08\ h^{-1}$, no significant change was found in the specific apple juice clarifying activity and the mycelium yield of the culture, while macerase, PE and e-PG activities decreased significantly, highly significantly and very highly significantly, resp.



D(h^{-1})	0.03	0.06	0.08	0.08	0.08
pH	4.5	4.5	4.5	4.5	5.0
Myc.	***	***	o	–	o
PL	***	o	–	**	o
PE	**	–	o	o	**
a-PG	***	–	o	o	***
e-PG	***	*	o	o	***
Mac.	***	***	o	o	***

Fig. 2. Effects of dilution rate and pH on the growth and specific pectolytic activities of the culture filtrate of *Aspergillus niger*. Levels of significance of differences in enzyme production caused by the dilution rate (* $\leq$ P = 5%; ** $\leq$ P = 1%; *** $\leq$ P = 0.1%)

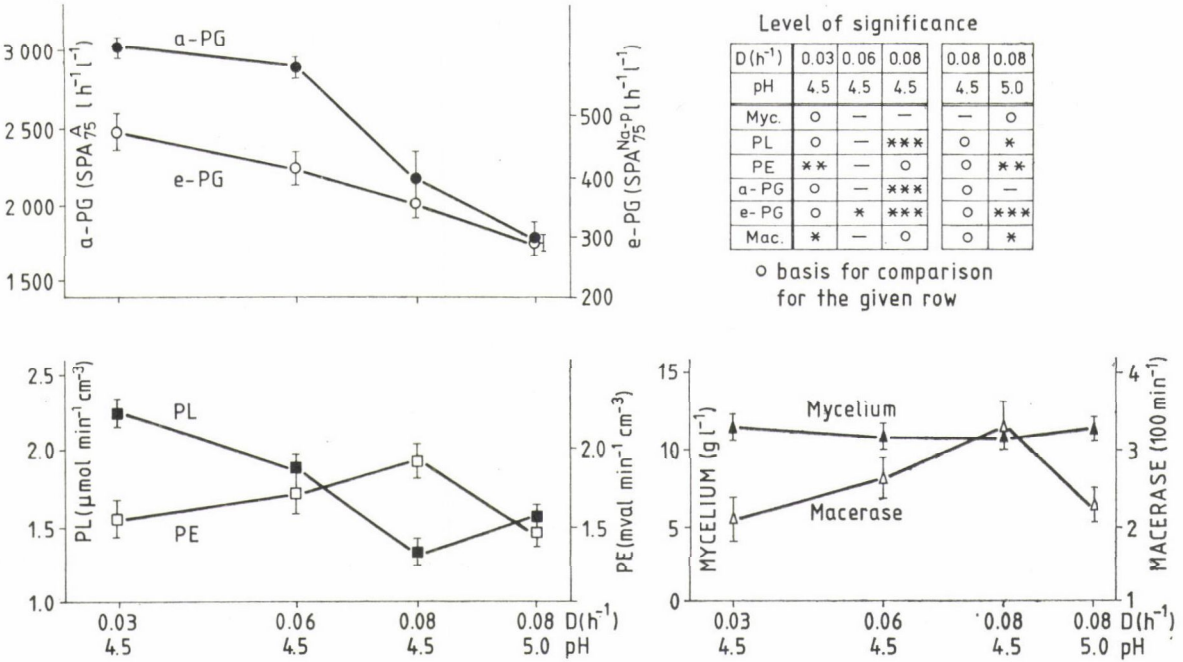


Fig. 3. Effect of dilution rate and pH on the mycelium and enzyme yield of *Aspergillus niger* (Culture volume: 10 l). Levels of significance of differences in enzyme production caused by the dilution rate (For symbols of significance level see Fig. 2)

The increase in pH from 4.5 to 5.0 showed a beneficial effect only on the biosynthesis of PL, as its activity increased significantly (from 1.338 to 1.579 μmol min^{-1} cm^{-3}).

Considering the yields in a 10–1 culture produced per h, the mycelium, macerase, endo-PG and PE showed the highest yield at $D = 0.08$ h^{-1} (Fig. 3).

The apple-juice-clarifying enzyme yield was about the same at both (0.06 and 0.08 h^{-1}) dilution rates, while the PL yield proved to be the highest at $D = 0.06$ h^{-1}, but it did not differ significantly from that obtained at $D = 0.08$ h^{-1}.

When the pH was adjusted to 5.0 instead of 4.5, a highly significant increase was found in the PL yield of the culture. With the above alteration of the pH, the other components of the pectolytic enzyme complex (a-PG, e-PG, macerase and PE, resp.) decreased very highly significantly and highly significantly, resp.

The increase of pH from 4.5 to 5.0 also resulted in an increase of mycelium yield of the culture, but not at a significant rate.

2.2. Effects of concentration of carbon source

The effect of the concentration of carbon source of the diluting medium on the formation of the various components of the pectolytic enzyme complex was also tested. The DM of the extract used, was 2 and 3%, with the following parameters of cultivation: D=0.036 h^{-1}, rpm=400, and air: 1.0 l^{-1} min^{-1} (Fig. 4).

The increase in the CCS of the diluting medium from 2 to 3% resulted in a very highly significant increase in the mycelium yield, the specific PL, e-PG and apple juice clarifying activity of the culture (from 3.8 to 4.9 g l^{-1}; 0.45 to 1 18 μmol min^{-1} cm^{-3}, from 230 to 420, and 450 to 820 l h^{-1} l^{-1}, resp.), while PE activity showed no remarkable change. The macerating activity of the culture filtrate increased also but only significantly ($P \leq 5\%$).

2.3. Effects of agitation speed

According to a number of batch experiments, made previously in our laboratory the oxygen transfer rate (OTR, as a result of various agitation and aeration conditions) had a considerable effect on the pectolytic enzyme formation of *Aspergilli* (ZETELAKI-HORVÁTH, 1972; ZETELAKI-HORVÁTH, 1978; ZETELAKI-HORVÁTH & BÉKÁSSY-MOLNÁR, 1975). An OTR of 60 mmol l^{-1} h^{-1}, was found to be optimal for the PL synthesis of *Aspergillus niger* in batch culture (ZETELAKI-HORVÁTH & VAS, 1981) carried over in the 10–l Kutesz fermentor (made in Hungary).

To obtain a thorough mixing of the filamentous culture, an agitation speed lower than 400 rpm could not be used in the Biostat system. This is why

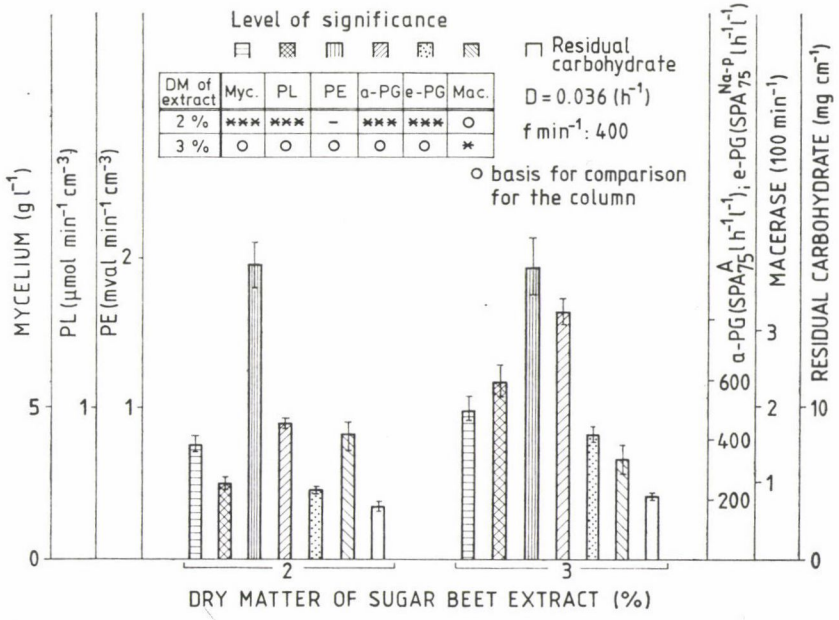


Fig. 4. The effect of carbon source concentration on the growth and pectolytic enzyme formation of *Aspergillus niger*. Levels of significance of differences in enzyme production caused by the CCS. (For symbols of significance level see Fig. 2)

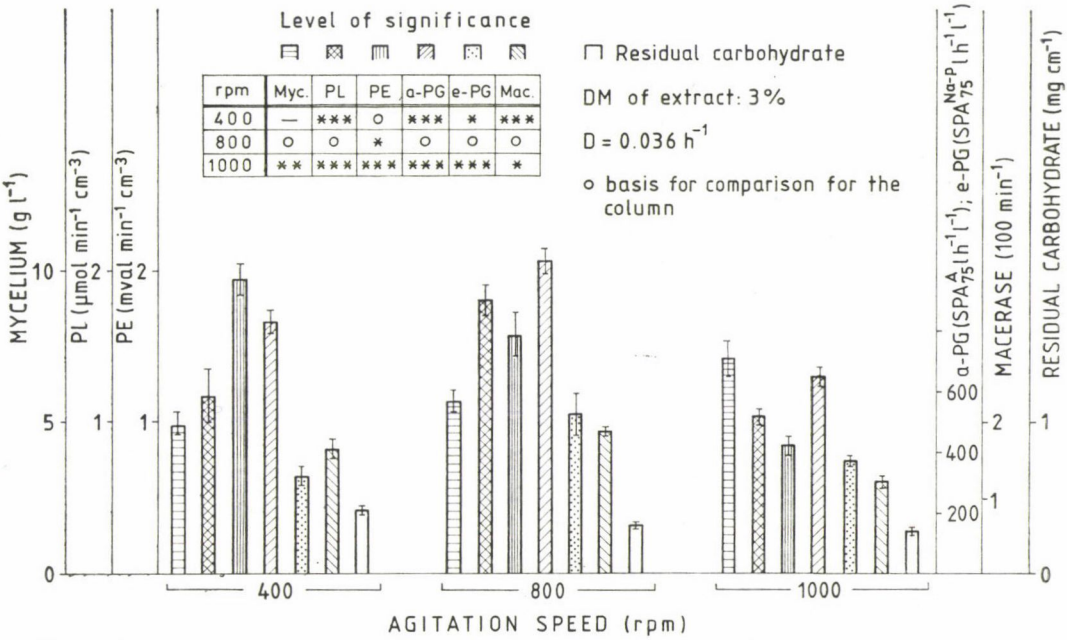


Fig. 5. The effect of agitation speed on the growth and pectolytic enzyme formation of *Aspergillus niger*. Levels of significance of differences in enzyme production caused by the agitation speed. (For symbols of significance level see Fig. 2)

agitation speeds of 400, 800 and 1 000 rpm were used, ensuring the following OTR values: 34, 140 and 197 mmol l^{-1} h^{-1} (measured by the method of COOPER et al., 1944) (Fig. 5).

With the increase of the agitation speed from 400 to 800 rpm the growth of the culture did not change significantly, while a further increase to 1 000 rpm, resulted in a highly significant increase in the mycelium yield.

Among the three tested speeds of agitation, the use of 800 rpm resulted in the highest specific a-PG and e-PG activities and the highest macerating activity as well. The specific PE activity proved to be the highest at the lowest rpm tested (34 mmol l^{-1} h^{-1}), and decreased significantly with the increase of agitation speed from 400 to 800 rpm. The formation of PL showed again a different response from that of PE to the oxygen supply of the culture. It was the highest at 800 rpm, and decreased very highly significantly at 400 rpm.

3. Discussion

As PL can split the highly esterified pectin molecule itself, the presence of PE in the enzyme complex is not necessary.

This is why the main intention of this work was to increase the PL synthesis of *Aspergillus niger* at the expense of PE. This aim, however, was accomplished to a lesser extent than it was hoped.

A certain increase in PL synthesis at the expense of PE was attained with the alteration of several parameters.

The decrease of the dilution rate from 0.08 to 0.03 h^{-1} increased the specific PL and decreased the specific PE activity of the culture. This means that, at $D = 0.03$ h^{-1}, PL production of the culture predominated over that of PE (PL: 34 740 μmol h^{-1}, PE: 28 800 mval h^{-1}), while at $D = 0.08$ h^{-1}, PE synthesis became preponderant (PE: 92 640 mval h^{-1}, PL: 64 320 μmol h^{-1}).

As the pH optimum for PL activity of *Aspergillus niger* proved to be 6.0 (ZETELAKI-HORVÁTH, 1982), increasing the pH of the culture medium towards the activity optimum of the enzyme (from 4.5 to 5.0) increased the PL yield from 54 000 to 76 000 μmol h^{-1}, while the PE yield decreased from 93 000 to 72 000 mval h^{-1}, resp. In the case of a long term continuous cultivation, pH higher than 5.0, is not advisable from the point of view of avoiding contamination.

The increase in agitation speed from 400 to 800 rpm, increased the specific PL activity of the culture from 1.18 to 1.81 μmol min^{-1} cm^{-3}, and decreased that of PE from 1.95 to 1.5 mval min^{-1} cm^{-3}. This means that, at 800 rpm, the PL synthesis was predominant over PE (PL: 39 100 μmol h^{-1}, 32 400 mval h^{-1}), while at 400 rpm the order was reversed (PE: 42 100 mval h^{-1}, PL: 25 490 μmol h^{-1}).

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CHANGES IN THE QUALITY OF GROUND BLACK PEPPER PACKAGED IN DIFFERENT MATERIALS DURING STORAGE

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Changes in the quality of ground black pepper packaged in different materials was studied as a function of storage time at room temperature and of packaging material. Pouches made of poly(vinylidene chloride)-emulsion treated kraft paper and of aluminium foil–kraft paper–polyethylene laminate and jars fitted with plastic sprinkler were used as packagings.

The moisture content and volatile oil content of the black pepper samples was determined, the UV spectrum of the volatile oils was taken and changes in the sensory properties were followed up during storage. By mathematical statistical evaluation of the results it was established that changes in quality were reflected by changes in the volatile oil content.

As regards the packaging materials the quality of ground black pepper was maintained for the longest period in the jar, PVDC-coated paper being the second best and pouches made of the combined foil the poorest.

Seasonings are considered in trade as goods of long shelf life because changes in the quality or quantity of their components are very slow. Therefore, to study these changes chemical, physico-chemical methods of analysis and sensory evaluation methods sufficiently sensitive to follow up these slow changes, had to be used.

Generally the following parameters of quality are determined in the analysis of seasonings: moisture content, ash content (total and water-soluble and acid insoluble), alcohol and ether extract, volatile oil content, nitrogen, arsenic, lead, copper as well as starch content, physical contamination and microscopic characteristics (PEARSON, 1976).

Some of these characteristics are not at all affected by the conditions of storage (time, temperature, relative humidity, light, packaging) and are of importance only in the quality test upon receipt. As a first step, therefore, the properties, the changes of which are easily followed up and represent reliably the quality changes, had to be established.

The seasoning capacity of pepper depends on the quantity and quality of its volatile oil content and the quantity and quality of the pungent principle contained.

The volatile oil content of pepper is about 0.5–1.0% and consists mainly (to at least 95%) of hydrocarbons and to at most 4% of oxygen containing substances. The terpenes contained in pepper oil fall into two fractions: about

70–80% of them is monoterpene while 30–20% is sesquiterpene. The main components of the monoterpene fraction are α and β pinene, sabinene, Δ^3 carene and limonene; the most important sesquiterpene is β cariofillene. However, the aroma of pepper is highly affected by the relatively small amount of oxygen containing components. The most important of them are: linalool, 1-terpene-4-ol, α-terpineol, methyl-eugenol, nerolidol, myristicin.

Beside the above mentioned compounds about another 50–60 components were extracted from pepper oil, but not all of them were as yet identified. Volatile oils are analysed by gas chromatography or with large instruments (e.g. mass-spectrometry, infrared spectrometry) (MAARSE & NIJSSEN, 1980). The sensory differences existing between pepper varieties are determined by the quantity and proportion of the volatile oil components (SALZER, 1975a, b; HEATH, 1973).

Mainly responsible for hotness in pepper are piperine and piperylene, detectable in the extract; however, the sensation of pungency is caused by an alkaloid mixture the composition of which has not been completely elucidated, but 98% of which is definitely piperine. Originally piperine has been determined by colorimetry and spectrophotometry (SHANKARANARAYANA et al., 1970), now thin-layer chromatography may also be used (WIJESEKERA et al., 1972).

It has been established that the shelf life of seasonings is not affected by the extent of their microbiological contamination (KALMÁR & FEHÉR, 1981) therefore this topic is not discussed in this study. Under optimum conditions of storage (absence of subsequent contamination or increased water activity, etc.) contamination hardly changes at all and during a longer period rather decreases. The initial microbiological condition of a seasoning has to comply with the requirements as laid down in the regulation of the HUNGARIAN MINISTER OF HEALTH (EÜM 6/1978/VII. 14).

The aim of this study was to determine first the critical characteristic or characteristics, which, under the given conditions of storage, change most rapidly and thereby reflect changes in quality and secondly to find the packaging material, on the basis of technical and economic points of view, that ensures the best protection to the seasoning under the given conditions of trading.

1. Materials and methods

1.1. Materials used in the study

1.1.1. Pepper. Ground pepper sample was obtained from Compack Trading Packaging Enterprise, Budapest. The sample originated from the consignment arriving at Budapest from Brasil on 6 of February, 1979.

The consignment travelled about two and a half months. It was packaged in two-layer jute sacks. At the Compack enterprise the sacks were stored in a provisional store built of corrugated aluminium sheets, 8 sacks to one pallet, 3 pallets one on the top of the other. The average temperature of the store was 15.5 °C during the 4 months while the product remained there and the average relative humidity was 57.7 %.

The sample taken at random from the sacks was ground to a powder falling through a sieve of 0.8 mm mesh (HUNGARIAN STANDARD, 1975).

Samples were packaged partly by machine, partly by hand. The poly(vinylidene chloride)-coated paper pouches were filled with 50 g ground pepper, while the pouches made of the combined foil with 25 g and the jars with 60 g, each.

1.1.2.1. Pouches made of paper coated with poly(vinylidene chloride)-emulsion. – This is the packaging material used in retail trade at present. The wood-free kraft paper of 70 g m^{-2} mass was surface-coated with 22–28 g m^{-2} poly(vinylidene chloride)-emulsion. Hereafter the marking "paper + PVDC" will be used.

1.1.2.2. Pouches made of combined foil. – This is the laminate of an aluminium foil of 0.012 mm, a kraft paper of 40 g m^{-2} type and a polyethylene foil of 0.02 mm thickness. Hereafter the name "combined foil" will be used.

1.1.2.3. Glass jar with sprinkler. – The jar used was of colourless glass enveloped in paper and provided with a plastic cap. It was of 40 mm width and 100 mm height. The cap (sprinkler) was of polyethylene stained white. Hereafter the name "glass jar" will be used.

1.2. Methods

1.2.1. Storage. Samples packed in pouches were stored in cardboard boxes, 40 or 80 pouches to one box and kept on a laboratory stand. The temperature and relative humidity of the room was measured and recorded with a TZ 18 type thermohygrograph (made in Poland). During the storage period the temperature in the room varied between 18 and 27 °C (T_{av} = 22.1 °C).

The relative humidity varied between 45 and 65 %, with an average of 52.8 %. During the storage period of 15 months the samples were tested every 3 months.

1.2.2. Moisture content. The method laid down in HUNGARIAN STANDARD (1955) was used, specified for the essential oil content of spices.

Procedure: 20 g of the sample were weighed on an analytical balance. These were transferred without loss to a 500 cm^3 round-bottom flask. Some anti-bumping granules and about 300 cm^3 toluene previously saturated with water were added. The flask was then fitted with a Marcusson receiver. The

latter was also filled with toluene saturated with water and finally distillation was started.

During the determination the condenser was rinsed several times with water-saturated toluene to remove the drops of water adhering to the Marcusson receiver. Distillation was continued till the amount of water passing through the apparatus did not change. The volume of water accumulated in the receiver was read. Three parallel determinations were made.

1.2.3. Determination of the volatile oil. The method given in the HUNGARIAN PHARMACOPOEIA (1967), was used.

Twenty g of the seasoning were weighed in a round-bottom 1 000 cm^3 flask and filled up with 500 cm^3 distilled water. Anti-bumping granules were added and the condenser was mounted. Through the upper opening the V-shaped part of the condenser was filled to transflow with water. After starting the cooling water the heater was switched on. On reaching the boiling point the distillation was maintained for 4.5 h, shaking the flask occasionally in between to wash down the particles adhering to the wall. The volatile oil was collected in the graduated cylinder separated from the watery phase. Twenty to thirty minutes after stopping distillation the volume of oil was read. Distillation was restarted and continued for 1 h and the volume of the oil was read again. If the increase between the two measurements was below 5% of the total volume the determination was considered finished, if not, distillation was continued. When distillation was finished the final volume of the oil was read.

1.2.4. UV spectrum of the volatile oil. The oil sample obtained by steam distillation was kept in closed vials, in the dark, at room temperature till it was used for determining the UV spectrum.

Photometric measurements were carried out in the UV range between 190 to 360 nm. The spectrophotometer used was Beckman Model 26 type. For the measurement 50 nm^3 of the oil were dissolved in 250 cm^3 of 96% ethyl alcohol. Of each sample three parallel master solutions were prepared. One cm^3 of the master solution was diluted to 25 cm^3. In order to reduce error due to dilution further dilutions were made in 5 parallels, each. The 1:125 000 dilution was used to measure light absorption. The 96% ethyl alcohol was used for reference.

To be able to evaluate the UV spectrum the determination of the relative number of showing the percentage of volatile oil related to the value at the beginning of the storage experiment, was necessary. This relative number was marked by the letter S and was calculated by the following equation:

$$S(\%) = \frac{I \cdot A}{I_0 \cdot A_0} \cdot 100$$

where

I = volatile oil content (cm^3 per 100 g seasoning),

A = light absorption,

I_0 = volatile oil content of the original sample (cm^3 per 100g seasoning),

A_0 = light absorption as measured in the original sample.

1.2.5. Sensory tests. Sensory tests were carried out according to the taste profile analytical method elaborated by MIHÁLYI and ZUKÁL (1972) taking into account the specifications of selecting the panel members as laid down in HUNGARIAN STANDARD (1976).

The samples for sensory test were prepared as follows. One g of the seasoning was shaken in an iodine number flask of 100 cm^3 with 20 g water clarified with active carbon. It was then extracted over a water bath at 60 °C. Subsequently 40 cm^3 clarified water were added and a suspension was prepared by mixing at 1 500 rpm for 2 min in a turmix apparatus. The suspension thus obtained was used to prepare a series of dilutions corresponding to an exponential sequence.

Ten-member panels carried out the sensory tests starting at the most dilute solution. Their task was to establish whether the taste may be sensed in the given solution and its character (characteristic flavours: slightly sour, aromatic, pungent, spicy). Scores were recorded. Not more than 8 samples were tested at a time by one panelist.

1.2.6. Evaluation of the results. Results were evaluated by mathematical statistical methods (SVÁB, 1967). The effect of the individual factors (storage period, packaging material) was tested by two-way analysis of variance. To judge the differences between values the significance of differences was calculated (SD). The results of sensory tests were evaluated after summarizing the scores by means of the contingency tables. Conditions of evaluation were: the expected frequency should be at least 2, the value of the empirical frequency should be higher than that of the expected frequency and the value of χ^2 for one field should be at least 1 or above. The characteristic flavour belonging to each dilution, or the boundary of dilution belonging to each flavour, in other words the dilution value at which the flavour tested may still be sensible, was established from the tables. This method of evaluation does not permit the determination of deviations belonging to limit dilutions. Thus to evaluate the results the limit values of dilution belonging to the "spicy flavour", characterizing the seasoning capacity of the samples, were given.

To fit and analyse the curves of two variables methods of regression analysis were used.

The differences significant at the $P \leq 5\%$ level were considered *significant*, those significant at the $P \leq 1\%$ level *highly significant* and those significant at the $P \leq 0.1\%$ level *very highly significant* and were marked with 1, 2 or 3 asterisks, respectively.

2. Results

2.1. Changes in the moisture content

The average values of moisture content as measured in the ground pepper samples packaged in different materials as a function of storage time and the deviation, as well as the significance levels of differences as established vs. packaging material, are contained in Table 1.

It was found that while the differences in the moisture content of the samples as a function of storage time were very highly significant, as a function of packaging material were not significant.

Table 1

Average values ($\bar{x}$) and standard deviations ($\pm s$) of the moisture content of ground black pepper samples in different packaging materials determined vs. *storage time and analysis of variance of the results*

Storage time (months)	Moisture content (%)					
	(1) Paper + PVDC		(2) Combined foil		(3) Glass jar	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
0	9.75	0.25	9.75	0.25	9.75	0.25
3	9.92	0.14	9.58	0.14	9.58	0.14
6	8.25	0.66	8.25	0.43	7.67	0.58
9	8.75	0.43	9.00	0.25	8.92	0.38
12	8.17	0.38	8.42	0.38	8.67	0 14
15	8.75	0.25	9.08	0.38	8.28	0.25

Source of variance	Square sum of differences	Degree of freedom	Square sum of mean differences	*F* (calculated)	*F* (table)	*P* (%)
Packaging material	0.12	2	0.062	0.94	4.10	5
Storage time	6.99	5	1.398	21.22	10.48	0.1
Residue	0.66	10	0.066	—	—	—
Total	7.77					

$SD_{5\%} = 0.47$; $SD_{1\%} = 0.66$; $SD_{0.1\%} = 0.96$

Mark of the packaging material	Significance level of differences									
	Storage time (months)									
	3		6		9		12		15	
	Mark of the packaging material									
	1	2	1	2	1	2	1	2	1	2
2	Ø		Ø		Ø		Ø		Ø	
3	Ø	Ø	*	*	Ø	Ø	*	Ø	*	**

Ø: $P \geq 5\%$; *: $P \leq 5\%$; **: $P \leq 1\%$;

It was of interest to note that the moisture content of the samples kept in glass jars changed similarly to those packaged in pouches. The reason for this may be found in the insufficient impermeability of the plastic caps used for closing. However, this did not form the subject of this study.

2.2. Changes in the essential oil content

The average values and standard deviations of essential oil content as measured in samples stored in different packaging materials as a function of time and the significance of differences as established as a function of packaging material, are summarized in Table 2.

Table 2

Average values ($\bar{x}$) and standard deviations ($\pm s$) of the essential oil content of ground black pepper samples in different packaging materials vs. *storage time and analysis of variance of the results*

Storage time (month)	Essential oil content (cm^3 per 100 g spice)					
	(1) Paper + PVDC		(2) Combined foil		(3) Glass jar	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
0	1.10	0.02	1.10	0.02	1.10	0.02
3	0.82	0.10	0.69	0.04	1.03	0.07
6	0.80	0.07	0.67	0.04	0.99	0.05
9	0.73	0.06	0.69	0.08	0.95	0.10
12	0.68	0.15	0.60	0.09	0.92	0.08
15	0.56	0.07	0.58	0.06	0.81	0.06

Source of variance	Square sum of differences	Degree of freedom	Square sum of mean differences	F (calculated)	F (table)	P (%)
Storage time	0.35	5	0.07	14.47	4.76	0.1
Packaging material	0.20	2	0.10	20.29	7.76	0.1
Residue	0.04	10	5.10^{-3}	—	—	—
Total	0.59					

$SD_{5\%} = 0.10$; $SD_{1\%} = 0.14$; $SD_{0.1\%} = 0.20$

Mark of the packaging material	Significance level of differences									
	Storage time (months)									
	3		6		9		12		15	
	Mark of the packaging material									
	1	2	1	2	1	2	1	2	1	2
2	**		**		Ø		Ø		Ø	
3	***	***	***	***	***	***	***	***	***	***

Ø: $P \geq 5\%$; *: $P \leq 5\%$; **: $P \leq 1\%$; ***: $P \leq 0.1\%$

The essential oil content diminished continuously in all three packaging materials during storage but the difference in degree was highly significant. The loss was lowest in the glass jars while highest in the pouches made of combined foil.

2.3. UV spectrum of the volatile oil

The essential oil obtained by steam distillation was photometrically investigated in the UV range of the spectrum. The maximum of the essential oil dissolved in ethyl alcohol was found to be at a wavelength of 202 nm.

Table 3

Average values ($\bar{x}$) and standard deviations ($\pm s$) of S values calculated from the UV absorption of the essential oil of ground black pepper samples in different packaging materials as well as results of the analysis of variance

Storage time (months)	S(%)					
	(1) Paper + PVDC		(2) Combined foil		(3) Glass jar	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
0	100	6.0	100	6.0	100	6.0
3	46.2	6.2	46.5	4.2	85.8	6.6
6	57.9	6.1	55.2	5.5	77.7	5.7
9	61.2	6.3	60.1	7.9	78.0	12.6
12	65.3	16.9	55.7	8.9	84.0	8.6
15	47.8	6.4	53.0	9.5	67.8	6.1

Source of variance	Square sum of differences	Degree of freedom	Square sum of mean differences	F (calculated)	F (table)	P (%)
Packaging material	1 574.68	2	787.34	13.22	7.56	5
Storage time	3 753.84	5	750.77	12.60	10.48	0.1
Residue	595.65	10	59.57	—	—	—
Total	5 924.18					

$SD_{5\%} = 0.47$; $SD_{1\%} = 0.66$; $SD_{0.1\%} = 0.96$

Mark of the packaging material	Significance level of differences									
	Storage time (months)									
	3		6		9		12		15	
	Mark of the packaging material									
	1	2	1	2	1	2	1	2	1	2
2	Ø		Ø		Ø		Ø		Ø	
3	***	***	*	**	*	*	*	**	**	*

Ø: $P \geq 5\%$; *: $P \leq 5\%$; **: $P \leq 1\%$; ***: $P \leq 0.1\%$

The average and deviation values of S as calculated from the absorption maxima vs. packaging material and storage time, as well as the significance of differences as a function of packaging materials, are shown in Table 3.

The S values differed at a very highly significant degree as a function of both storage time and packaging material. The highest S value was measured at every length of storage time in samples kept in glass jars. The difference between the other two packaging materials was non-significant.

2.4. Sensory tests

The changes in the pepper samples during storage were followed up by sensory tests. The limits of dilution related to "spicy flavour" characteristic of the changes were established by means of contingency tables. The values

Table 4

Values of limit dilutions of spicy flavour obtained from sensory tests carried out to establish changes during storage at room temperature in the ground black pepper samples packaged in various materials as well as the results of analysis of variance

Storage time (months)	Limit dilution (kg water per g spice)		
	(1) Paper + PVDC	(2) Combined foil	(3) Glass jar
0	48	48	48
3	24	36	48
6	12	24	24
9	24	12	24
12	24	12	24
15	24	12	12

Source of variance	Square sum of differences	Degree of freedom	Square sum of mean differences	F (calculated)	F (table)	P (%)
Packaging material	112	2	56	1.00	4.10	5
Storage time	2 358	5	474	8.46	5.64	1
Residue	560	10	56	—	—	—
Total	3 040					

$SD_{5\%} = 23.6$; $SD_{1\%} = 33.5$; $SD_{0.1\%} = 48.6$

Mark of the packaging material	Significance level of differences									
	Storage time (months)									
	3		6		9		12		15	
	Mark of the packaging material									
	1	2	1	2	1	2	1	2	1	2
2	Ø		Ø		Ø		Ø		Ø	
3	*	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø

Ø: $P \geq 5\%$; *: $P \leq 5\%$

thus established and the differences in significance levels as a function of packaging materials are contained in Table 4.

The seasoning capacity of ground pepper was found to diminish during storage. The difference between values as measured at start and in the 3rd month of storage, was significant. However, the seasoning capacity of samples stored in different packaging materials did not differ significantly.

3. Conclusions

On the basis of this study it may be established that out of the different quality characteristics the change of the essential oil content follows most closely the change in the quality of pepper.

It was found by means of covariance analysis that the change in the essential oil content of pepper samples stored in three different packaging materials may not be described by a single spoilage curve, thus, it may be concluded that the shelf life varies with the packaging material.

The change in time of the essential oil content of the stored pepper samples was of an exponential character. This is proven by the correlation coefficients belonging to the curves describing the change.

The curves plotted on the basis of the equations for each packaging material, showing the measuring points, are illustrated in Figs. 1, 2 and 3.

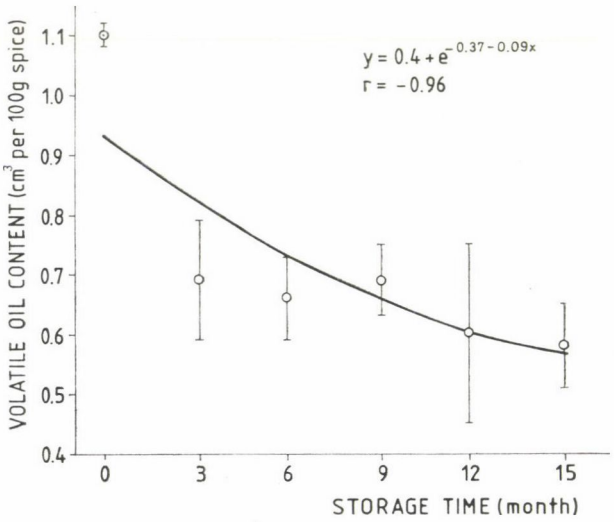

Fig. 1. Changes in the essential oil content of ground black pepper packaged in PVDC coated paper as a function of storage time. The curve represents the change in the essential oil content calculated by the equation given. $\overset{\top}{\underset{\perp}{\circ}}$ stands for the average values and their standard deviations as measured at different points of time

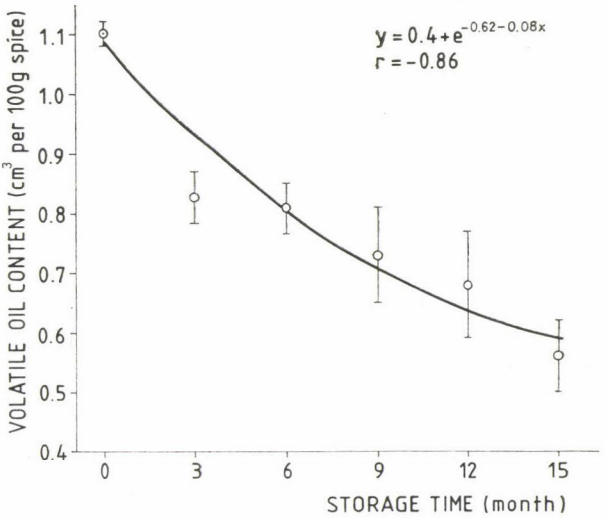

Fig. 2. Changes in the essential oil content of ground black pepper packaged in combined foil as a function of storage time. The curve represents the change in the essential oil content calculated by the equation given. ⊺ stands for the average values and their standard deviations as measured at different points of time

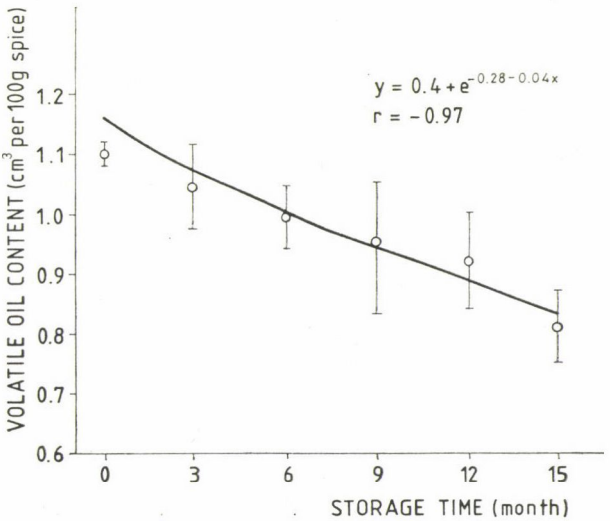

Fig. 3. Changes in the essential oil content of ground black pepper packaged in glass jars as a function of time. The curve represents the changes in the essential oil calculated by the equation given. ⊺ stands for the average values and their standard deviations as measured at different points of time

Using the results of measurements the inverted solutions of the equations given in the Figures were established and therefrom the storage stability and the confidence intervals belonging to them at the probability level of $P \leq 1\%$.

Out of the characteristics of quality the essential oil content was found to be the one most rapidly changing. The quality of ground black pepper is maintained during the longest period in glass jars (16.8 ± 0.23 months); the next best protection is ensured by the PVDC-coated paper pouches (6.4 ± 1.16 months) and the last ones are pouches made of the combined foil (4.8 ± 1.08 months). In accordance with general practice the period during which the quality is maintained is understood to mean the period during which the loss in the essential oil reaches 33%.

Sensory tests have shown the seasoning capacity of ground black pepper to be reduced to 50% of the original in the 3rd months of storage when packaged in the PVDC-coated paper pouches, while in glass jars and in pouches of combined foil this occurred only in the 6th months of storage.

It may be concluded that the method used proved to be suitable to follow up changes in the quality of black pepper. The evaluation method was suitable to establish the shelf life of spice. Results enable the development of the economically most feasible packaging.

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EXPERIMENTAL METHOD FOR THE DETERMINATION OF THE THERMAL DEATH PARAMETERS OF MICROORGANISMS IN A CONTINUOUS SYSTEM

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The determination of the thermal death parameters of microorganisms in a continuous system is carried out with accounting for the residence time distribution in a flow reactor mixed to perfection.

In a reactor of controlled temperature mixed to perfection the suspension of microorganisms of known viable cell density is fed in a flow of constant volume. The viable cell count is determined in the out-going fluid. In the case of the applied experimental layout the death rate coefficient may be determined from the quotient of the in-going and out-going viable cell density in contrast to traditional methods, where the basis of calculation is formed by the logarithm of the quotient of the initial and surviving cell counts. Thus, this new method is suitable to determine experimentally death rate coefficients 1–1.5 orders of magnitude higher than the traditional ones, while at lower temperatures the results correspond to those obtained by the traditional methods.

The test organisms used in the experiments were the yeast strains *Saccharomyces cerevisiae* and *Rhodotorula rubra* and the bacteria *Escherichia coli* and *Streptococcus faecalis*. The temperature range applied was 323–349 K (50–76 °C). In this range the logarithm of death rate coefficients increased linearly with increasing temperature.

The lowest decimal reduction times determined in the experiments were about 0.003 min.

The basis for calculating thermal treatments as applied in the industry to kill microorganisms (pasteurization, sterilization) is the knowledge of the correlation between death rate and change of temperature. This correlation is generally determined experimentally by thermal death studies under isothermal conditions. The thermal death curve is plotted in the knowledge of death rate coefficients and decimal reduction times determined at different temperatures. This forms then the basis of calculation of thermal treatment.

The industrial equipments for heat treatment operate, in order to achieve sufficiently high reduction of the viable cell count, generally at a higher temperature than that applied in the experimental determination of the thermal death curve. This carries the danger of estimating erroneously the value of the effective death rate coefficients and decimal reduction times when extrapolating the correlation determined experimentally to industrial heat treatment. This uncertainty is counterbalanced in practice by overestimating the heat treatment requirement.

In evaluating thermal death experiments a kinetic of the first order is usually assumed and the calculation of decimal reduction time determined

at constant temperature is based on this. Stumbo's formula (STUMBO, 1948) is used mostly:

$$D = \frac{U}{\log N_0 - \log N_u}$$

where

D = decimal reduction time,
U = period of heat treatment,
N_0 = initial viable cell concentration,
N_u = viable cell density after U time.

Since there is not much likelihood of the viable cell count to exceed the value of 10^8–10^9 cell cm^{-3} and the time still measurable exactly is taking into account the conditions of heat transfer, practically 0.5 min, the shortest decimal reduction time measurable in this way is about 0.06 min or 3–4 s.

This value is valid not only for experiments carried out in a static system but is valid for decimal reduction times as experienced in isothermal tube reactors in continuous systems. AIBA and co-workers (1965) described some examples of this kind of experimental scheme. Measuring the thermal death parameters of *Escherichia coli* in a continuous system the highest death rate coefficient found by them was 25 min^{-1} and this corresponds to about 0.09 min decimal reduction time.

When higher decimal reduction times are determined at lower temperatures any number of points on the thermal death curve may be established. If it is possible to exclude the disturbing factors of individual measurements (different media of heat treatment, different nutrient media, changes in the physiological conditions of the test organism, etc.) the thermal death curve may be established with high exactitude.

However, the upper limit of the experimentally measurable temperature range is generally lower than the operating temperature of pasteurization or sterilization equipments.

The uncertainty originating from the extensive extrapolation of the thermal death curve as determined from the data measured in the narrow interval can only be counterbalanced by an exaggerated heat treatment. This solution, however, requires much energy and reduces at the same time the quality of the product (hedonic value, vitamin content, etc.). Thus, it seems desirable to determine also, by experiments, the thermal death parameters of microorganisms in a wider interval, extended in the direction of the temperature of heat treatment because thereby the heat treatment may be determined with greater accuracy. To achieve this, a method was developed which enables the experimental determination of death rate coefficients of the order of 10^3 min^{-1} or of corresponding decimal reduction times of 0.002 min.

1. Materials and methods

1.1. Microbial strains

1.1.1. Saccharomyces cerevisae. The strain used was obtained from the Microbiological Gene Bank, Budapest, and was marked KE 150.

It was maintained and cultivated in a nutrient medium of the following composition: 40 g glucose; 10 g peptone; 10 g yeast extract; 20 g agar; 1000 cm^3 water, at a pH of 5.8. The storage temperature was 288 K (15 °C); cultivation temperature: 303 K (30 °C).

1.1.2. Rhodotorula rubra. Isolated from a fruit processing plant and identified at the Microbiological Department Group, University of Horticulture, Budapest.

The nutrient medium used for maintaining and cultivating the strain and the temperatures applied in storage and cultivation were the same as under 1.1.1.

1.1.3. Escherichia coli. Obtained from the Microbiological Gene Bank, Budapest, marked B 200. It was maintained and cultivated in a nutrient medium of the following composition: 1 g glucose; 5 g peptone; 2.5 g yeast extract; 20 g agar; 1 000 cm^3 water, at pH 7.0.

1.1.4. Streptococcus faecalis. The strain was isolated by the Department for Food Hygiene of the University of Veterinary Sciences, Budapest.

Nutrient medium used for maintaining and cultivating the strain: 2.5 g glucose; 10 g yeast extract; 17 g tryptone; 3 g soya-peptone; 5g NaCl; 5.5 g K_2HPO_4; 20 g agar; 1 000 cm^3 water, at pH 7.3.

The storage temperature was 288 K (15 °C) and the cultivation temperature 310 K (37 °C).

1.2. The heat treatment medium

was sterile physiological saline solution.

1.3. Determination of the viable cell count

The viable cell count was determined by diluting aliquots of the samples with a solution of 9 g l^{-1} NaCl + 1 g l^{-1} peptone, applying the decimal dilution assay technique and subsequent plating. The nutrient medium as described under 1.1. was used. For the yeasts an incubation period of 3–4 days, for bacteria that of 2–3 days was applied.

1.4. Methods of analysis

1.4.1. Isothermal heat treatment in a batch system. Four test tubes of slant agar cultures were added in the form of a dense suspension of about 10 cm^3 to 400 cm^3 heat treatment medium set at the appropriate temperature. Temperature was adjusted by the hot water flowing in the heating spiral

immersed in the medium. The temperature of the heating water was set with a Medingen U-10 type ultrathermostat. The suspension of microbs was mixed with the magnetic mixer Radelkisz (Hungary) OP 912/3 type. At predetermined points of time samples were taken from the suspension and the viable cell count was determined. The death rate coefficient and the decimal reduction time were calculated from the regression equation of the linear section of the survival curve.

1.4.2. Anisothermal heat treatment in a batch system. The essence of the method is that by it becomes possible to construct the thermal death curve by a series of measurements in a single identical system (REICHART, 1979).

The temperature of the microbial suspension to be investigated is continuously increased and the data of viable cell count determined point by point and the temperature are plotted against time. The death rate coefficients and the decimal reduction times belonging to different temperatures are determined from the difference quotients according to time of the anisothermal survival curve. Since neither the age of the culture, nor the heat treatment medium, nor the composition of the nutrient medium changes in the course of the experiment the results reflect changes occurring solely as consequence of temperature.

The experimental equipment corresponded to that described in section 1.4.1. The temperature of the heating water was set at 343 K (70 °C) and this was circulated in the heating spiral. The temperature of the heat treatment medium containing the suspension of microbes and originally of room temperature, increased in accordance with the transitional function of the control-technically first order elements. Upon reaching 323 K (50 °C) temperature samples were taken from the suspension mixed to perfection, every 0.5 min and the temperature of the medium was recorded. The death rate coefficients and decimal reduction times belonging to given temperatures were calculated from the change in the viable cell count of the samples.

1.4.3. Heat treatment in a continuous system. In determining the death rate coefficient in a continuous system the residence time distribution of an ideal stirred tank reactor formed the starting point.

The density function of the residence time distribution in a reactor tank mixed to perfection (DENBIGH & TURNER, 1971):

$$f(t) = \frac{1}{\bar{t}} e^{-\frac{t}{\bar{t}}} \tag{1}$$

where $\bar{t} = \frac{V}{W}$ is the average residence time.

In case of a reaction of the first order the value of the concentration of the material leaving the reactor:

$$C = \int_0^t C(t)\, f(t)\, \mathrm{d}t, \tag{2}$$

where $C(t)$ stands for the dependence of concentration on time. This applied to the kinetics of thermal death of microorganisms at constant temperature:

$$N(t) = \int_0^t N_0 \, e^{-kt} \tag{3}$$

Substituting the density function (1) of residence time distribution in the tank mixed to perfection, the survival cell density in case of an isothermal ideal mixed flow reactor:

$$N(t) = \int_0^t N_0 \, e^{-kt} \cdot \frac{1}{\bar{t}} \, e^{-\frac{t}{\bar{t}}} \, dt \tag{4}$$

In so far as $N_0 =$ constant, that is, the suspension fed in the reactor is of constant viable cell density and the flow rate of the entering suspension is constant (W):

$$N = N_0 \frac{1}{\bar{t}} \int_0^t e^{-\left[kt + \frac{t}{\bar{t}}\right]} \, dt \tag{5}$$

$$\frac{N}{N_0} = \frac{1}{\bar{t}} \int_0^t e^{-[k\bar{t}+1]\frac{t}{\bar{t}}} \, dt \tag{6}$$

Integrated:

$$\frac{N}{N_0} = \frac{1}{k\bar{t}+1} \left[1 - e^{-(k\bar{t}+1)\frac{t}{\bar{t}}}\right] \tag{7}$$

Insofar as $t = \infty$

$$\frac{N}{N_0} = \frac{1}{k\bar{t}+1} \tag{8}$$

Relationship (8) corresponds to the solution for the steady-state condition of the differential material balance equation of an isothermal tank reactor mixed to perfection.

Value k from equation (8)

$$k = \frac{\frac{N_0}{N} - 1}{\bar{t}} \tag{9}$$

It can be seen from equation (9) that on determining the value of k the quotient of the initial and steady-state viable cell concentrations is used in contrast to all traditional methods, calculating the death rate coefficient from the logarithm of this quotient.

In practice a sufficiently accurate result is obtained when, instead of determining the steady-state value ($t = \infty$) measurements are started when the out-going viable cell count reaches 99% or exceeds 99% of the steady-state value. The time required is calculated from equations (7) and (8):

$$\frac{1}{k\bar{t}+1}\left[1 - e^{-(k\bar{t}+1)\frac{t_{99}}{\bar{t}}}\right] = 0.99\,\frac{1}{k\bar{t}+1} \tag{10}$$

$$\frac{t_{99}}{\bar{t}} = \frac{-\ln 0.01}{k\bar{t}+1} \tag{11}$$

Relationship (11) is illustrated in Fig. 1. Here the value $\bar{t} = 8$ min was used for calculation, which is an average value characteristic of the experimental equipment.

The experimental equipment is shown in Fig. 2.

Useful volume of the reactor is $V = 370$ cm^3 and the in-going flow rate is $W = 46$ cm^3 min^{-1}.

The continuously mixed microbial suspension to be treated was introduced by means of a Zalimp 304 type peristaltic feeding pump. The temperature of the reactor was kept under control by the water flowing in the glass heating spiral. The temperature of the water was set with the Medingen U 10 type ultrathermostat. To mix the microbial suspension and the reactor to perfection the Radelkisz (Hungary) OP 912 type magnetic stirrers were used.

Whether the apparatus behaved like a tank mixed to perfection was controlled experimentally.

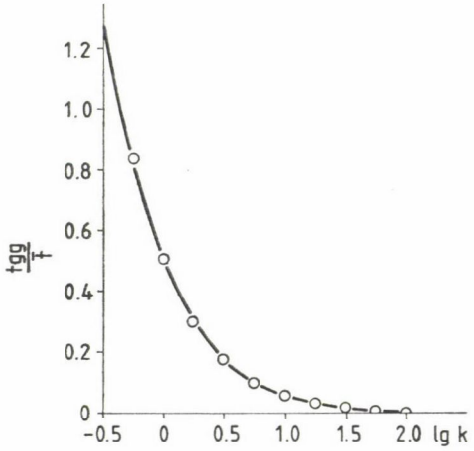

Fig. 1. Relationship between the time required to the 99% approximation of the steady-state value and the death rate coefficient for an isothermal tank reactor
$\bar{t} = 8$ min; k = min^{-1}

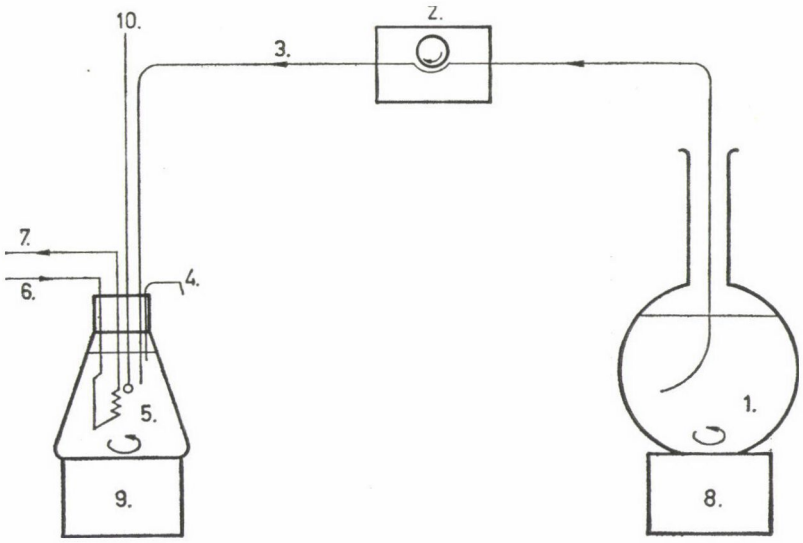

Fig. 2. Experimental apparatus for the determination of thermal death
1: Microbial suspension to be treated; 2: peristaltic feeding pump; 3: feeding the reactor; 4: sampling cock; 5: reactor; 6: introduction of heating water; 7: outlet of heating water; 8: magnetic stirrer; 9: magnetic stirrer; 10: thermometer

In case of a tank mixed to perfection, on changing suddenly the concentration of the in-going liquid, the concentration of the out-going liquid changes according to the transient function of first order elements.

In the tank reactor filled with a safranin pigment solution, water was introduced and the change of the optical density of the liquid in time was followed up with VSU 2-P type photometer at a wavelength of 520 nm. (In the range measured – below 0.8 OD – the optical density is a strictly linear function of concentration.)

In case of a tank perfectly mixed, if in the in-going liquid $C = 0$, the change in the concentration of the out-going liquid:

$$\frac{C}{C_0} = e^{-\frac{t}{\bar{t}}}$$

thus, the logarithm of the out-going concentration changes linearly in time: The experimentally determined relationship is shown in Fig. 3.

The equation describing the change in concentration:

$$\ln \frac{C}{C_0} = 0.0256 - 0.12446\, \bar{t}$$

$\bar{t} = 8.03$ min, reciprocal of the slope of the line.

The correlation coefficient $(r) = 0.9998$.

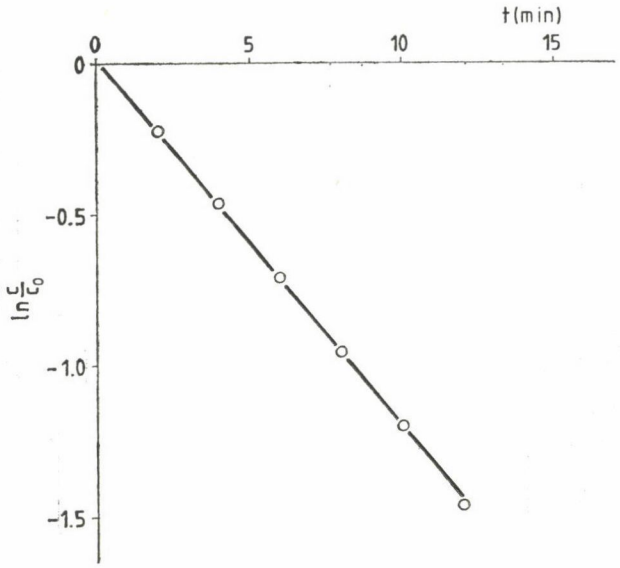

Fig. 3. Linearized form of the transient function of the ideal mixed flow tank reactor used in the thermal death experiments

In Fig. 3 the correlation coefficient of the relationship describing the linearized intermediate function shows a very close correlation thus, the reactor may be assumed to be mixed to perfection.

Samples of the heat treated suspension were taken at the overflow sampling cock of the apparatus in sterile test tubes cooled previously by immersion in an ice-aceton mixture.

In the course of these experiments sampling cocks of three different sizes were applied. The on-temperature-keeping times at the sampling cocks as calculated from the geometrical dimensions were as follows:

$$t_h = 0.014 \text{ min},$$

$$t_h = 0.006 \text{ min},$$

$$t_h = 0.0014 \text{ min}.$$

Considering that in the overflow sampling cock (s. c.) the temperature remains practically unchanged, at higher temperatures the death taking place in the sampling cock has to be taken into account, too, since this process is of exponential character:

$$\left[\frac{N}{N_0}\right]_{\text{s. c.}} = e^{-kt_h} \tag{12}$$

Taking this into account the viable cell density at the outlet is:

$$\frac{N}{N_0} = \frac{1}{k\bar{t} + 1} e^{-kt_h} \tag{13}$$

Relationship (13) is illustrated in Fig. 4, accounting for $\bar{t} = 8$ min and the three on-temperature-keeping times. The relationship describing the mixed tank is also shown in the Figure.

As it can be seen, the on-temperature-keeping effect of the overflow sampling cock is significant at k values higher than 10 min^{-1} or at reductions in viable cell counts higher than 2 orders of magnitude.

Theoretically this effect may be substantially reduced by reducing the residence time in the sampling cock.

In the knowledge of the experimentally determined reduction in the viable cell count the death rate coefficient was graphically determined from Fig. 4. To determine the value of k at a death rate below one and a half orders of magnitude relationship (9) may also be used since in that case the on-temperature-keeping effect of the sampling cock is negligible.

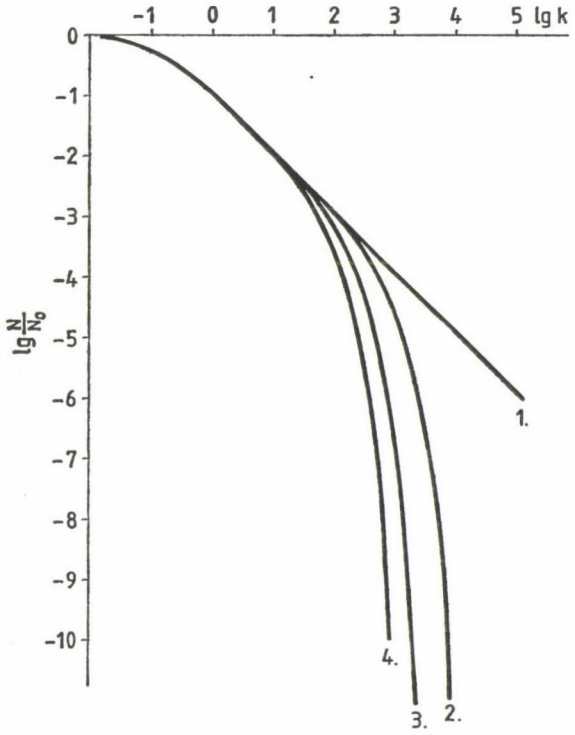

Fig. 4. Change in cell density as a function of the death rate coefficient for the perfectly mixed tank (1) and taking into account the effect of the sampling cock (2) $t_h = 0.0014$ min; (3) $t_h = 0.006$ min; (4) $t_h = 0.014$ min. Value of $\bar{t} = 8$ min for every case

In relation to the new experimental method the theoretical error calculation was also carried out in order to see the degree of viable cell count reduction at which the value of the death rate coefficient was reliable.

Presuming a death kinetic of the first order, on calculating the relative error of the death rate coefficient the inaccuracy in viable cell counting by plating, and in measuring time and temperature, was taken into account. The disturbing factors due to the nutrient medium and to differences in the physiological state of the test strains could not be accounted for.

In studying the temperature dependence of death rate the following relationship was used as starting point:

$$\lg k = a + \frac{1}{z} T \tag{14}$$

Transforming relationship (13):

$$\frac{N_0}{N} = (k\bar{t} + 1)\, e^{kt_h} \tag{15}$$

The theoretical standard deviation as expected in the course of the determination of value k, taking into account the laws of error transmission, is as follows:

$$\sigma_k = \sqrt{\left[\frac{\delta k}{\delta \dfrac{N_0}{N}} \sigma_{N_0/N}\right]^2 + \left[\frac{\delta k}{\delta \bar{t}} \sigma_{\bar{t}}\right]^2 + \left[\frac{\delta k}{\delta t_h} \sigma t_h\right]^2 + \left[\frac{\delta k}{\delta T} \sigma_T\right]^2} \tag{16}$$

where $\sigma_{N_0/N}$ = standard deviation of the ratio of initial and steady-state viable cell density,

$\sigma_{\bar{t}}$ = standard deviation of the average residence time,

σ_{t_h} = standard deviation of the residence time in the sampling cock,

σ_T = standard deviation of the temperature in the reactor.

Calculating the partial derivatives

$$\frac{\delta \dfrac{N_0}{N}}{\delta_k} = e^{kt_h}[k\bar{t}\, t_h + t_h + \bar{t}] \tag{17}$$

$$\frac{\delta k}{\delta \dfrac{N_0}{N}} = \frac{1}{[k\bar{t}\, t_h + t_h + \bar{t}]} e^{-kt_h} \tag{18}$$

$$\frac{\delta k}{\delta \bar{t}} = \frac{k}{[k\bar{t}\, t_h + t_h + \bar{t}]} \tag{19}$$

$$\frac{\delta k}{\delta t_h} = \frac{k(kt_h + 1)}{[k\bar{t}\,t_h + t_h + \bar{t}]} \tag{20}$$

$$\frac{\delta k}{\delta T} = \frac{1}{z} k \tag{21}$$

Standard deviation of individual factors are as follows:

Standard deviation of viable cell count determination

Assuming a 20 % coefficient of variation and N_0 and N being determined by the same number of parallel measurements (variance is minimal in this case):

$$\sigma_{N_0/N} = \sqrt{\frac{0.08}{n}}\,\frac{N_0}{N}$$

where n is the number of parallel viable cell count determinations.

Standard deviation of the average residence time

Determined by 30 measurements

$$\sigma_{\bar{t}} = 0.1 \text{ min}$$

Standard deviation of the on-temperature-keeping time

Taking into account the fluctuation in the feeding rate of the peristaltic pump and the accuracy of the determination of the dimensions of the sampling cock

$$\sigma_{t_h} = 2 \cdot 10^{-4} \text{ min}$$

Standard deviation of the temperature

Value experimentally determined:

$$\sigma_T = 0.1 \text{ K}$$

Substituting derivatives (18) to (21) into relationship (16) and dividing them by the appropriate value of k, the theoretically expected value of the coefficient of variation of the death rate coefficient is:

$$\frac{\sigma_k}{k} = \sqrt{\frac{(k\bar{t} + 1)^2\left[\frac{0.08}{nk^2} + \sigma_{t_h}^2\right] + \sigma_{\bar{t}}^2}{(k\bar{t}\,t_h + t_h + \bar{t})^2} + \frac{\sigma^2 T}{z^2}} \tag{22}$$

Calculating with standard deviations of the parameters accounted for and assuming a value of z of the order af 5.0 k the relative error of the new experimental method was determined for $n = 1$, 2 and 5. The residence times accounted for in the calculations:

$$\bar{t} = 8 \text{ min}; \; t_h = 0.0014 \text{ min}.$$

The relationship is shown in Fig. 5.

As it can be seen, above a given viable cell count reduction (about the order of magnitude of 5) the reduction achieved in the relative error by increasing the number of parallels, is non-significant. Of the two sections as observed in the function of relative error, the first one $\left(\lg \frac{N_0}{N} < 2\right)$ is characteristic of the reactor mixed to perfection. The second section accounting for a higher range of death is an error function taking into account the on-temperature-keeping time of the sampling cock. Determining in 5 parallels both the initial and the steady-state viable cell counts ($t > t_{99\%}$), at a reduction higher than 2 orders of magnitude the relative error of the k values remains practically unchanged and its minimum is about $\lg \frac{N_0}{N} = 4$. Carrying out 1 or 2 parallel viable cell count determinations the minimal error is obtained at $\lg \frac{N_0}{N}$ values above 4 or 5.

Taking into account the relationships obtained in the course of the theoretical error calculations, the viable cell density of the in-going and out-going cell suspensions was determined in 5 parallel determinations each. To establish the steady-state value subsequent to the point of time $t_{99\%}$ as previously estimated, sample was taken at the sampling cock every minute. The $t_{99\%}$ times were determined from the relationship illustrated in Fig. 1 by previously estimating the death rate coefficient to be expected.

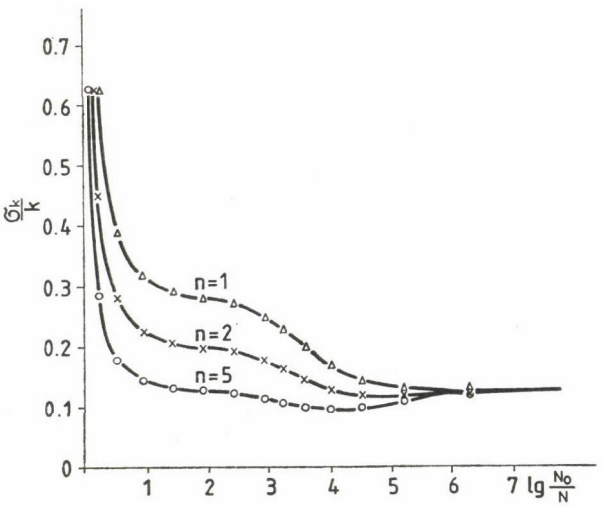

Fig. 5. Theoretically expected standard deviation of the death rate coefficient determined in the isothermal tank reactor as a function of decreasing cell density, accounting for the on-temperature-keeping effect of the sampling cock
$\bar{t} = 8$ min; $t_h = 0.0014$ min

Table 1

Results of the thermal death experiments with Saccharomyces cerevisiae

Date of measurement	Age of culture (days)	Method[a]	Temperature (K)	Temperature (°C)	t_h (min)	$\lg \frac{N_0}{N}$	$\lg k$	k (min^{-1})	D (min)
20-10-1980	4	1	326.0	53.0	—	3.50	−0.724	0.189	12.2
30-10-1980	4	1	326.3	53.3	—	1.50	−0.595	0.254	9.08
		3	333.7	60.7	0.014	3.05	1.78	60.3	0.038
		3	334.9	61.9	0.014	3.80	2.10	125	0.018
		3	335.6	62.6	0.014	4.25	2.25	178	0.013
		3	336.3	63.3	0.014	5.00	2.45	282	0.0082
4-11-1980	1	2	324.7	51.7	—		0.025	1.06	2.70
		2	326.2	53.2	—		0.344	2.21	1.04
		2	327.5	54.5	—		0.655	4.52	0.51
		2	328.7	55.7	—		0.928	8.47	0.27
		3	326.7	53.7	0.006	1.37	0.441	2.76	0.83
		3	329.2	56.2	0.006	2.04	1.13	13.5	0.17
		3	332.3	59.3	0.006	3.07	1.93	85	0.027
		3	333.4	60.4	0.006	3.41	2.12	132	0.017
		3	334.4	61.4	0.006	3.87	2.35	224	0.010
		3	335.3	62.3	0.006	4.78	2.65	447	0.0051
12-1-1981	3	1	329.1	56.2	—	3.50	0.362	2.30	1.00
		3	329.2	56.2	0.0014	1.26	0.334	2.16	1.07
		3	331.1	58.1	0.0014	1.88	0.969	9.31	0.25
		3	333.1	60.1	0.0014	2.48	1.58	38.0	0.061
		3	335.5	62.5	0.0014	3.38	2.34	219	0.011
		3	337.0	64.0	0.0014	4.36	2.94	871	0.0026

[a] See para. 1.4.

Table 2

Results of thermal death experiments with Rhodotorula rubra

Date of measurement	Age of culture (days)	Method[a]	Temperature (K)	Temperature (°C)	t_h (min)	$\lg \frac{N_0}{N}$	$\lg k$	k (min^{-1})	D (min)
6-2-1981	2	1	326.3	53.3	—	3.50	−0.900	0.126	18.3
		1	330.2	57.2	—	3.00	0.029	1.07	2.14
		3	331.0	58.0	0.0014	1.31	0.386	2.43	0.95
		3	332.9	59.9	0.0014	1.78	0.880	7.59	0.30
		3	334.9	61.9	0.0014	2.31	1.408	25.6	0.090
		3	336.0	63.0	0.0014	2.92	1.95	89	0.026
		3	337.0	64.0	0.0014	3.20	2.19	115	0.015
		3	338.0	65.0	0.0014	3.55	2.45	282	0.0082
		3	339.0	66.0	0.0014	3.92	2.70	501	0.0406

2. Results

In the course of the experiments the thermal death of cultures of different ages was studied by different methods.

In the study of continuous systems by changing the geometrical dimensions of the sampling cock the effect of the on-temperature-keeping time was

Table 3

Results of thermal death experiments with Escherichia coli

Date of measurement	Age of culture (days)	Method[a]	Temperature (K)	Temperature (°C)	t_h (min)	$\lg \frac{N_0}{N}$	$\lg k$	k (min^{-1})	D (min)
18-11-1980	1	1	327.0	54.0	—	5.7	−0.67	0.211	10.91
		1	327.4	54.4	—	6.7	−0.507	0.311	7.41
		1	329.0	56.0	—	5.7	−0.192	0.642	3.59
		1	331.3	58.3	—	3.5	0.300	1.994	1.15
		1	333.2	60.2	—	6.7	0.814	6.52	0.353
		2	330.3	57.3	—		0.111	1.29	1.78
		2	331.2	68.2	—		0.307	2.03	1.t4
		2	332.0	59.0	—		0.496	3.13	0.735
		2	332.6	59.6	—		0.685	4.84	0.476
		3	337.8	64.8	0.006	2.74	1.70	50.1	0.046
		3	339.4	66.4	0.006	3.47	2.15	141	0.0163
		3	340.9	67.9	0.006	4.10	2.45	282	0.0082

[a] See para. 1.4.

Table 4

Results of thermal death experiments with Streptococcus faecalis

Date of measurement	Age of culture (days)	Method[a]	Temperature (K)	Temperature (°C)	t_h (min)	$\lg \frac{N_0}{N}$	$\lg k$	k (min^{-1})	D (min)
12-3-1981	2	2	335.9	62.9	—		−0.638	0.230	10.0
		2	337.0	64.0	—		−0.336	0.461	5.00
		2	338.1	65.1	—		−0.036	0.921	2.50
		2	339.1	66.6	—		0.207	1.612	1.43
		2	339.9	66.9	—		0.362	2.30	1.00
		2	340.6	67.6	—		0.538	3.45	0.667
		2	341.2	68.2	—		0.732	5.39	0.427
		2	341.7	68.7	—		0.877	7.53	0.306
		3	346.2	73.2	0.0014	2.68	1.78	60.3	0.038
		3	349.3	76.3	0.0014	3.72	2.57	371	0.0062

also taken into account. Results were summarized in Tables in which the experimental techniques were marked as follows:

1. Method described in para. 1.4.1.: Isothermal heat treatment in a batch system.
2. Method described in para. 1.4.2.: Anisothermal heat treatment in a batch system.
3. Method described in para. 1.4.3.: Continuous isothermal heat treatment in a mixed flow reactor.

In case of the measurements carried out in a continuous system the averages of 5 parallel viable cell count determinations were used. The standard deviations of individual samples varied between 10–20 %. They have never exceeded 20 %. In the isothermal batch system heat treatments (Method 1), the death rate coefficients and the decimal reduction times were determined from the equation of the curve constructed from 4–6 points of measurement. The values of the correlation coefficients varied around −0.98 and −0.99.

2.1. Thermal death studied with Saccharomyces cerevisiae

Table 1 contains the results and death rate coefficients and decimal reduction times calculated from the results.

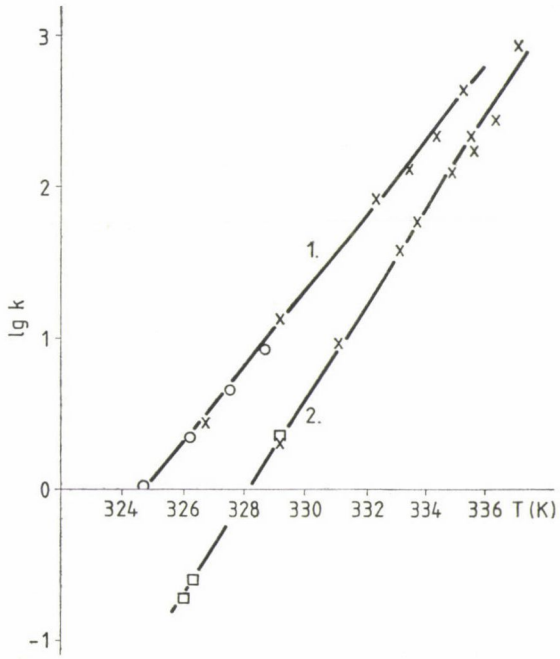

Fig. 6. Temperature dependence of the death rate coefficient for *Saccharomyces cerevisiae*
1. Age of culture: 1 day; 2. age of culture: 3 and 4 days;
—□— isothermal measurements in a batch system
—○— anisothermal measurements in a batch system
—×— continuous isothermal measurements

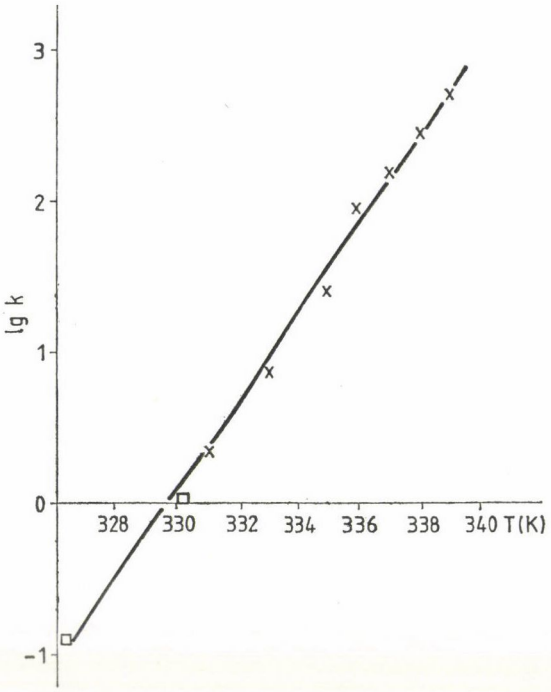

Fig. 7. Temperature dependence of the death rate coefficient for *Rhodotorula rubra*
—□— isothermal measurements in batch system
—×— continuous isothermal measurements

2.2. Thermal death studies on Rhodotorula rubra

Table 2 contains the results of the experiments and death rate coefficients and decimal reduction times calculated from the results.

2.3. Thermal death studies on Escherichia coli

Table 3 contains the results of the experiments and the death rate coefficients and decimal reduction times calculated from the results.

2.4. Thermal death studies on Streptococcus faecalis

Table 4 contains the results of the experiments with *Streptococcus faecalis* and the death rate coefficients and the decimal reduction times calculated from the results.

3. Conclusions

On evaluating the experimental results by plotting of logarithm of the death rate coefficients against time has shown the result to be a straight line in the case of all four microorganisms (Figs. 6–9).

The relationships derived by regression calculations and the z values characteristic of the thermal death of the strains are summarized in Table 5.

Table 5

Summary of thermal death experiments. Temperature dependence of death rate coefficients

Microbial strain	Number of data pairs	Regression equation (Temperature dependence)	Correlation coefficient (r)	z (K)
Saccharomyces cerevisiae 1 (Age: 1 day)	10	$\lg k = -80.914 + 0.2491\,T$ (324 K – 336 K)	0.9991	4.01
Saccharomyces cerevisiae 2 (Age: 3–4 days)	12	$\lg k = -103.795 + 0.3163\,T$ (326 K – 337 K)	0.9984	3.16
Rhodotorula rubra	9	$\lg k = -96.501 + 0.2927\,T$ (326 K – 339 K)	0.9977	3.42
Escherichia coli	12	$\lg k = -73.095 + 0.2216\,T$ (327 K – 341 K)	0.9991	4.51
Streptococcus faecalis	10	$\lg k = -79.472 + 0.2349\,T$ (336 K – 350 K)	0.9987	4.26

The equations in Table 5 describe the relationships between death rate coefficients determined by various methods and the temperatures. The correlation coefficients representing an extremely close linear correlation prove that the death rate coefficients determined by the new method fit thoroughly the values obtained by other traditional methods. Since the new method permits

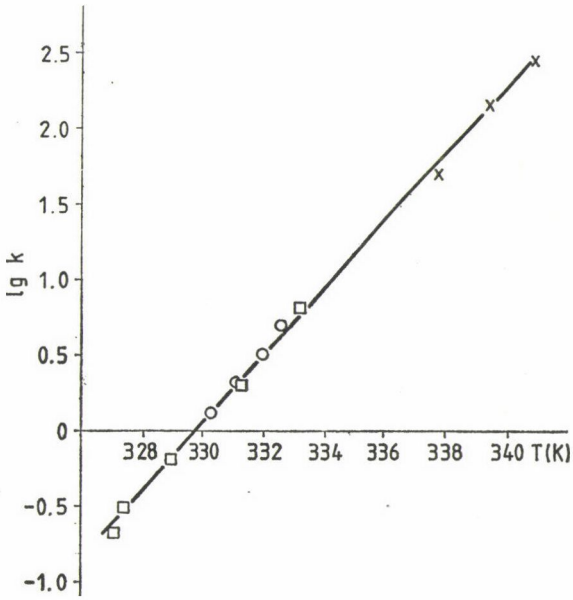

Fig. 8. Temperature dependence of the death rate coefficient for *Escherichia coli*
—□— isothermal measurements in batch system
—○— anisothermal measurements in batch system
—×— continuous isothermal measurements

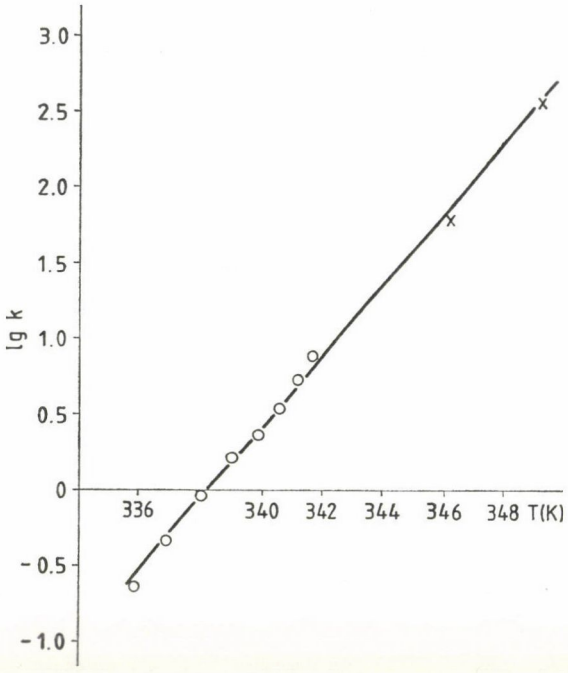

Fig. 9. Temperature dependence of the death rate coefficient for *Streptococcus faecalis*
—○— anisothermal measurements in batch system
—×— continuous isothermal measurements

the determination of the death rate coefficient and the decimal reduction time at temperatures very close to the pasteurization temperatures as used in the industry as well as the taking into account a much wider interval for the calculation of the z value than earlier, the thermal death parameters thus obtained enable the calculation of heat treatment with greater safety.

Symbols

D decimal reduction time (min)
k death rate coefficient (min^{-1})
t time (min)
$\bar{t}$ average residence time in the flow reactor (min)
V volume of the ideal mixed flow reactor (cm^3)
W flow rate ($cm^3\ min^{-1}$)
C concentration
N cell density (cell cm^{-3})
N_0 cell density at $t = 0$ point of time, cell density entering the tank reactor mixed perfectly (cell cm^{-3})

$t_{99\%}$ time required to reach 99 % of the steady-state cell density in the reactor
t_h holding time in the sampling cock of the flow reactor (min)
T temperature (K i.e. °C)
z temperature increase required to the ten-fold increase of the death rate coefficient (K i.e. °C)
a intersection of the axis of the line describing the temperature dependence of the logarithm of the death rate coefficient
σ standard deviation of the parameter in the index. The unit of measure corresponds to that of the parameter
r correlation coefficient

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INFLUENCE OF IONIZING RADIATION AND STORAGE CONDITIONS UPON SOME QUALITY PARAMETERS OF SOYBEANS

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It was established in the course of preliminary experiments that soyflour produced for animal feeding in a pilot plant was occasionally contaminated to an extent exceeding the microbiological standard set for human consumption.

Thus, it was concluded that to reduce microbiological contamination was a necessity and the suitability of ionizing radiation for this purpose was investigated. In practice conditions prevailing in the storage space – particularly temperature and humidity – determine the microbiological quality of the product. Soybeans of different moisture content (9 and 13%) were, therefore, irradiated and stored in spaces of different relative humidity (55 and 75%) at 5–15 °C or 20–25 °C temperature. The soybean samples were irradiated with 1 and 5 kGy, resp.

The storage experiments have shown the humidity of the storage room to be of extreme importance to the propagation of moulds. The inhibitory effect of a temperature of 5–10 °C in itself is not sufficient, neither is the effect of the radiation dose applied sufficient during a longer storage period. The presence of aflatoxin B_1 in mildewy soybeans was not proven. But toxins have to be reckoned with as potential sources of danger. Further experiments are necessary to support results hitherto obtained.

It was established that the moisture content of the seeds affected strongly the quality of the extracted oil. With increased moisture content increases the acid number as the mark of rancidity and the peroxide value, the amount of carbonyls and the extent of conjugation. These unfavourable effects increase at higher temperatures. The radiation doses applied in the experiments did not affect the quality of the oil.

The conclusion drawn from the experiments was that the microbial contamination of soybeans can be kept at the initial low value by treatment with 1 kGy even under unfavourable storage conditions for a period of 2 months. Thus, irradiation may be applied as a temporary solution to stabilize the microbiological state of soybeans.

The oil-free residue of soybeans, soybean meal, because of its high nutrient content, is one of the most widely used basic materials for feed protein. The yearly yield of soybean cultivation amounts to cca 60 million tons. Since it is rich in essential amino acids it is used to set the protein level of various animal diets. Hungary imports for feeding purposes several hundred thousand tons of soybean meal per year. In recent years, in order to be able to reduce the imports, efforts were made to propagate its cultivation. At present about 30 thousand tons of soybeans are processed in Hungary and thus, arouse the problems of storage and maintenance of quality.

Soybeans play an increasing role in up-to-date human nutrition, too. Modern processing technology permits its use for a great variety of food products (KRALOVÁNSZKY, 1975).

Although soybeans are not attacked by mould fungi prior to harvest, moulds are present and if storage conditions favour their multiplication, they contaminate the seeds. A moisture content of about 13 % is sufficient to start the propagation of the fungi (CHRISTENSEN & DORWORTH, 1966). The species predominant in mould formation during storage are: *Aspergillus candidus* and *Aspergillus flavus*. At high moisture contents the intense propagation of fungi is followed by the multiplication of bacteria. The activity of thermophilic bacteria may even cause the spontaneous ignition of soybeans (CHRISTENSEN & KAUFMANN, 1972).

Table 1

Moulds most frequently occurring on soybeans and their toxins

Microorganism	Toxin
Aspergillus glaucus	
A. flavus	aflatoxin B_1, B_2, G_1, G_2, M_1, M_2
A. candidus	citrinin
A. versicolor	sterigmatocystin
A. nidulans	sterigmatocystin
A. niger	
A. fumigatus	fumitremorogen
A. ochraceus	ochratoxin A
Bipolaris sp.	sterigmatocystin
Penicillium rubrum	rubratoxin A, B
Penicillium sp.	ochratoxin
Trichoderma viride	gliotoxin
Fusarium oxysporum	zearalenon (F-2)
F. roseum	fusariogenin

Beside the *Aspergillus* species about another 20 fungal species occur regularly and 34 species occasionally on soybeans (MISLIVEC & BRUCE, 1977). In case of fungal contamination the potential danger of micotoxin formation arises. Several of the fungus species occurring on soybeans are known to produce toxin as it is seen in Table 1 (MOREAU, 1972; INCZE, 1977).

The most dangerous fungus is *Aspergillus flavus* and the toxin produced by it. However, although it multiplies rapidly on soybeans, toxin was rarely found on the contaminated beans and in those cases, too, the amount present was small (HESSELTINE et al., 1966; SHOTWELL et al., 1969a, b). According to HESSELTINE and co-workers (1966) soybeans do not supply satisfactory medium for toxin production.

The presence of sterigmatocystin was not detected either in soybeans contaminated with *Aspergillus versicolor*, *A. nidulans* or *Bipolaris sp.* (SHANNON & SHOTWELL, 1976). EMEH and MARTH (1976) stated that under natural conditions *Penicillium rubrum* is rarely found on soybeans but it occurs on foodstuffs or fodder prepared with soybean. Soybeans were contaminated intentionally with *P. rubrum* and found that the fungus started to grow at 8 °C and produced toxin at 13 °C.

CHRISTENSEN and KAUFMANN (1972) maintain that toxins, particularly aflatoxin do not form a great problem in relation to soybeans, because they are present only in very low amounts and in the course of extraction they do not pass into the oil but remain in the cake. Since in the animal feeds prepared with soy meal the latter constitutes only a small part, therefore, its mycotoxin content is negligible or it is not even toxic. However, the health damaging effect of the consumption of these subtoxic amounts is not sufficiently elucidated.

Under field conditions soybeans have several pests, which however, are not dangerous during storage. In the temperate zone soybeans have no significant storage pests, only occasionally *Plodia interpunctella* (ANON, 1975) but even that does not cause significant damage. Under tropical climate, however, the presence of *Ephestia cautella* forms a serious problem (COX & SIMMS, 1978).

The use of radiation treatment for the desinfestation of cereals and flour was permitted in several countries. Generally a dose of 0.30–0.75 kGy was suggested (LORENZ, 1975). The immediate death of all pests may be achieved by treatment with 3–5 kGy, however, the application of 1 kGy is sufficient to kill all the pests present in the cereal within a few days after treatment (CORNWELL, 1966). At a conference held in Geneva in 1976 it was suggested by FAO, WHO, and IAEA to permit a treatment of 1 kGy for the desinfestation of cereals (WHOLESOMENESS OF IRRADIATED FOOD, 1977). The dose of 0.30–0.75 kGy permitted in several countries is also effective against insects in every stage of development, inhibits their growth and within a larger or shorter period leads to their total destruction (GOLUMBIC & DAVIS, 1966; BECZNER & FARKAS, 1972).

The aim of this study was to assess the microbial contamination in soybeans and all the products manufactured from them and to establish the extent of the problem of toxin production by fungi. It was intended to clarify also the danger of insect infestation. Parallel to the microbiological tests some of the chemical parameters as a function of storage conditions, time and radiation dose were followed up during storage. At the end of the storage period the soybeans were tested for the presence of mycotoxin.

1. Materials and methods

1.1. Materials

The samples investigated in the years 1976 through 1978 were of different place of origin. The soyflour was supplied by the Enterprise for Cereal Industry, the other products and the soybeans by the Research Institute for the Vegetable Oil and Detergent Industry and were of commercial quality.

1.2. Methods

1.2.1. The moisture content of soybeans used in the storage experiment was 6%. The original moisture content was adjusted to 9 and 13%, resp. The samples were treated with 1 kGy and stored in a space of 55 and 75% ERH, resp. Samples were stored individually in linen bags and placed in jars. To achieve the predetermined humidity sulfuric acid at different concentrations was used. Beans of 9% moisture content were stored above sulfuric acid of 0.55 water activity and those of 13% moisture content above 0.75 water activity. The samples were stored either at room temperature (20–25 °C) or at 5–15 °C.

1.2.2. Irradiation was carried out with an RH-γ type laboratory ^{60}Co radiation source of 0.30 PBq nominal activity at a dose rate of 4.8 kGy h^{-1}.

1.2.3. For the microbiological tests 10 g of soybeans or soy product were shaken in 90 cm^3 pepton solution containing Tween 80 (Reanal, Hungary) for 15 min at 30 °C (KISS, 1977). To determine the total mesophilic aerobic cell count liquid Nutrient (Oxoid) was inoculated in three parallels. The anaerobic spores were determined in liver broth. The fungal count was determined on slant malt nutrient. Results were evaluated on the basis of Hoskin's table by the most probable viable cell count method. Microbiological tests were carried out four times during storage, each time by two separate weighings.

1.2.4. The following chemical tests were carried out in the course of the storage period:

The oil content was determined in a Soxhlet extractor according to HUNGARIAN STANDARD (1976).

The acid number was established by alcoholic potassium hydroxide titration (HUNGARIAN STANDARD 1973a).

To establish the peroxide value the iodometric method was applied (HUNGARIAN STANDARD 1973b).

By measuring the UV absorption of the extracted oil the extent of conjugation (C) could be inferred. Absorption (A) was measured at 270 nm (3 mg cm^{-3}) against iso-octane. For calculation the following equation was used:

$$C = \frac{A_{270}}{bc}$$

where

b = thickness of the cuvette (cm),

c = oil concentration (g l^{-1}).

1.2.5. The mycotoxin (aflatoxin B_1) analysis was carried out at the Microbiological Department of the National Feed Inspectorate, Budapest in October 1978. At this time the samples stored at room temperature in a space

of high ERH were visibly covered by mould. The determination was carried out according to HUNGARIAN STANDARD (1980).

1.2.6. Mathematical statistical evaluation. The changes of the mesophilic aerobic cell count were evaluated by variance analysis, while the mould count by t test.

2. Results

2.1. Results of the microbiological tests

2.1.1. The results of the microbiological tests of soybean products are shown in Table 2. In the Table beside the values obtained for soya flour the values as decreed in relation to human consumption are also given (ORDER OF THE MINISTER OF HEALTH, 1978).

Table 2

Microbial contamination on soybeans during processing
(August 1976)

Microorganism tested	Cell count (g^{-1})[a]				
	Soy flour[b,c]			Soybeans	
	Cell count found	Satisfactory	Acceptable	Whole	Milled
Total mesophilic aerobic cell count	9.8×10^5	10^4	10^5	9.3×10^3	2.4×10^6
Aerobic spores	2.7×10^4	—	—	—	—
Anaerobic spores	—	—	—	4.6×10^2	2.4×10^2
Moulds	2.4×10^3	10^2	10^3	4.6×10^3	1.5×10^2

Microorganism tested	Cell count (g^{-1})[a]			
	Soy cake		Soy meal	
	Gritty	Milled	Disintegrated I.	Disintegrated II.
Total mesophilic aerobic cell count	9.3×10^3	9.4×10^4	2.0×10^5	4.6×10^5
Aerobic spores	—	—	—	—
Anaerobic spores	9.3×10^3	2.4×10^3	2.4×10^3	2.8×10^2
Moulds	3.6×10^2	$<3.6\times10$	$<3.6\times10$	$<3.6\times10$

[a] Cell counts are the averages of two separate parallel weighings
[b] March 1976
[c] Made of extracted meal. Microbiological standard exists only for soy flour

As it can be seen the mesophilic aerobic cell count of soybeans reaches or exceeds the acceptable level and the mould count is high, too. It can be seen that during processing the mesophilic aerobic cell count was not substantially reduced, the anaerobic cell count did not change, either. The fungal cell count is reduced, however, fungi are still present at a provable density. Some means of reducing the cell count is inevitably necessary, because the initial cell count is determinative and there is no substantial reduction during processing. At the same time the infection of soybeans and their products during a storage period of longer or shorter duration has to be accounted for, too.

2.1.2. Microbiological conditions of the soybeans during storage depends on the conditions prevailing in the storage room, particularly on temperature and humidity. Thus, in the storage experiment soybeans of different moisture

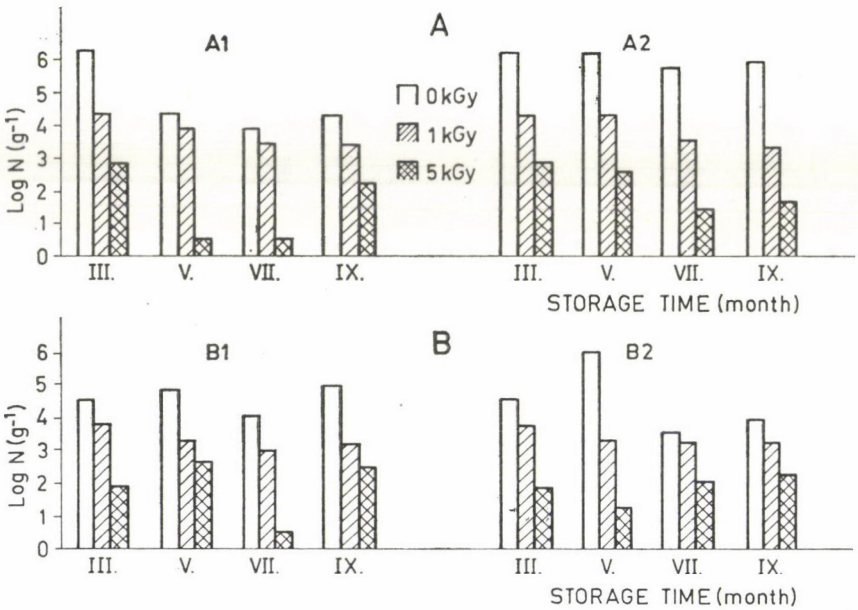

Fig. 1. The mesophilic aerobic cell count in soybeans as a function of radiation dose, moisture content, storage temperature and storage time (1978)

A: Moisture content of soybeans: 9–11%, ERH: ~ 55%
A1: Storage temperature: March–May: 5–10 °C
May–September: 15–20 °C
A2: Storage temperature: 20–25 °C

B: Moisture content of soybeans: 13–16%, ERH: ~ 75%
B1: Storage temperature: March–May: 5–10 °C
May–September: 10–20 °C
B2: Storage temperature: 20–25 °C

content were placed in storage spaces of different humidity and kept at two different temperatures.

2.1.2.1. Mesophilic aerobic cell count. The initial moisture content of the beans was 6% and the mesophilic total aerobic cell count $4.5 \times 10^4\ g^{-1}$. The moisture content of the beans was raised to 9.5% and 13%, respectively, and they were subjected to radiation treatment. The total mesophilic aerobic cell count and the mould count were determined twice during the storage period.

In Fig. 1 the mesophilic aerobic cell count is shown as a function of radiation dose, humidity, temperature and storage period.

Simultaneously with the microbiological test the moisture content of the beans was also established and it was found to increase with storage time. To determine the moisture content of the beans an Ultra–X apparatus was used. Of the moisture contents recorded in the Figure the first one is the initial, the other one as measured at the end of the storage period. The cell count was reduced by the radiation treatment as found directly after treatment. A treatment with 1 kGy reduced it by 1–1.5 order of magnitude, 5 kGy at least

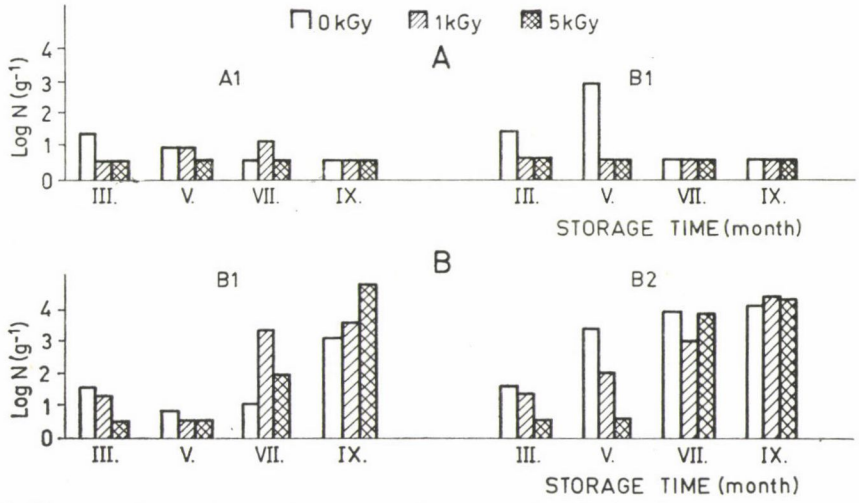

Fig. 2. The mould count in soybeans as a function of radiation dose, moisture content, storage temperature and storage time (1978)

A: Moisture content of soybeans: 9–11%, ERH: ~ 55%
A1: Storage temperature: March–May: 5–10 °C
May–September: 15–20 °C
A2: Storage temperature: 20–25 °C

B: Moisture content of soybeans: 13–16%, ERH: ~ 75%
B1: Storage temperature: March–May: 5–10 °C
May–September: 10–20 °C
B2: Storage temperature: 20–25 °C

by 1.5 order in addition. The differences in the effect of treatments were maintained during the storage period. At higher temperature and greater humidity the differences in the samples tended to equalize.

The statistical evaluation of the results is presented in Table 3.

Analysis of variance was carried out for each storage condition and treatment from 8 parallel data by considering the logarithms of cell counts as determined during storage, as parallel measurements. This is not an error because the cell count does not change substantially as a function of time. Significant correlation was not found between storage time and logarithms of cell counts whether it was based on the calculated r values or on analysis of variance. This is proven also by the mean values and standard deviation values calculated from the 8 data. The difference between the cell counts of samples stored at different conditions of storage, was not significant either. Cell count seems to depend primarily on the radiation dose. A dose of 1 kGy reduced the cell count at least by one order of magnitude and the reduced cell count for beans of low moisture content differed from the control at a $P = 5\%$ level, for beans of higher moisture content at a $P = 1\%$ or $P = 0.1\%$ level. The cell count reducing effect of 5 kGy is much more intense and the difference between the untreated and samples treated with 5 kGy is highly significant ($P = 0.1\%$). The difference between samples treated with 1 and 5 kGy is also significant independently from conditions of storage (at the $P = 1\%$ or the $P = 0.1\%$ level).

Summing up, it may be said that cell count is reduced by irradiation, however, the favourable effect of radiation treatment becomes effective only in storage at low temperature and in a space of low ERH during a longer storage period.

2.1.2.2. Mould count. Mould count as a function of radiation dose, humidity, storage temperature and storage period is presented in Fig. 2.

It can be seen in the Figure that low moisture content and low ERH affect favourably the mould count. The favourable effect of maintaining the beans at a low moisture content is not affected by higher temperature during a long period, or the increase of temperature, not even an 1–2% increase in the moisture content of the beans. A slight mould count reducing effect of radiation treatment is observed directly upon treatment or after two months storage but after 4 months of storage the difference between the treated and untreated samples disappears completely. In a few cases the mould count of treated samples was even higher than that of the untreated ones.

The statistical evaluation of mould counts is summed up in Table 4.

Since, as it can be seen in Figure 2, the mould count of bean samples of low moisture content does not change much with radiation dose, nor with storage time or storage temperature, in the statistical evaluation only the samples of 13–16% moisture content were taken into account. In bean samples

Table 3

Analysis of variance of the mesophilic aerobic cell count (log N) *in soybeans as a function of radiation dose*[d]

Radiation dose (kGy)	Cell count (log N)							
	Temperature: 5–20 °C Moisture content: 9–11%		Temperature: 20–25 °C Moisture content: 9–11%		Temperature: 5–20 °C Moisture content: 13–16%		Temperature: 20–25 °C Moisture content: 13–16%	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
0	$4.64^{a,x}$	0.94	4.90^{ab}	1.69	4.59^{x}	0.53	$4.56^{a,x}$	0.99
1	$3.60^{a,x}$	0.54	$3.60^{a,xy}$	0.65	3.29^{y}	0.44	$3.39^{b,x}$	0.42
5	$1.52^{b,y}$	1.92	$1.90^{b,xy}$	0.94	1.91^{z}	0.88	$1.64^{c,y}$	0.89
Significance of differences	$P_{5\%} = 0.91$ $P_{99\%} = 1.68$		$P_{5\%} = 1.21$ $P_{1\%} = 1.64$ $P_{0.1\%} = 2.22$		$P_{0.1\%} = 1.22$		$P_{1\%} = 1.02$ $P_{0.1\%} = 1.38$	

[d] As a function of radiation dose every treatment differs from the others at $P = 5\%$ level, therefore only probability levels differing from this were marked. Differences between values marked by letters (a, b, c) and (x, y, z) are significant at levels $P = 1\%$ and $P = 0.1\%$, resp.

Table 4

Significance test of the mould count of soybeans (log N) *as a function of storage time*

Storage time (month)	Treatments											
	Temperature: 5–10 or 15–20 °C Moisture content: 13–16%						Temperature: 20–25 °C Moisture content: 13–16%					
	0 kGy		1 kGy		5 kGy		0 kGy		1 kGy		5 kGy	
	x	±s	x	±s	x	±s	x	±s	x	±s	x	±s
0	1.50	0.19	1.47	0.22	0.55	0.28	1.50	0.19	1.47	0.22	0.55	0.28
2	0.87**	0.28	0.55*	0.28	0.47	0.28	3.02	0.91	1.93	0.07	0.47	0.28
4	1.16	0.29	3.07**	0.15	1.80*	0.24	3.71	0.95	2.78*	0.57	3.54**	0.71
6	3.57**	0.56	3.57**	0.56	4.47***	0.71	4.04***	0.03	4.38***	0.03	4.17***	0.25

The significance test (t test) as a function of time was carried out in comparison with the initial condition (0 storage time) for each treatment

Significant at probability level: * P = 10%; ** P = 5%; *** P = 1%

of high moisture content irradiation did not exert a substantial count reducing effect directly upon treatment. At room temperature, however, the mould count of the radiation treated samples did not change much after 2 months storage, but it was with at least 1 order lower than that of the control. After 4 months this "protective" effect disappears. In the 4th months of storage the mould count of all the samples was higher at a probability level of P = 5% or P = 10% than the initial value. In the 6th months it was higher at the P = 1% probability level. In samples of high moisture content but stored at low temperature the mould count is primarily affected by the storage temperature in the 2–4th months of storage, here the protective effect of irradiation does not assert itself. The mould count increases with increasing ambient temperature. At the end of storage the mould count of all the samples was significantly higher at P = 5% probability level than the initial count. It should be noted, however, that although the mould count of samples of high moisture content changed at both storage temperatures no close correlation was found between storage time and mould count by correlation calculations. This is due to the low number of data (n = 4, degree of freedom = 2).

2.2. Chemical analysis

Chemical analysis was carried out only twice during storage. Results are shown in Figs. 3 and 4.

The acid number shows that the hydrolysis of the oil depends mostly on the moisture content of the beans and on the storage temperature. There was no significant difference between the acid numbers of the irradiated and

the control bean samples. A similar trend is observable in the peroxide values as a function of temperature and humidity (Fig. 3). At room temperature a striking change was observable in the samples stored at high ERH. The parameters of the irradiated samples were worse than those of the control samples.

The UV absorption values relating to the extent of conjugation were affected, too, primarily by the moisture content of beans and the ERH of the storage space (Fig. 4). At low moisture content an unfavourable effect was observable directly upon treatment with 5 kGy, this, however, later disappeared as all the samples underwent an unfavourable conjugation change against storage time.

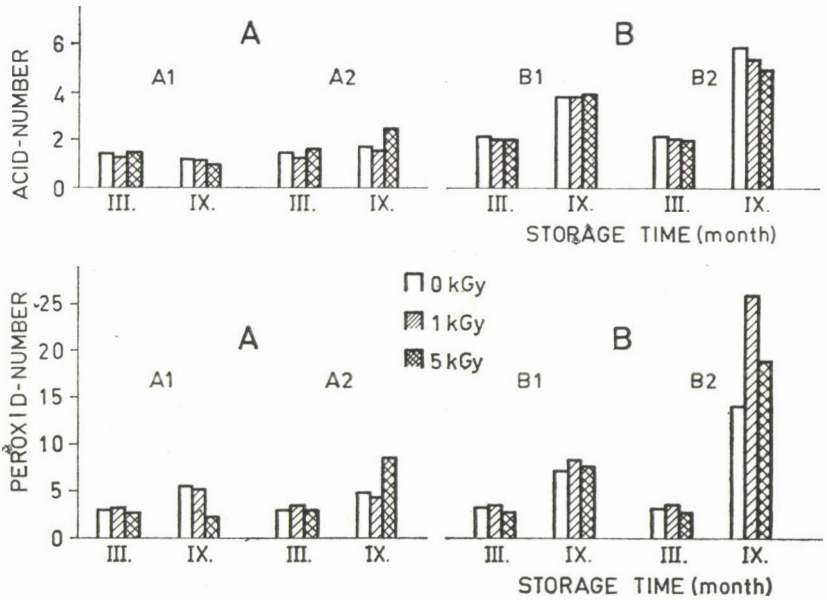

Fig. 3. The acid number and peroxide value of the oil extracted from soybeans as a function of radiation dose, moisture content, storage temperature and storage time (1978)

A: Moisture content of soybeans: 9–11%, ERH: ~ 55%
A1: Storage temperature: March–May: 5–10 °C
May–September: 15–20 °C
A2: Storage temperature: 20–25 °C

B: Moisture content of soybeans: 13–16%, ERH: ~ 75%
B1: Storage temperature: March–May: 5–10 °C
May–September: 10–20 °C
B2: Storage temperature: 20–25 °C

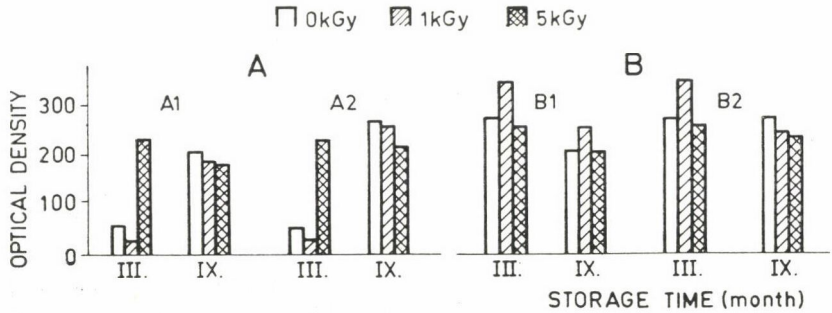

Fig. 4. UV absorption values (270 nm) of the oil extracted from soybeans as a function of radiation dose, moisture content, storage temperature and storage time (1978)

A: Moisture content of soybeans: 9–11%, ERH: ~ 55%
A1: Storage temperature: March–May: 5–10 °C
May–September: 15–20 °C
A2: Storage temperature: 20–25 °C

Moisture content of soybeans: 13–16%, ERH: ~ 75%
B1: Storage temperature: March–May: 5–10 °C
May–September: 10–20 °C
B2: Storage temperature: 20–25 °C

From the point of view of industrial processing the samples of 13–16% moisture content, stored at room temperature and low or varying temperature, judged by their chemical properties, proved to be poor. These samples were all very mouldy and this may have contributed to the unfavourable chemical changes.

2.3. Mycotoxin tests

The samples were tested for mycotoxin at the Microbiology Department of the National Feed Inspectorate in 1978. At this time the part of the samples of high moisture content (13–16%) was visibly covered by mould.
In spite of this none of the samples contained aflatoxin B_1.

3. Conclusions

The experiments have shown the humidity of the storage space to be of importance in the multiplication of fungi. The unfavourable effect of low water activity on the growth of certain *Aspergillus* species is known from related literature (ORTH, 1976; NORTHOLT et al., 1976). A temperature of 5–10 °C is in itself not sufficiently inhibitory because it is higher than the minimum temperature of growth for several fungi. Thus, multiplication of fungi at this temperature is steady, even if slow (Fig. 2, Table 4).

A radiation dose of 1 kGy is sufficiently effective particularly under tropical climate, against the storage pest, *Ephestia cautella* and against *Plodia interpunctella*, a potential pest under climatic conditions in Hungary. However, it does not seem efficient, particularly in itself to reduce cell count. A radiation dose of 5 kGy can only retard the growth of fungi and this effect extends over a short storage period only (Figs. 1, 2, Tables 3, 4).

It should also be noted that according to certain data in the literature radiation treatment may increase the toxin production of certain fungi (*Aspergillus flavus*, *A. parasiticus*, *A. orchraceus*, *Fusarium culmorum*, *Penicillium urticae*) in pure cultures (APPLEGATE & CHIPLEY, 1974; BULLERMAN et al., 1973; JEMMALI & GUILBOT, 1970; SELLYEY et al., 1979; SCHINDLER et al., 1980). The cause of the increased toxin production is not as yet elucidated, it may be traced back probably to the formation of mutants. In the opinion of INGRAM and FARKAS (1977) the mycotoxin production of *Aspergillus* species is not activated by radiation treatment.

It has been shown by several authors, however, that the combined heat treatment and irradiation exert a synergistic effect upon the destruction of conidia and/or mycelia of *A. flavum* (MOHYUDDIN & SKOROPAD, 1975; PADWAL–DESAI et al., 1976a, b). By a pretreatment of 35–50 °C the conidia may be sensitized to irradiation. Then by a treatment with 1 kGy the survival of the conidia may be reduced to a minimum. Although the mould species show different resistance to this treatment it would be possible to reduce the microbial contamination of soybeans to a very low level by combined heat and radiation treatment.

Recent data in related literature prove that soybeans, apparently in sound condition, are contaminated by a variety of fungi (MISLIVEC & BRUCE, 1977). Contamination affects mainly the outer surface of the beans, but after decontamination of the surface a substantial degree of "inner contamination" remains. The fungi listed in Table 1 occur regularly on the surface of beans but in addition other mould species forming toxin appear occasionally. It is known, however, that a mixed contamination goes usually with a reduced toxin production (CALVERT et al., 1978) or sometimes with a completely discontinued toxin production (MIROCHA & CHRISTENSEN, 1974; BOLLER & SCHROEDER, 1974). It is probably due to this fact that the presence of mycotoxin could not be demonstrated even in highly contaminated soybeans (CHANG et al., 1966; SHOTWELL et al., 1969 a, b). On the other hand, other authors demonstrated toxin in artificially infected soybeans (DAVIS & DIENER, 1970; NAGARAJAN et al., 1973). The contradictory data in the literature are explained by the experiments of GUPTA and VENKITASUBRAMANIAN (1975) carried out with zinc and phytinic acid. They established that free zinc is required by *Aspergillus parasiticus* to produce aflatoxin and this is liberated in soybeans only by heat treatment from the complex formed with phytinic acid. These

results draw attention to the circumstance that soybeans and soy products may become nutrient media for the toxin producing fungal species after heat treatment (i.e. industrial drying, soy meal and cake after extraction) which liberates zinc and thereby induces toxin production.

Chemical analysis has shown the high moisture content of the seeds to affect disadvantageously the quality of the extracted oil. In this case increases the acid number and the peroxide value marking the advance of rancidity. The extent of conjugation is also increased. This unfavourable effect is enhanced by increasing temperature and the microbial degradation (moulding) of the samples. Radiation treatment does not affect rancidity, not even a treatment with 5 kGy (Figs. 3 and 4). A dose of 5 kGy increases the extent of conjugation directly after irradiation against that of the control and that treated with 1 kGy, this difference, however, disappears in the course of storage. All the characteristics are affected by the condition of the beans and the storage conditions.

The results of the microbiological and chemical tests show that the storage stability of beans and the quality of the oil extracted from them depends mainly on the moisture content of the seeds and the relative humidity of the storage room. Increased temperature affects unfavourably the microbiological and chemical conditions. A moisture content below 10% and an equilibrium relative humidity below 70% and a temperature below 10 °C are favourable conditions of storage. The initial contamination of the seeds is also an important characteristic. Since the surface of soybeans is always heavily contaminated (mostly by fungi) and this cannot be reduced by treatment with radiation, application of the latter does not seem expedient, the more so as under the temperate zone soybeans have no storage pest causing serious damage. In the tropical zone *Ephestia cautella* causes great damage and this can be reduced by treatment with 1 kGy. The oil gained from the beans thus treated is not affected by the treatment. In the continental zone to maintain the quality of soybeans it is sufficient to ensure optimal storage conditions and treatment with 1 kGy becomes necessary only if this is not possible. By treatment with this dose the mould infection of the beans can be kept at the initial, usually low, level for 2 months, even in the case of beans with high moisture content. Thus this is a satisfactory temporary solution till sufficient suitable storage room is available. A further study of mycotoxins seems important, since in the course of processing infection may increase to a level dangerous for human health.

*

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BOOK REVIEW

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(Ed.)

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The book contains the proceedings of the 1st International Symposium on Cyclodextrins (Budapest, Hungary, 30 September — 2 October, 1981). The cyclodextrin symposium was held under the auspices of the Medical Chemistry Section of the Hungarian Pharmacological Society and the Chemical Division of the Hungarian Academy of Sciences, organized by the Congress Bureau of MOTESZ (Association of the Hungarian Medical Societies) and sponsored by the Chinoin Pharmaceutical- and Chemical Works, Budapest.

Sixty three papers are published in the book. Seven of them treat the chemical properties and production methods of cyclodextrins. Methods for the spectrophotometric determination of micro-amounts of cyclodextrins are described and for their separation by gel chromatography or by HPLC. The results of the investigation of the enzymatic, toxicologic and metabolic properties of cyclodextrins are reviewed in 10 papers. The research results have shown cyclodextrins to be extremely resistant to the hydrolytic effect of starch hydrolyzing enzymes. Apart from a few exceptions they are not suitable for fermentation, they are not attacked directly by yeasts.

Information on their toxicologic and metabolic characteristics was gained in experiments with mice, rabbits, rats and dogs. Results show that cyclodextrins, within the permitted limits, have no toxic effects. To study their metabolism ^{14}C-labelled beta-cyclodextrin and starch were used. Glucose metabolism was studied on rats by feeding them with labelled glucose. The radioactivity in blood was measured by liquid scintillation technique. It was established that a part of cyclodextrins was metabolized by rats, though slowly.

Cyclodextrin complexes were discussed in 16 papers. Methods were described for the determination of guest molecules in the presence of beta-cyclodextrins.

The types and possibilities of utilization of cyclodextrin derivatives and polymers were discussed in 8 papers. Cyclodextrin polymers used as specific sorbents and their characteristics were described. Thirteen papers treated the subject of the use of cyclodextrins in the pharmaceutical industry and 9 their use in the food and other industries. Beta-cyclodextrin is one of the most frequently used. The most important of its properties is that its outer surface is polar because the hydroxyl groups of the glucose molecules are located here. Therefore the outer coat is soluble in water. Inside the molecule the cave is apolar and so non-soluble in water. It resists fermentation and is non-toxic. These characteristics make it suitable for enclosing, "packaging" solid, fluid and gaseous materials. In the food industry and agriculture they are most useful in the maintenance of aromatic substances. Beta-cyclodextrin complexes of aromatic substances are stable and resist heat, light and oxygen effects. They can be utilized in product diversification, inasmuch the same basic product can be aromatized with a variety of aroma substances. They can also be used in the production of dietary preparations and for the stabilization of the aroma substances of seasonings, coffee, tea, fruit, meat, tobacco, etc. It was found by some of the researchers that beta-cyclodextrin is capable of eliminating the unpleasant taste and odour of certain products. Beta-cyclodextrin can be used as foaming and foam stabilizing agent, it can be utilized in cosmetics. Research results have shown its inhibitory capacity in enzymatic browning processes, in the liquefaction of sugars. According to certain results it can also be used as a sweetening agent.

É. SZÁNTÓ

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DETERMINATION OF THE SHELF LIFE OF FOOD PRODUCTS

I. VARSÁNYI and L. SOMOGYI

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The determination of how long nutritional raw materials, partially cooked foods and ready-to-serve products can be stored is an extremely important task. The mechanism and dynamics of the quality changes taking place in food products during storage depend not only on the hygienic conditions, but also on the storage temperature and the method of packaging. The aim of the present study was to elaborate an objective method of evaluating the optimum conditions for quality maintenance.

A continuous deterioration model was set up in order to solve the problem and it was found that the quality changes of the studied products can be described by functions approximating to linear, quadratic and hyperbolic curves. The applicability of the method is illustrated for one product each from the poultry, frozen foods and meat industries.

The quality characteristics of food products change, usually for the worse, as a function of the period and conditions of storage. The identification and description of the dynamics of the deterioration processes and the subsequent determination of the shelf life is important for both the producer and the consumer. Taking into account various points of view, shelf life is taken here to mean the period during which the most rapidly deteriorating of the important characteristics changes to a just tolerable degree if the foodstuff is stored under the given conditions (FARKAS et al., 1975).

Thus, in order to interpret the shelf life it is necessary to determine the degree of deterioration which is just acceptable, the most rapidly deteriorating property or properties characteristic of the quality of the product (the so-called critical characteristic(s)) and the storage conditions influencing the maintenance of quality. Two types of models are generally used to determine shelf life: these can be referred to as discrete and continuous models.

Discrete models are employed primarily for studying the quality-influencing effect of the storage conditions. The procedure basically consists of comparing the characteristics of the product at various intervals during storage using statistical methods (mainly variance analysis) and determining the degree of quality change from the results (GACULA & KUBALA, 1975).

The continuous models are based on a determination of the supposedly stochastic relationship between the storage period and the quality of the foodstuff. Continuous models are characterised by the numerical determination and statistical analysis of so-called deterioration curves (GACULA, 1975).

Considering the process as a whole, the discrete models provide less information with a relatively high degree of reliability, while the continuous models provide more information, but with a parallel decrease in the reliability of the conclusions.

There is still no universal agreement on the so-called critical value which is judged to be just acceptable or on methods for determining this value. Some standards take a 25% reduction in the maximum number of quality points as the critical value (HUNGARIAN STANDARD, 1977), while others give a figure of 35% reduction (HUNGARIAN STANDARD, 1971). On the −5 to +5 scale employed by DALHOFF and JUL (1965) the critical value was +2, i.e. 80% of the maximum scale value. PALMER and co-workers (1953) defined the critical value as 3 on a 1 to 5 scale, which is 60% of the maximum. During sensory evaluations at the RESEARCH LABORATORY OF THE POULTRY INDUSTRY (1979) a 50% reduction in the maximum was designated as the just permissible value. It can be seen that the critical values employed in practice fall between 50% and 80% of the maximum number of points.

The critical characteristic, i.e. the most rapidly deteriorating of the characteristic(s), can be determined after statistical analysis from either discrete or continuous models. The majority of authors, however, draw attention to the fact that the critical characteristic should be determined taking the various storage conditions into account (VAS, 1971; JOHNSON et al., 1974; WOOTEN et al., 1974; DETHMERS, 1979).

It is for this reason that research into the shelf life of food products deals not only with the effects of the technology used to produce the foodstuffs, but also includes studies on the effect of storage conditions. The composition, and relative humidity of the atmosphere, the temperature, the packing material, and the method of packing and the light are considered to be the most important of the conditions tested (PALMER et al., 1953; PERYAM, 1964; ANON., 1974; VARSÁNYI, 1980). The present study was carried out using a continuous deterioration model. Among the storage conditions, the effect of the storage temperature and the type of packaging material were examined. The deterioration processes were traced in identical samples stored under different conditions and the characteristic which changed to the greatest extent, taking into account the different storage conditions, was considered to be critical for the characterisation of deterioration.

In this paper certain products of the poultry, meat and frozen food industries are used as examples to illustrate the method developed for calculating shelf life, the results obtained and the conclusions to which they lead.

1. Materials and methods

1.1. Materials

Among the *poultry products*, the determination of the storage period for ready-to-fry chickens will be described. The products were stored at temperatures of −20 °C or −15 °C for 12 months in bags made of poly(vinylidene chloride)(PVdC) or shrinking polyethylene foil.

Chemical procedures (peroxide number, fatty acid number, ammonium-nitrogen) and sensory evaluations (odour test for raw materials, cooking and frying test) were used to follow changes in quality. The sensory evaluation was carried out by 8 panelists on a 5 points system, where the just acceptable quality was equivalent to 2.5.

The storing and testing was carried out in the Research Laboratory of the Poultry Industry on 3 parallel samples each. The samples were taken randomly every two months for the first 12 months and then every 3 months (Research Laboratory of the Poultry Industry, 1979).

Of the *meat products*, calculations on the shelf life of raw leg of pork are presented. The meat was stored at 5–7 °C or at 20–22 °C. The storage and testing were carried out at the National Meat Research Institute (1978).

The meat was stored in approx. 500 g pieces in bags made of laminated cellophane + polyethylene (cellothene) foil or on polystyrene plates covered with shrinking polyethylene film for 10 days. Before packaging the meat was stored at 8 °C for 24 hours after slaughtering.

The determination of quality changes was carried out using chemical (water, fat and protein contents, pH) and microbiological (viable spore count) tests and a sensory evaluation (colour, taste) implemented by five people using a 0–7 point system on 3 samples each. The just acceptable quality was given 4 points in this system.

Among the *frozen food* products, calculations on the storage stability of quick-frozen beefburger rolls are presented. The product was stored at −18 °C or at +5 °C. The storage and testing were carried out in the Central Quality Control Laboratory of the Hungarian Refrigeration Industry (1980).

The product was stored in bags made of polyethylene foil or laminated polyethylene + polyamide film at −18 °C for 9 months or at +5 °C for 6 days. Sampling took place once a month or daily.

As a function of the storage period, chemical (total SO_2 content, pH), microbiological (coliform, total spores) and sensory tests (colour, odour, taste, firmness) were carried out on samples taken from five packets each. The sensory evaluations were made by five people using a 100 point system, where the just acceptable quality was awarded 66 points.

1.2. Methods of evaluation

The evaluation consisted basically of selecting the quality characteristic which changes most rapidly in time and the mathematical modelling of the change.

The continuous deterioration models were constructed by determining the deterioration curves using numerical curve fitting. When discussing mathematical methods for calculating shelf life GACULA (1975) recommends approaching the function on the principle of least squares. The estimation of shelf life from the deterioration curve is carried out by substituting the critical value back into the equation. The error of estimation is determined graphically, knowing the confidence belt of the deterioration curve. In order to select the critical characteristic and identify the effect of the storage conditions, the parameters of the calculated deterioration curves are evaluated using the t-test.

Considering the fact that the majority of deterioration curves approximate the function $f(x) = thx$, MAJOROS and NÉMETH (1977) use this type of curve in every case. Their main purpose in analysing deterioration curves was to study the economic effect of modifying shelf life and their method is basically of a graphic nature.

A similarly simplified model was used by FARKAS and co-workers (1975). Assuming that the deterioration curve was sigmoid, they determined the parameters of the curve using a running regression calculation. The effect of storage conditions was determined by statistically analysing the calculated deterioration constants using variance analysis.

In the course of the present study the principle of least squares was used to fit the curves to the measured points. Where necessary linear transformation was carried out.

The accuracy of curve fitting was characterised by the residual variance for the linear form (SVÁB, 1973).

$$s_y^2 = \frac{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2}{n-2}$$

while the standard error of parameters a and b in the function $f(x) = a + bx$, which gives a general description of deterioration, was estimated using the following correlations:

the standard error of regression constant a was estimated from the expression

$$s_a^2 = \frac{\sum_{i=1}^{n} e_i^2 \cdot \sum_{i=1}^{n} x_i^2}{n(n-2) \sum_{i=1}^{n} (x_i - \bar{x})^2} \qquad (1)$$

while that of the regression coefficient b was estimated from the expression

$$s_b^2 = \frac{\sum_{i=1}^{n} e_i^2}{(n-2)\sum_{i=1}^{n}(x_i - \bar{x})^2} \tag{2}$$

where s_y^2 = value of residual variance (standard error of dependent variable),
y_i = measured value of dependent variable at time i,
$\hat{y}_i$ = estimated value of dependent variable at time i,
s_a^2 = standard error of regression coefficient,
n = total number of measured points,
x_i = value of independent variable at time i,
$\bar{x}$ = mean value of independent variable,
e_i^2 = residue: $(y_i - \hat{y}_i)^2$,
s_b^2 = standard error of regression coefficient.

The deterioration curves obtained in linear form as a result of curve fitting were compared in pairs using covariance analysis in order to select the critical properties characteristic of deterioration.

The F values obtained from the analysis were compared with the F_t values taken from tables to determine the significance of differences between the curves. The characters which gave significantly steeper deterioration curves were taken as critical characteristics.

Various storage conditions (temperature, packaging) influence the quality in different ways; thus the rate of deterioration is not always the same.

In order to determine these differences linear forms of the curves were used for examination with statistical methods. The basis of the evaluation was a comparison of the slope of the transformed curves, on the grounds that the steepness expresses the rate at which the product deteriorates. The following equations can then be set up (GUEST, 1961):

in order to compare the steepness (b_k and b_l are the regression coefficients) of the kth and lth curves the random variable was formed, which follows a t-distribution with (2n–4) degrees of freedom

$$\frac{b_k - b_l}{s_d} = t \tag{3}$$

where n = number of measured points, s_d = standard error for the difference between the steepness of the two curves:

$$s_d = \sqrt{s_{b_k}^2 + s_{b_l}^2} \tag{4}$$

while

$$s_{b_l}^2 \text{ i.e. } s_{b_k}^2 = \frac{\Sigma y^2 - \frac{(\Sigma y)^2}{n} - \frac{\left(\Sigma xy - \frac{\Sigma x \Sigma y}{n}\right)^2}{\Sigma x - \frac{(\Sigma x)^2}{n}}}{\left[\Sigma x - \frac{(\Sigma x)^2}{n}\right][n-2]} \tag{5}$$

where s_{b_k} and s_{b_l} are the standard error of regression coefficients of kth and lth curves which are being compared.

The significance of the difference between the rates of deterioration was calculated by comparing the calculated t values to the t_l values from tables.

Significant differences in the flow rate or steepness of the curves were attributed to variations in the storage conditions, whereas in non-significant cases the differences were explained by random fluctuations in the determination of the characteristic.

Assuming identical initial quality, the rate of deterioration is the most characteristic indication of shelf life. Thus, by comparing the steepness of the deterioration rate, information can be obtained on what storage conditions result in the maintenance of quality for the shortest or longest period.

2. Results

The examination of the critical characteristics of the investigated food products made it possible to draw the conclusion that, as a function of time, these characteristics change according to one of three types of equations. Changes in the model products examined can be expressed approximately by linear, quadratic and hyperbolic functions. The choice of function was carried out with the help of transformation or by trial graph plotting.

2.1. Linear correlations

Changes in certain characteristics of ready-to-fry chickens were chosen as a model to demonstrate quality characteristics which change uniformly as a function of time.

The quality characteristics studied were the following: raw smell, (cooked) meat aroma and roasted flavour. Figure 1 shows the mean and standard error values calculated on the basis of sensory evaluation of chickens packed in bags made of shrinking polyethylene foil and stored at −20 °C; it also shows the curves determined from these values, the characteristic data of the curves and

the results of covariance analysis carried out in order to determine the critical characteristic.

It was found that changes in the raw smell differ very significantly from changes in the (cooked) meat aroma and the roasted flavour, while no significant difference could be demonstrated between changes in the (cooked) meat aroma and the roasted flavour. Consequently, it can be stated that the raw smell is the most rapidly changing characteristic, thus this can be regarded as the critical characteristic.

In a similar manner the critical characteristic was determined in the case of each storage condition.

The deterioration curves, the characteristic data of the curves and the results of covariance analysis based on sensory evaluations of ready-to-fry chickens stored at the same temperature but packed in PVdC foil are presented in Fig. 2.

It can be seen from the results that time changes in the raw smell differ very significantly from changes in the meat aroma and roasted flavour. No significant difference could be pointed out between changes in the meat aroma and the roasted flavour. On the basis of significance analysis the raw smell was again taken as the critical characteristic in this case.

The deterioration curves, the characteristic data of the curves and the results of covariance analysis carried out in order to determine the critical characteristic calculated from the data of sensory evaluations carried out on ready-to-fry chickens packed in bags made of shrinking polyethylene foil and stored at −15 °C, are summarized in Fig. 3.

It can be seen that the changes in time in the raw smell were very significantly different from time changes in the meat aroma and roasted flavour. There was no significant difference between time changes in the meat aroma and the roasted flavour. Thus, it can be stated on the basis of the results that the critical characteristic is the raw smell.

Figure 4 shows the deterioration curves calculated for the results of sensory evaluations on ready-to-fry chickens stored at −15 °C as before, but wrapped in PVdC foil, together with the characteristic data of the curves and the results of covariance analysis.

It can be seen from the results that time changes in the raw smell differed highly significantly from time changes in the meat aroma and roasted flavour. No significant difference could be found between time changes in the meat aroma and roasted flavour.

Summarising the results, it can be seen that the critical characteristic for ready-to-fry chickens is always the raw smell, irrespective of the storage conditions.

The effect of different storage conditions on shelf life was determined by comparing the deterioration rates characteristic of the storage conditions

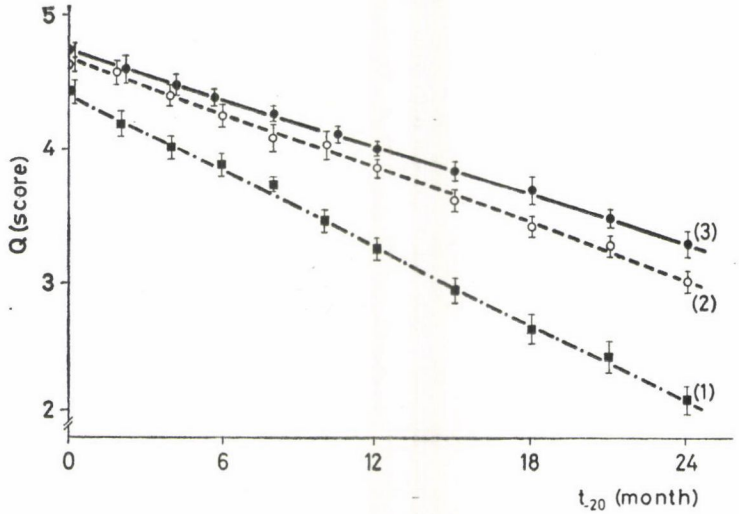


Fig. 1. Deterioration curves and characteristic data of the curves for ready-to-fry chickens wrapped in shrinking polyethylene bags and stored at −20 °C

Symbol	Quality characteristic	Equation of deterioration curve	Residual standard error	Standard error of regression coefficient	Standard error of regression constant	Significance of difference in the regression coefficients
—·—·— (1)	raw smell	$Q = 4.41–0.0963\, t_{-20}$	0.12	0.0036	0.52	***
– – – – – (2)	meat aroma	$Q = 4.70–0.0706\, t_{-20}$	0.09	0.0041	0.54	***
——— (3)	roasted flavour	$Q = 4.72–0.0617\, t_{-20}$	0.13	0.0012	0.36	Ø

*** Very highly significant difference at the $P \geq 99.9\%$ probability level
Ø Not significantly different at the $P \leq 95\%$ probability level

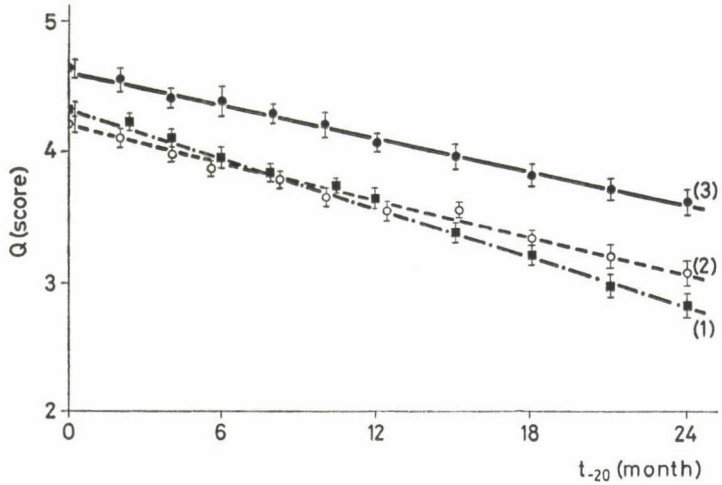


Fig. 2. Deterioration curves and characteristic data of the curves for ready-to-fry chickens wrapped in bags made of PVdC foil and stored at −20 °C

Symbol	Quality characteristic	Equation of deterioration curve	Residual standard error	Standard error of regression coefficient	Standard error of regression constant	Significance of difference in the regression coefficients
—·—·— (1)	raw smell	$Q = 4.36–0.0635\, t_{-20}$	0.12	0.0054	0.19	**
– – – – – (2)	meat aroma	$Q = 4.44–0.0492\, t_{-20}$	0.15	0.0024	0.11	**
——— (3)	roasted flavour	$Q = 4.59–0.0363\, t_{-20}$	0.11	0.037	0.23	Ø

** Highly significant difference at the $P \geq 99\%$ probability level
Ø Not significantly different at the $P \leq 95\%$ probability level

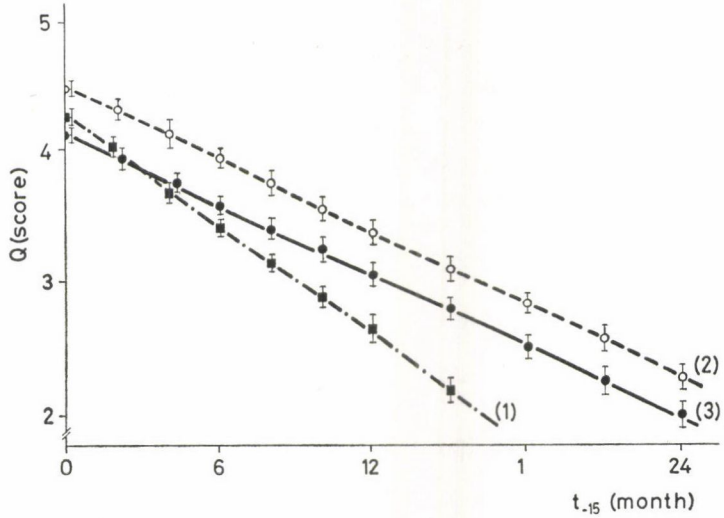


Fig. 3. Deterioration curves and characteristic data of the curves for ready-to-fry chickens wrapped in shrinking polyethylene bags and stored at −15 °C

Symbol	Quality characteristic	Equation of deterioration curve	Residual standard error	Standard error of regression coefficient	Standard error of regression constant	Significance of difference in the regression coefficients
— · — · — (1)	raw smell	$Q = 4.29–0.1345\, t_{-15}$	0.34	0.0061	0.12	***
— — — — — (2)	meat aroma	$Q = 4.36–0.0912\, t_{-15}$	0.15	0.0033	0.25	***
———— (3)	roasted flavour	$Q = 4.14–0.0906\, t_{-15}$	0.12	0.0047	0.14	Ø

*** Very highly significant difference at the $P \geq 99.9\%$ probability level
Ø Not significantly different at the $P \leq 95\%$ probability level

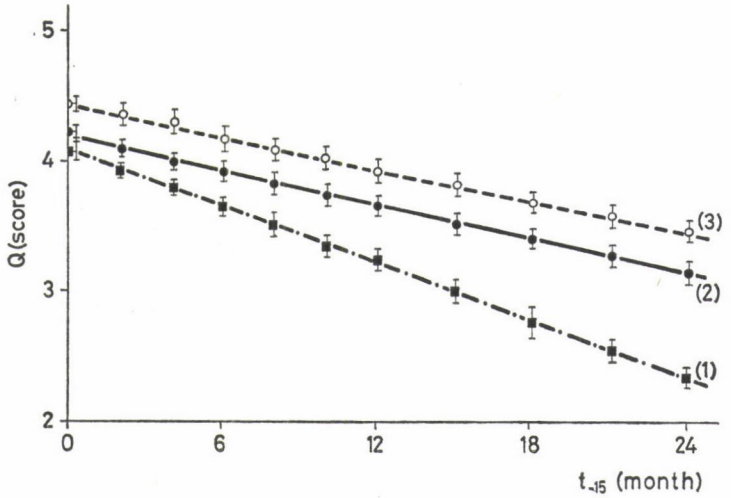


Fig. 4. Deterioration curves and characteristic data of the curves for ready-to-fry chickens wrapped in bags made of PVdC foil and stored at −15 °C

Symbol	Quality characteristic	Equation of deterioration curve	Residual standard error	Standard error of regression coefficient	Standard error of regression constant	Significance of difference in the regression coefficients
−·−·− (1)	raw smell	$Q = 4.10 - 0.0698\, t_{-15}$	0.27	0.0024	0.07	***
– – – – – (2)	meat aroma	$Q = 4.23 - 0.0426\, t_{-15}$	0.24	0.0020	0.14	*** Ø
——— (3)	roasted flavour	$Q = 4.45 - 0.0384\, t_{-15}$	0.13	0.0035	0.12	

*** Very highly significant difference at the $P \geq 99.9\%$ probability level
Ø Not significantly different at the $P \leq 95\%$ probability level

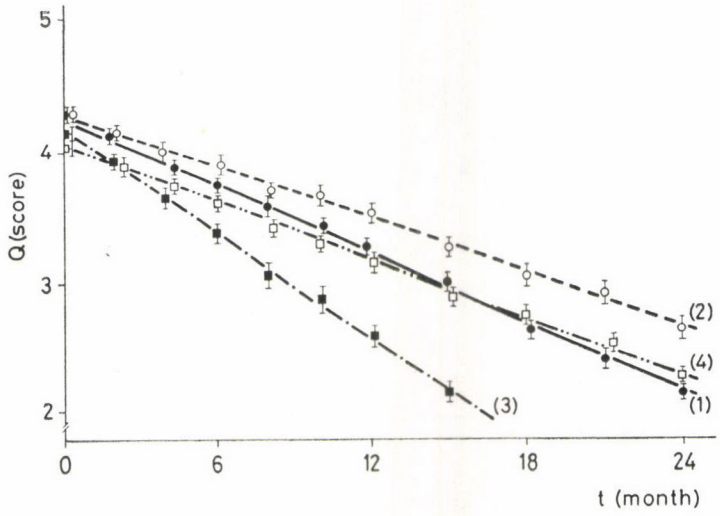
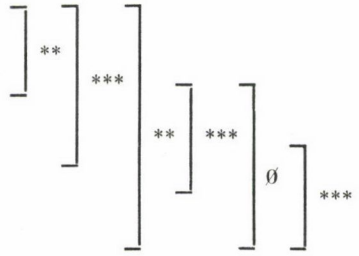


Fig. 5. Results of a statistical comparison of deterioration curves determined on the basis of a raw smell test for ready-to-fry chickens stored under various conditions

Symbol	Equation of deterioration curve	Storage temperature °C	Packaging material	Significance of difference in the regression coefficients
——— (1)	$Q = 4.41 - 0.0963\, t_{-20}$	−20	polyethylene foil	
– – – – – (2)	$Q = 4.36 - 0.0635\, t_{-20}$	−20	PVdC foil	
– · – · – (3)	$Q = 4.29 - 0.1345\, t_{-15}$	−15	polyethylene foil	
– ·· – ·· – (4)	$Q = 4.10 - 0.0698\, t_{-15}$	−15	PVdC foil	



*** Very highly significant difference at the $P \geq 99.9\%$ probability level
** Highly significant difference at the $P \geq 99\%$ probability level
Ø Not significantly different at the $P \leq 95\%$ probability level

using mathematical and statistical methods. The calculations were carried out using equation (3). In Fig. 5 the four deterioration curves, the characteristic data of the curves and the results of mathematical and statistical comparisons of the deterioration rates are summarized.

It can be seen from the results that there is no significant difference between the deterioration rates of products wrapped in PVdC foil. The deterioration rates of products wrapped in bags made of shrinking polyethylene foil, however, differ from each other at the P = 95 % probability level, and from products wrapped in PVdC foil at the P = 99.9 % probability level.

In summary, it can be concluded that the rate of quality change in ready-to-fry chickens stored by freezing is greatly influenced by the packaging material in the −20 °C −15 °C temperature range examined. In agreement with theoretical considerations, the use of PVdC foil, which lets considerably less (100 times less) gas through than polyethylene foil, proved to be more favourable. The deterioration rates for products wrapped in this manner were not significantly different from one another at −20 °C and −15 °C. This fact proves that PVdC packing is equally good for a temperature reduction of at least −5 °C in the temperature range examined, which could lead to a considerable saving in energy.

2.2. Quadratic correlations

Partially cooked beefburger rolls, which were shown in preliminary experiments to have a deterioration process which could be approximated with a quadratic equation, were chosen as the model on which to illustrate characteristics which influence quality and which change according to a quadratic correlation as a function of storage time.

The characteristics examined were as follows: aroma, flavour and firmness.

The results of sensory evaluations of quick-frozen partially cooked beefburger rolls wrapped in polyethylene bags and stored at −18 °C are shown in Fig. 6, together with the deterioration curves calculated from the results, the characteristic data of the curves and the results of covariance analysis carried out after linear transformation.

It can be seen from the figure that the change in the firmness differed from changes in the aroma and flavour at the P = 99.9 % level of probability. No significant difference could be observed between changes in the aroma and the flavour. Consequently, it can be stated that the characteristic which changes most rapidly as a function of the storage period is the firmness, so this can be regarded as the critical characteristic.

For quick-frozen partially cooked beefburger rolls wrapped in bags made of a laminated polyethylene + polyamide foil and again stored at

−18 °C, the results of sensory evaluations, the deterioration curves calculated from these results, and the results of covariance analysis are summarized in Fig. 7.

It can be seen from the figure that the changes in the firmness differed very significantly from changes in the aroma and flavour, while there was no significant difference in the rates of change for the aroma and the flavour. Thus, the results prove that the critical characteristic is the firmness.

The results of sensory evaluations for beefburger rolls wrapped in polyethylene bags and stored at +5 °C, together with the deterioration curves calculated from the results, are shown in Fig. 8 which also summarizes the characteristic data of the curves and the results of covariance analysis.

It can be seen from the results that the rate of change in the firmness differed at the P = 99% level of probability from the rate of change in the aroma and the flavour. There was no significant difference between the rates of change in the aroma and the flavour. It can thus be stated that the critical characteristic is the firmness.

For quick-frozen partially cooked beefburger rolls wrapped in bags made from a laminated polyethylene + polyamide foil and stored at +5 °C the results of sensory evaluation, the deterioration curves calculated from the results, the characteristic data of the curves and the results of covariance analysis carried out in order to determine the critical characteristic, are summarized in Fig. 9.

It can be seen from the results that the rate of change in the firmness differed significantly from the rate of change in the aroma and flavour. No significant difference could be demonstrated between the rates of change in the aroma and the flavour. Thus, on the basis of the results the firmness can be regarded as the critical characteristic.

Summarizing the results of the calculations it can be stated that, irrespective of the storage conditions, the firmness is the critical characteristic of quick-frozen partially cooked beefburger rolls and is particularly important when the frozen product is thawed.

The effect of different storage conditions on quality maintenance was determined by comparing the deterioration rates characteristic of the storage conditions. The curves which were compared, the characteristic data of the curves and the mathematical and statistical comparisons of the deterioration rates are presented in Fig. 10.

The results of the comparison show that the deterioration rates of products stored at the same temperature but in different packaging materials do not differ significantly from each other, while the deterioration rates of products stored in the same packaging material but at different temperatures differ significantly at the P = 99.9% level of probability.

From the point of view of quality maintenance a storage temperature of

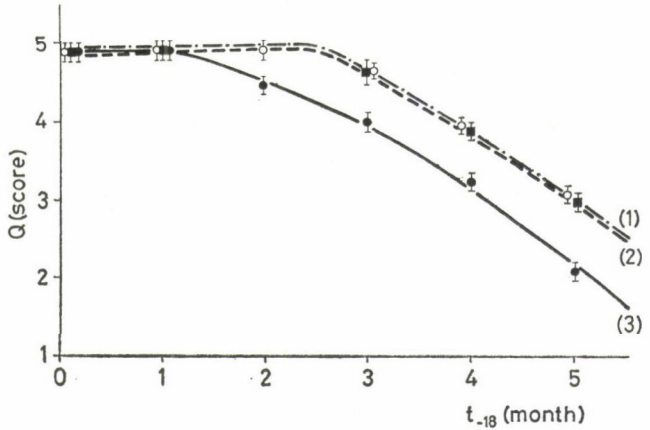


Fig. 6. Deterioration curves and characteristic data of the curves for quick-frozen partially cooked beefburger rolls wrapped in polyethylene bags and stored at −18 °C

Symbol	Quality characteristic	Equation of the deterioration curve	Residual standard error	Standard error of regression coefficient	Standard error of regression constant	Significance of difference in the regression coefficients
— . — . — (1)	aroma	$Q = \begin{cases} 4.90, & \text{if } t_{-18} \leqslant 2.34 \\ 4.90-0.08\, t_{-18}^2, & \text{if } t_{-18} \geqslant 2.34 \end{cases}$	0.12	0.004	0.12	Ø
— — — — — (2)	flavour	$Q = \begin{cases} 4.90, & \text{if } t_{-18} \leqslant 2.34 \\ 4.90-0.08\, t_{-18}^2, & \text{if } t_{-18} \geqslant 2.34 \end{cases}$	0.18	0.006	0.14	***
——————— (3)	firmness	$Q = \begin{cases} 4.90, & \text{if } t_{-18} \leqslant 1.16 \\ 4.90-0.11\, t_{-18}^2, & \text{if } t_{-18} \geqslant 1.16 \end{cases}$	0.17	0.003	0.11	***

*** Very highly significant difference at the $P \geq 99.9\%$ probability level
Ø Not significantly different at the $P \leq 95\%$ probability level

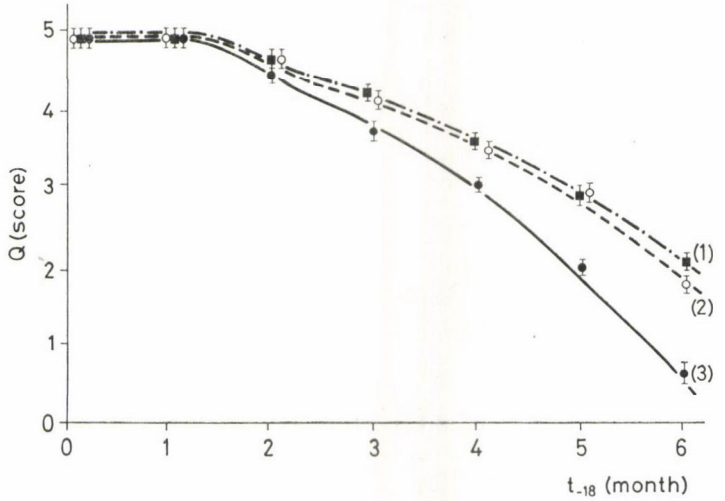


Fig. 7. Deterioration curves and characteristic data of the curves for quick-frozen partially cooked beefburger rolls wrapped in bags made of a laminated polyethylene + polyamide film and stored at −18 °C

Symbol	Quality characteristic	Equation of the deterioration curve	Residual standard error	Standard error of regression coefficient	Standard error of regression constant	Significance of difference in the regression coefficients
— · — · — (1)	aroma	$Q = \begin{cases} 4.90, & \text{if } t_{-18} \leq 1.25 \\ 4.90-0.08\, t^2_{-18}, & \text{if } t_{-18} \geq 1.25 \end{cases}$	0.15	0.01	0.12	Ø, ***, ***
– – – – – (2)	flavour	$Q = \begin{cases} 4.90, & \text{if } t_{-18} \leq 1.25 \\ 4.90-0.07\, t^2_{-18}, & \text{if } t_{-18} \geq 1.25 \end{cases}$	0.15	0.01	0.10	
——— (3)	firmness	$Q = \begin{cases} 4.90, & \text{if } t_{-18} \leq 1.25 \\ 4.90-0.12\, t^2_{-18}, & \text{if } t_{-18} \geq 1.25 \end{cases}$	0.14	0.01	0.10	

*** Very highly significant difference at the $P \geq 99.9\%$ probability level
Ø Not significantly different at the $P \leq 95\%$ probability level

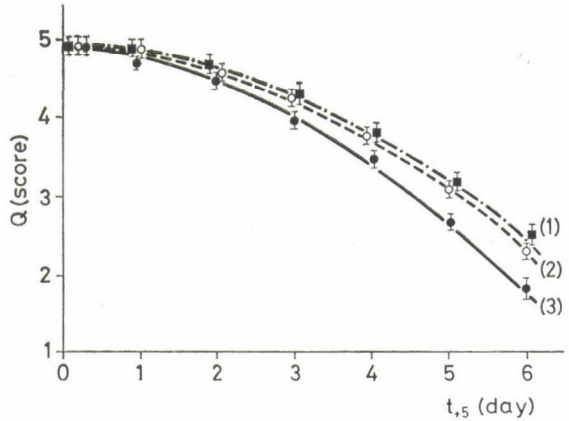


Fig. 8. Deterioration curves and characteristic data of the curves for quick-frozen partially cooked beefburger rolls wrapped in polyethylene bags and stored at +5 °C

Symbol	Quality characteristic	Equation of the deterioration curve	Residual standard error	Standard error of regression coefficient	Standard error of regression constant	Significance of difference in the regression coefficients
— · — · — (1)	aroma	$Q = \begin{cases} 4.90, & \text{if } t_{+5} \leq 0.5 \\ 4.90-61.7\, t_{+5}^2, & \text{if } t_{+5} \geq 0.5 \end{cases}$	0.17	0.09	0.83	Ø, ***, ***
– – – – – (2)	flavour	$Q = \begin{cases} 4.90, & \text{if } t_{+5} \leq 0.5 \\ 4.90-62.1\, t_{+5}^2, & \text{if } t_{+5} \geq 0.5 \end{cases}$	0.16	0.10	0.71	
——— (3)	firmness	$Q = \begin{cases} 4.90 & \text{if } t_{+5} \leq 0.1 \\ 4.90-72.3\, t_{+5}^2, & \text{if } t_{+5} \geq 0.1 \end{cases}$	0.22	0.11	0.24	

*** Very highly significant difference at the $P \geq 99.9\%$ probability level
Ø Not significantly different at the $P \leq 95\%$ probability level

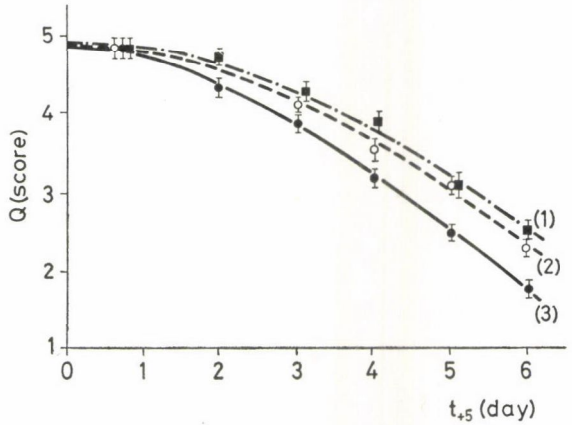


Fig. 9. Deterioration curves and characteristic data of the curves for quick-frozen partially cooked beefburger rolls wrapped in bags made of a laminated polyethylene + polyamide film and stored at +5 °C

Symbol	Quality characteristic	Equation of the deterioration curve	Residual standard error	Standard error of regression coefficient	Standard error of regression constant	Significance of difference in the regression coefficients
— · — · — (1)	aroma	$Q = \begin{cases} 4.90, & \text{if } t_{+5} \leq 0.4 \\ 4.90 - 60.5\, t_{+5}^2, & \text{if } t_{+5} \geq 0.4 \end{cases}$	0.21	0.08	0.92	Ø, ***, ***
— — — — — (2)	flavour	$Q = \begin{cases} 4.90, & \text{if } t_{+5} \leq 0.5 \\ 4.90 - 63.2\, t_{+5}^2, & \text{if } t_{+5} \geq 0.5 \end{cases}$	0.18	0.08	0.81	
——— (3)	firmness	$Q = \begin{cases} 4.90, & \text{if } t_{+5} \leq 0.1 \\ 4.90 - 73.5\, t_{+5}^2, & \text{if } t_{+5} \geq 0.1 \end{cases}$	0.16	0.09	0.90	

*** Very highly significant difference at the $P \geq 99.9\%$ probability level
Ø Not significantly different at the $P \leq 95\%$ probability level

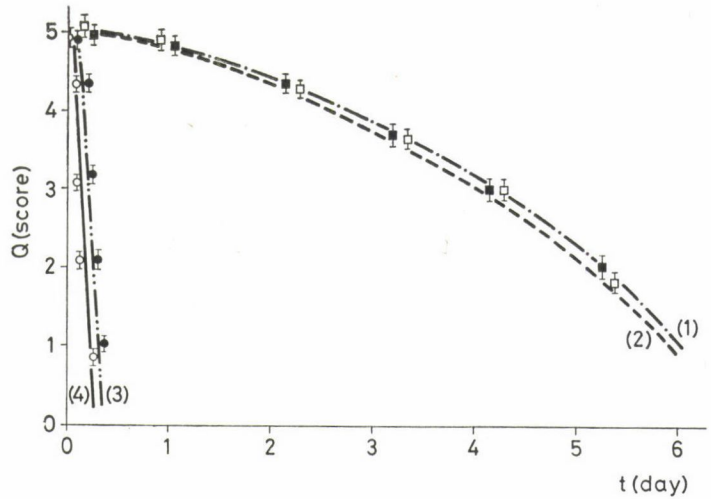


Fig. 10. Results of a statistical comparison of deterioration curves determined on the basis of changes in the firmness of quick-frozen partially cooked beefburger rolls stored under various conditions

Symbol	Equation of the deterioration curve	Storage temperature °C	Packaging material
— · — · — (1)	$Q = \begin{cases} 4.90, & \text{if } t_{-18} \leq 1.16 \\ 4.90 - 0.11\, t_{-18}^2, & \text{if } t_{-18} \geq 1.16 \end{cases}$	−18	polyethylene foil
— — — — — (2)	$Q = \begin{cases} 4.90, & \text{if } t_{-18} \leq 1.25 \\ 4.90 - 0.12\, t_{-18}^2, & \text{if } t_{-18} \geq 1.25 \end{cases}$	−18	polyethylene + polyamide film
— · · — · · — (3)	$Q = \begin{cases} 4.90, & \text{if } t_{+5} \leq 0.10 \\ 4.90 - 72.3\, t_{+5}^2, & \text{if } t_{+5} \geq 0.10 \end{cases}$	+5	polyethylene foil
——————— (4)	$Q = \begin{cases} 4.90, & \text{if } t_{+5} \leq 0.10 \\ 4.90 - 73.5\, t_{+5}^2, & \text{if } t_{+5} \geq 0.10 \end{cases}$	+5	polyethylene + polyamide film

Significance of differences in the regression coefficients

ø *** *** *** *** ø

*** Very highly significant difference at the $P \geq 99.9\%$ probability level
ø Not significantly different at the $P \leq 95\%$ probability level

below 0 °C is favourable. In the positive temperature range deterioration processes accelerate very rapidly and consequently the shelf life is very quickly reduced. This fact draws particular attention to the necessity for correct storage of frozen foods in households.

In summary, it can be stated that the deterioration rate of quick-frozen beefburger rolls, and consequently the shelf life, can be fundamentally influenced by reducing the storage temperature. As regards the wrapping material, no effect of this type can be demonstrated on the basis of the experimental results.

2.3. Correlations which approximate to a hyperbola

Raw leg of pork was chosen as the model on which to demonstrate quality-influencing characteristics which change in a hyperbolic manner as a function of time.

The sensory characteristics studied were colour and smell.

The results of sensory evaluation on pork wrapped in cellothene foil and stored at 20–22 °C are summarized in Fig. 11 together with the deterioration curves calculated from the results, the characteristic data of the curves and the results of covariance analysis carried out in order to select the critical characteristic.

It can be seen from the results that the change in smell differed significantly at the $P = 99\%$ probability level from the rate of change in the colour, so the smell was taken as the critical characteristic.

For pork stored at the same temperature but wrapped in shrinking polyethylene foil the results of sensory evaluation, the calculated deterioration curves, the characteristic data of the curves and the results of comparison between the curves using covariance analysis are presented in Fig. 12.

It can be seen from the results that the rate of change in the smell is significantly different from that of the colour. Consequently, the smell can be regarded as the critical characteristic.

The results of sensory evaluation on pork wrapped in bags made of cellothene foil and stored at 5–7 °C, the deterioration curves calculated from the results and the characteristic data of the curves are presented in Fig. 13, which also summarizes the results of covariance analysis.

It can be seen from the results that the rate of change in the smell differed significantly from the rate of change in the colour, so the smell is the critical characteristic.

For pork stored at 5–7 °C but wrapped in shrinking polyethylene foil, the mean values and standard deviations obtained from sensory evaluation, the deterioration curves calculated from these values, the characteristic data of the curves and the results of covariance analysis are summarized in Fig. 14.

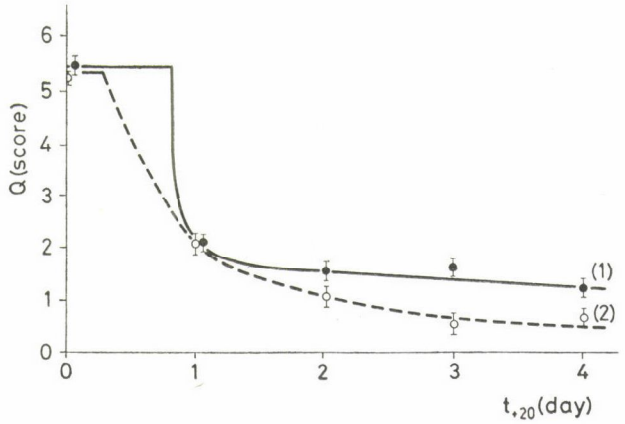


Fig. 11. Deterioration curves and characteristic data of the curves for raw leg of pork wrapped in bags made of cellothene and stored at 20–22 °C

Symbol	Quality characteristic	Equation of the deterioration curve	Residual standard error	Standard error of regression coefficient	Standard error of regression constant	Significance of difference in the regression coefficients
——— (1)	colour	$Q = \begin{cases} 5.4, & \text{if } t_{+20} \leq 0.8 \\ 2.0\ t_{+20}^{-0.3}, & \text{if } t_{+20} \geq 0.8 \end{cases}$	0.15	0.09	0.03	***
– – – – – (2)	smell	$Q = \begin{cases} 5.3, & \text{if } t_{+20} \leq 0.3 \\ 2.1\ t_{+20}^{-0.9}, & \text{if } t_{+20} \geq 0.3 \end{cases}$	0.13	0.07	0.02	

*** Very highly significant difference at the $P \geq 99.9\%$ probability level

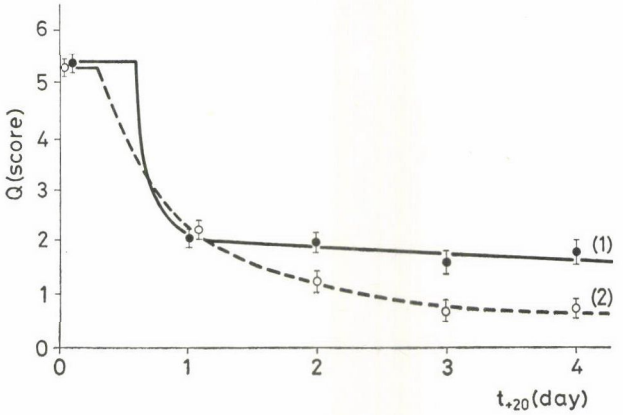


Fig. 12. Deterioration curves and characteristic data of the curves for raw leg of pork wrapped in shrinking polyethylene film and stored at 20–22 °C

Symbol	Quality characteristic	Equation of the deterioration curve	Residual standard error	Standard error of regression coefficient	Standard error of regression constant	Significance of difference in the regression coefficients
——— (1)	colour	$Q = \begin{cases} 5.4, & \text{if } t_{+20} \leq 0.6 \\ 2.1\, t_{+20}^{-0.2}, & \text{if } t_{+20} \geq 0.6 \end{cases}$	0.08	0.12	0.05	***
– – – – – (2)	smell	$Q = \begin{cases} 5.3, & \text{if } t_{+20} \leq 0.3 \\ 2.2\, t_{20}^{-0.9}, & \text{if } t_{+20} \geq 0.3 \end{cases}$	0.10	0.08	0.07	

*** Very highly significant difference at the $P \geq 99.9\%$ probability level

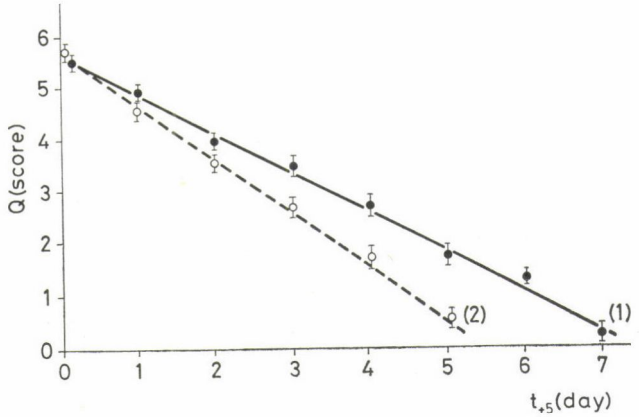


Fig. 13. Deterioration curves and characteristic data of the curves for raw leg of pork wrapped in bags made of cellothene and stored at 5–7 °C

Symbol	Quality characteristic	Equation of the deterioration curve	Residual standard error	Standard error of regression coefficient	Standard error of regression constant	Significance of difference in the regression coefficients
——— (1)	colour	$Q = 5.6–0.6871\, t_{+5}$	0.17	0.0020	0.06	***
– – – – – (2)	smell	$Q = 5.7–0.9436\, t_{+5}$	0.26	0.0018	0.09	

*** Very highly significant difference at the $P \geq 99.9\%$ probability level

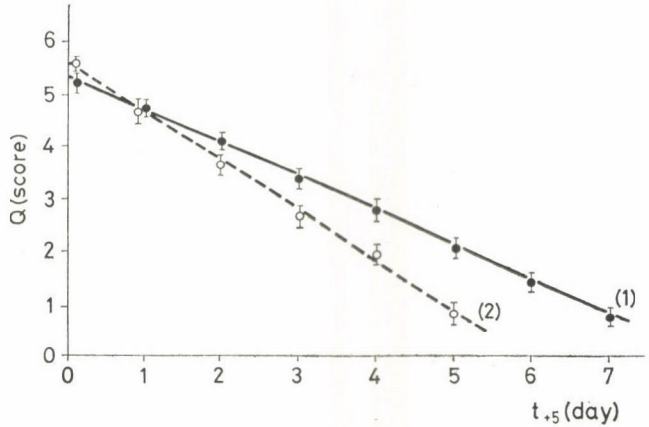


Fig. 14. Deterioration curves and characteristic data of the curves for raw leg of pork wrapped in shrinking polyethylene film and stored at 5–7 °C

Symbol	Quality characteristic	Equation in the deterioration curve	Residual standard error	Standard error of regression coefficient	Standard error of regression constant	Significance of difference in the regression coefficients
——— (1)	colour	$Q = 5.4–0.6415\, t_{+5}$	0.11	0.0011	0.08	***
– – – – – (2)	smell	$Q = 5.6–0.9294\, t_{+5}$	0.14	0.0012	0.09	

*** Very highly significant difference at the $P \geq 99.9\%$ probability level

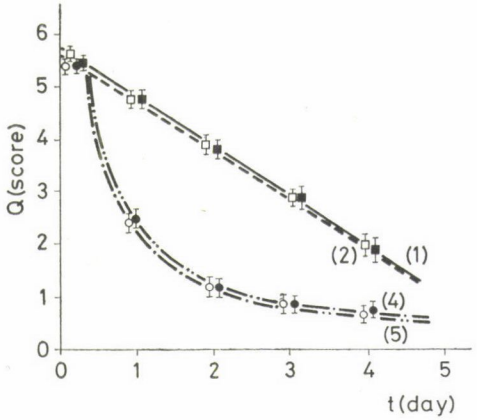


Fig. 15. Results of a statistical comparison of deterioration curves determined on the basis of a smell test on raw leg of pork stored under various conditions

Symbol	Equation of deterioration curve	Storage temperature (°C)	Packaging material	Significance of difference in the regression coefficients
——— (1)	$Q = 5.7 - 0.9436\ t_{+5}$	5–7	cellothene film	
– – – – – (2)	$Q = 5.6 - 0.9294\ t_{+5}$	5–7	shrinking polyethylene film	
— · — · — (4)	$Q = \begin{cases} 5.3, & \text{if } t_{+20} \leq 0.3 \\ 2.3\ t_{+20}^{-0.9}, & \text{if } t_{+20} \geq 0.3 \end{cases}$	20–22	cellothene film	
— · · — · · — (5)	$Q = \begin{cases} 5.3, & \text{if } t_{+20} \leq 0.3 \\ 2.2\ t_{+20}^{-0.9}, & \text{if } t_{+20} \geq 0.3 \end{cases}$	20–22	shrinking polyethylene film	

Ø *** *** *** *** Ø

*** Very highly significant difference at the $P \geq 99.9\%$ probability level
Ø Not significantly different at the $P \leq 95\%$ probability level

It can be seen from the results that the degree of change in the smell again differed significantly from the degree of change in the colour, so in this case, too, the smell was taken as the critical characteristic.

In summary, it can be stated that the critical character of raw leg of pork, irrespective of the storage conditions, is the smell.

A statistical comparison of the regression coefficients of the deterioration curves describing changes in the critical characteristic under different storage conditions makes it possible to determine the effect of the storage conditions on quality maintenance. The curves which were compared, the characteristic data of the curves and the results of the statistical comparison of deterioration rates are summarized in Fig. 15.

It can be seen from the results that the deterioration rates of pork stored at the same temperature but in different packaging do not differ significantly from one another. However, the deterioration rates of pork stored at different temperatures in the same type of packaging differ very significantly from one another.

It can be seen that the rate of deterioration is influenced to a very great degree by the storage temperature, while this type of effect could not be established on the basis of the experiments for the type of packaging material.

The decisive role played by the storage temperature is also proved by the fact that the nature of the deterioration curve changes from hyperbolic to linear when the storage temperature is reduced from 20–22 °C to 5–7 °C. This leads to the important practical conclusion that in order to prolong the shelf life, raw leg of pork should definitely be stored under refrigerated conditions.

3. Conclusions

The present research was aimed at elaborating a mathematical method for objectively evaluating the results of measurements on raw materials and other food products in order to trace changes in the quality and calculate the shelf life.

Poultry, meat and frozen food products stored at various temperatures and packed in different materials were chosen as models, with the aid of which a statistical analytical procedure suitable for the selection of the critical characteristic which deteriorates most rapidly, and an analytical method for the comparison of differences in the rate of deterioration due to the different storage conditions, were described.

On studying changes in the quality of the products it was found that the change in quality characteristics as a function of time can be approximately described with linear, quadratic and hyperbolic functions. By substituting the

so-called critical value which is just acceptable into the equation, it is possible to calculate the shelf life.

In summary, it can be stated that the evaluation method elaborated for tracing time changes in quality is well suited for determining the characteristic with the greatest influence on the quality of the product, for selecting the best storage conditions, including the type of packaging, and, last but not least, for calculating the shelf life.

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EFFECT OF CHLORFLURENOL ON THE YIELD AND TECHNOLOGICAL VALUE OF RED PEPPER (*CAPSICUM ANNUUM L.*)

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It was found that treatment with chlorflurenol on individual flowers and whole plants of pepper in full bloom caused an increase in the total number and weight of fruit. However, individual fruit from plants treated with chlorflurenol had a smaller weight than fruit from control plants. Under the influence of chlorflurenol fruit maturity time was shortened by 3 to 5 days as compared to control plants. On plants treated with chlorflurenol seedless fruits were developed. In fruit from plants treated with chlorflurenol there was an increase in the content of vitamin C, protein, dry matter, total extract and total sugar. Chlorflurenol caused a decrease in the content of β-carotene in peppers.

Chlorflurenol has already been used in practice in the production of pickling cucumbers (Schneider, 1974). It seems that it is possible to use this compound in tomato production, especially with the once-over, mechanical harvest technique used for the processing industry (Rudich & Rabinowitch, 1974).

In this paper an attempt was made to use chlorflurenol to increase the yield of sweet pepper. The technological value of the fruit obtained was evaluated with a view to utilizing it in processing.

1. Materials and methods

The experiments were carried out on the Poznańska Słodka variety in the greenhouse (Lublin–Felin) in the years 1978 and 1980.

In 1978 the temperature in the greenhouse (non-heated) was very unfavourable for the growth and crop of pepper, as it fluctuated between 5–15 °C at night and 10–30 °C during the day. In 1980 the air temperature in the greenhouse was at the normal level, being 15–18 °C at night and 22–28 °C during the day. The plants were grown in Mitscherlich pots filled with hotbed soil in the period from April to September.

Chlorflurenol (CME 74050 P) was applied in solutions having concentrations of 0.25, 0.50 and 1.00 $cm^3\ dm^{-3}$. In 1978 individual fully open flowers were immersed once in chlorflurenol immediately after blooming, while in 1980 the whole plants in full bloom were sprayed with the preparation. 0.001 %

Tween 80 (Reanal, Hungary) was added to the chlorflurenol solution. The control plants were treated only with Tween 80.

The experiments were arranged in a completely randomized design. Each combination included 10 plants in 1978 and 26 plants in 1980. Paper labels with the date of hormon treatment were put on the peduncles during the chlorflurenol treatment of the individual flowers. Fruits from both series of experiments were harvested individually as they matured, and a record was made of their weight and the date of harvest. On the basis of the data of chlorflurenol treatment and the harvest date, the number of days elapsing from hormon treatment of the flowers to the fruit harvest was calculated for all the fruit in 1978.

In the cross-sectioned fruit, the colour of the flesh and the extent to which the chambers were filled with seeds were determined.

In the peppers obtained in 1980 from plants treated with chlorflurenol and from the control plants, the content of dry matter, total extract, total sugar, protein, vitamin C and β-carotene, fibre and ash were determined. Six analyses were carried out for each of the chemical components in each treatment (3 times from each of the first two fruit harvests), except for protein, which was determined in 4 replicates (twice for each of the first two harvests). Three-four fruit were taken for each analysis. The dry matter was determined in a desiccator at 105 °C. The total extract was determined with the help of a refractometer. The total content of sugar was determined by Samogyi–Nelson's method, and protein by Kjeldahl's method. Tilman's method was used to determine vitamin C, while β-carotene was examined according to the Polish Standard PN 71/1 7510 (CHARŁAMPOWICZ, 1966). The fibre was determined by Hemmberg–Stohman's method and total ash by sample burning.

The total number and weight of fruit and the results of the chemical tests were statistically analysed by Duncan's multiple range test.

2. Results

The highest total number of fruit was obtained after the application of chlorflurenol solutions with concentrations of 1.00 $cm^3\,dm^{-3}$ in 1978 and 0.25 $cm^3\,dm^{-3}$ in 1980. In comparison with the control plants, the number of fruit in these treatments increased by 6.3 fruit per plant on the average in 1978 and by 4.3 fruit per plant in 1980. An increase in the total number of fruit was also found in other experiments after a single treatment of flowering plants with 1.00 mg dm^{-3} solutions of methyl-2-chloro-9-hydroxyfluorene-(9)-carboxylate (ŁUKASIK & HORTYNSKA, 1977). Under the influence of this compound the number of fruit with greater weights was increased in comparison to the control plants. As a result of this, the commercial and total yields were higher. Similarly, in toma-

toes treated with chlorflurenol methyl ester the total and early yield of fruit increased (ŁUKASIK, 1977). In these experiments, however, in spite of a considerable increase in the fruit number on plants treated with chlorflurenol, no significant increase in the total weight of fruit was observed. This was caused by the fact that in plants treated with chlorflurenol there were many fruits with very small weights (Table 2).

The highest total weight of fruit was obtained with a chlorflurenol concentration of 0.50 $cm^3\ dm^{-3}$. In this treatment there was an increase of over 61 g per plant on the average in 1978 in comparison with the control plants, and an increase of over 37 g per plant in 1980. In the remaining treatments, in spite of the increase in the total number of fruit, the total fruit weight was on the level of the fruit for the control plants (Table 1).

Table 1

Effect of chlorflurenol applied to individual blooming flowers (1978) and to whole plants in bloom (1980) on the yield of Poznańska Słodka pepper. Average for one plant

Concentration of chlorflurenol ($cm^3\ dm^{-3}$)	Years			
	1978		1980	
	Total number of fruit[b]	Total weight of fruit (g)[a]	Total number of fruit[c]	Total weight of fruit (g)[a]
0.00	2.9	87.0	6.7	267.9
0.25	6.2	114.2	10.4	281.7
0.50	6.5	128.7	9.8	305.7
1.00	9.2	78.3	8.4	256.7

[a] Differences are non-significant
[b] Differences are significant at $D_{0.05} = 1.09–1.18$
[c] Differences are significant at $D_{0.05} = 1.09–1.19$

Table 2

Percentage of fruit of different weights in total number of fruit in Poznańska Słodka pepper treated with chlorflurenol in solutions of different concentrations in 1978 and 1980

Concentration of chlorflurenol ($cm^2 dm^{-3}$)	Weight of single fruit (g)							
	0–19		20–34		35–50		>50	
	Year		Year		Year		Year	
	1978	1980	1978	1980	1978	1980	1978	1980
0.00	51.72	18.13	31.03	17.03	17.24	33.52	0.00	31.32
0.25	61.29	48.33	30.64	20.45	8.06	14.87	0.00	16.36
0.50	52.31	47.25	46.15	14.28	1.54	11.35	0.00	27.11
1.00	40.34	38.71	46.34	24.88	7.32	14.28	0.00	22.12

Table 3

Average number of days from chlorflurenol treatment of single flowers to fruit harvest in Poznańska Słodka pepper in 1978

Concentration of chlorflurenol ($cm^3\ dm^{-3}$)	Average number of days to fruit maturity (days)
0.00	68.9
0.25	65.6
0.50	64.2
1.00	63.3

Table 4

Percentage of parthenocarpy in total number of fruit in Poznańska Słodka pepper treated with chlorflurenol at different concentrations in 1978 and 1980

Concentration of chlorflurenol ($cm^3\ dm^{-3}$)	Percentage of parthenocarpy	
	1978	1980
0.00	0.0	3.9
0.25	45.8	74.0
0.50	44.6	66.7
1.00	58.5	75.6

Under the influence of chlorflurenol there was a shortening of the time required for fruit maturity. When flowers were treated with solutions of 1.00 $cm^3\ dm^{-3}$ the fruit matured 5 days earlier than in the control plants. Using a concentration of 0.25 $cm^3\ dm^{-3}$ fruit were harvested 3 days earlier than in the control treatment (Table 3). An increase in early yield was also noticed when fully developed peppers were treated with etephon (ŁUKASIK & HORTYNSKA, 1976) or methyl-2-chloro-9-hydroxyfluorene-(9)-carboxylate (ŁUKASIK & HORTYNSKA, 1977).

The fruit on plants treated with chlorflurenol were red in colour, similar to the control fruit. At higher concentrations of chlorflurenol a slight deformation of the fruit was noticed. A similar deformation in the fruit was noticed in tomatoes at high concentrations of morphactin (ŁUKASIK, 1975).

Under the influence of chlorflurenol seedless fruit developed. The highest percentage of parthenocarpic fruit in the total number of fruit was noticed when chlorflurenol was used at a concentration of 1.00 $cm^3\ dm^{-3}$ (Table 4). It should be stressed that the development of parthenocarpic fruit due to the influence of morphactins was frequently noticed in earlier experiments on pepper (JAYAKARAN, 1973), tomatoes (SCHNEIDER, 1964; 1970, and ŁUKASIK & HUSZCZA,

1974) and cucumbers (ROBINSON et al., 1971; CANTLIFFE et al., 1972; CANTLIFFE, 1974).

The highest amount of dry matter was found in peppers harvested from plants treated with chlorflurenol at the lowest concentration (0.25 cm^3dm^{-3}). In comparison with the control plants the content of dry matter in this treatment increased by about 2%. Similarly, fruit harvested from plants treated with chlorflurenol at concentrations of 0.50 and 1.00 cm^3dm^{-3} contained 1.15% and 0.60% more dry matter than the control (Table 5).

In fruit harvested from plants treated with chlorflurenol a higher content of total extract (about 1%) and a higher total sugar content (about 0.50%) were observed. However, the content of fibre in fruit treated with chlorflurenol was slightly lower than in the control fruit.

It should be stressed that parallel to the increase in chlorflurenol concentration there was a significant increase in vitamin C. Thus, in fruit harvested from plants treated with chlorflurenol at concentrations of 1.00 and 0.50 $cm^3\,dm^{-3}$ the vitamin C content was about 25 mg % and 21 mg % higher, respectively, than in the control fruit (Table 5). A higher content of vitamin C was also found in seedless fruit of tomatoes treated with chlorflurenol methyl ester (ŁUKASIK et al., 1978).

The highest amount of raw protein was observed in peppers treated with chlorflurenol. The difference in the raw protein content proved to be statistically significant (Table 5). The content of total ash in peppers was similar to that in the control. Chlorflurenol considerably decreased the β-carotene content in peppers (Table 5).

Table 5

Effect of chlorflurenol on the content of some chemical constituents in Poznańska Słodka peppers treated with different chlorflurenol concentrations

Constituents	Concentration of chlorflurenol ($cm^3\ dm^{-3}$)			
	0,00	0.25	0.50	1.00
Total extract (%)[1]	6.60	7.55	7.38	7.30
Total dry matter (%)[1]	6.65	8.40	7.80	7.25
Total sugar (%)[1]	5.40	5.83	6.25	6.10
Raw protein (%)[2]	0.73	1.25	1.10	1.20
Fibre (%)[1]	1.52	1.18	1.41	1.40
Total ash (%)[1]	0.78	0.76	0.69	0.62
Vitamin C (mg%)[3]	110.55	119.10	132.08	135.82
β-carotene (mg%)[4]	0.94	0.87	0.86	0.40

[1] Differences are non-significant
[2] Differences are significant at $D_{0.05} = 0.08–0.90$
[3] Differences are significant at $D_{0.05} = 7.79–8.42$
[4] Differences are significant at $D_{0.05} = 0.17–0.19$

3. Conclusion

The following conclusions can be drawn on the basis of the experimental data:

- Treatment with chlorflurenol on the individual flowers or whole plant of peppers in full bloom considerably increased the number of fruit (Table 1).
- Individual fruit from plants treated with chlorflurenol had a smaller weight than fruit from control plants. Thus, the total weight of fruit from plants treated with chlorflurenol was only slightly higher than in the control plants (Tables 1 and 2).
- Under the influence of chlorflurenol, the fruit maturity time was shortened by 3 to 5 days as compared to the control plants (Table 3).
- On plants treated with chlorflurenol seedless fruit developed. The percentage of parthenocarpic fruit was highest at a chlorflurenol concentration of 1 cm^3dm^{-3} (Table 4).
- In fruit from plants treated with chlorflurenol there was a statistically significant increase in the content of vitamin C (Table 5).
- In all treatments chlorflurenol caused a statistically significant increase in the raw protein content of peppers (Table 5).
- In fruit harvested from plants treated with chlorflurenol a higher content of dry matter, total extract and total sugar was observed than in the control fruit (Table 5).
- The ash content in the fruit was similar to that in the control (Table 5).
- Chlorflurenol decreased the content of β-carotene in peppers (Table 5).

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

II. THE EFFECT OF CONSISTENCY UPON TASTE

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Four foods of plant origin were used in experiments aimed at establishing interaction of taste and consistency during sensory evaluation. The four foods investigated were: quick-frozen string beans, four varieties (Amanda, Amboy, Valja, Machine-harvested); quick-frozen raspberries; fresh potatoes and cauliflower. The samples prepared in different ways were subjected to scoring by a panel of 10 members. Scores were evaluated by Kramer's method and correlation coefficients were calculated. Generally the consistency of the samples influenced the evaluation of their taste, particularly with foods the consistency of which is an important quality parameter (raspberries). With foods prepared in various ways in general consumption this tendency was not observed. Neither was it observable when the consistency factor was eliminated by pulping.

In the previous paper of the author (URBÁNYI, 1982) the effect of colour on the evaluation of taste was investigated. In the present paper the effect of another important sensory property, that of consistency is discussed.

Since the sensory organs in the mouth are sensitive beside the taste to mechanical effects as well, thus, the mechanical effect of foods of different consistency probably affects the sensation of taste, too.

It is well known that foods suffering a change of consistency during consumption (e.g. icecream, chocolate, etc.) are very popular (SZCZESNIAK, 1971). It seems evident that this change affects the sensation of taste.

MACKEY and VALASSI (1956) studied the effect of the viscosity of foods upon the threshold value of sensation. They found that in systems of higher viscosity the threshold value of taste was significantly higher than that of pure aroma substances in less viscous aqueous solutions.

PAULUS and HAAS (1980) investigated the sensation of saccharose, sodium chloride, caffeine and citric acid in solutions of various viscosities and found that with higher viscosities a higher concentration of aroma substances was necessary to cause the same sensation of taste.

Similar observations were made in experiments carried out with different substances by MOSKOWITZ (1970), PANGBORN and SZCZESNIAK (1974), and STONE and OLIVER (1976).

All these data found in the literature prove that rheological properties affect the sensation of taste. Thus, to investigate the interaction between consistency and taste evaluation experiments were carried out with a variety of fruits and vegetables.

1. Materials and methods

1.1. Materials

Four varieties of quick-frozen string beans with green pod (Amanda, Amboy, Valja, Machine-harvested), quick-frozen raspberries, fresh potatoes and cauliflower were used in the experiments. Except for the beans, the rest was of unidentified variety bought in the market.

1.2. Methods

Evaluation was carried out by a scoring method. In view of the problem investigated only consistency and taste were scored. The maximum number of points in the case of beans and raspberries was 10, of potatoes and cauliflower 9 for each property. The panel consisted of 10 members. Retasting was made possible.

To evaluate the results Kramer's method was used as described in the first part of this study (URBÁNYI, 1982).

2. Results

First the bean samples were evaluated. The quick-frozen samples each were boiled for 25 min in a 1% common salt solution. The half of each cooked sample was turmixed. Thus, four pulped and four samples of beans cut in

Table 1

Evaluation according to Kramer of the sensory test by scoring of string bean samples by a 10-member panel

Number of sample	Taste	Consistency
1.a	37.5	33.0
1.b	56.5	28.5
2.a	45.5	59.0
2.b	61.5	48.0
3.a	41.5	49.5
3.b	49.5	43.5
4.a	26.0*	35.5
4.b	42.0	63.0

* Significant at P = 95% probability level (27–63)

First figure marks the variety: 1 = Amanda beans, 2 = Amboy beans, 3 = Valja beans, 4 = Machine-harvested beans. The second figure: a. cut in pieces; b. pulped

Table 2

Evaluation according to Kramer of string bean samples grouped according to consistency

Number of sample	Cut in pieces				Pulped			
	Taste		Consistency		Taste		Consistency	
	Rank sum	Order	Rank sum	Order	Rank sum	Order	Rank sum	Order
1	24.0	2	19.0	1	28.5	3	17.0	1
2	30.0	4	32.5	4	30.0	4	26.0	3
3	27.0	3	29.0	3	21.5	2	23.5	2
4	19.0	1	19.5	2	20.0	1	33.5*	4

For symbols see Table 3

Table 3

Evaluation according to Kramer of the sensory test by scoring of raspberries carried out by a 10-member panel

Number of sample	Taste	Order	Consistency	Order
1	20.5	1–2	11.0**	1
2	20.5	1–2	21.5	2
3	27.5	3	33.5*	3
4	31.5	4	34.0*	4

** highly significant at P = 99% probability level (15–35)
* significant at P = 95% probability level (17–33)
Symbols: 1 = whole fruits slowly thawed
2 = whole fruits rapidly thawed over steam
3 = fruit fragments slowly thawed
4 = fruit fragments rapidly thawed over steam

pieces were subjected to evaluation, all at the same time. The results, evaluated according to Kramer, are given in Table 1.

Significant difference was observed only with one of the samples.

In order to refine evaluation the pulped and lumpy samples were evaluated separately, too (Table 2).

Although only one of the samples differs from the rest significantly, as regards the order of the samples an interesting tendency may be observed. Of the cut bean samples No. 1 and 4 stand in the first place for taste as well as consistency. The rank sum of the two samples differs only slightly (19.0 and 19.5). If we disregard this the order of the four samples for both properties is identical. In case of the pulped samples the same tendency is not observable. The order of the samples – using the serial number of the samples – for taste: 4, 3, 1, 2 and for consistency: 1, 3, 2, 4.

Next the quick-frozen raspberries were tested. The samples were separated into whole fruits and broken fragments. Both parts were halfed. One half of each sample was spread in a thin layer and thawed at room temperature (20–22 °C), slowly. The other half was thawed rapidly over steam. From a single sample four samples of different consistency were, thus, obtained. These samples were then subjected to scoring. Results are shown in Table 3.

It can be seen that the whole fruits thawed slowly were found to be the best at 99 % probability, while the samples obtained from the fruit fragments were found worse than the rest at 95 % probability. Thus, there was a substantial difference in the consistency of the four samples. As regards the taste of the four samples originating from a single sample – which should be practically identical – significant difference could not be found between them. However, the order as established from the rank sums based on the taste scores is the same as the order of consistency. This shows, since the samples scored were of the same taste, that the evaluation of taste is highly affected by consistency.

The potato samples were prepared from the same batch. The potatoes were boiled in a 1 % common salt solution for 25 min. The four samples differing in consistency were prepared from the boiled potatoes:

- Whole potato,
- Potato cut in slices,
- Potatoes mashed with fork,
- Pulped potatoes.

The same series of samples was prepared a second time and for this the potatoes were boiled for 30 min. Evaluation of both series is given in Tables 4 and 5.

In neither of the two series was significant difference found in taste or in consistency. The method of preparation of the four samples corresponded

Table 4

Evaluation according to Kramer of the sensory test by scoring of potato samples by a 10-member panel
(First test)

Number of sample	Taste	Order	Consistency	Order
1	25.5	3–4	20.5	1
2	25.0	2	23.0	2
3	25.5	3–4	26.5	3
4	24.0	1	30.0	4

Symbols: 1 = whole potato
2 = cut in slices
3 = mashed
4 = pulped

Table 5

Evaluation according to Kramer of the sensory test by scoring of potato samples by a 10-member panel
(Second test)

Number of sample	Taste	Order	Consistency	Order
1	18.5	1	20.0	1
2	29.0	4	25.5	2
3	26.0	2	26.5	3
4	26.5	3	28.0	4

Symbols: 1 = whole potato
2 = cut in slices
3 = mashed
4 = pulped
It would be significant at P = 95% probability level (17–33)

to methods applied in everyday life, thus it was familiar to the panel members. Since neither of the samples was given preference there was no reason to discriminate between samples made of the same original material.

The cauliflowers selected from the same batch were cooked in a 2% common salt solution for 30 min. The following four samples were prepared, taking care to divide each cooked head of cauliflower evenly between samples:

- Heads divided in four,
- Heads separated into flowerets,
- Heads mashed with fork,
- Pulped heads.

The series of samples was repeated with the difference of using for boiling a 0.1% salt solution.

The results of the evaluation of the two series are given in Tables 6 and 7.

In both series panel members found better the taste and consistency of the samples divided in four and separated into flowerets than those comminuted to a higher degree. From the aspect of traditions in consumption these two samples do not differ substantially. Express difference was not found between these two samples.

In the first series cauliflower separated into flowerets was found better, in the second the quartered heads. The pulped sample differing from the habitual way of consumption obtained the lowest score for both taste and consistency. In the first series the consistency, in the second the taste obtained significantly lower scores.

A third series was also studied with cauliflower. In this differences were caused in the taste of the samples of the same and of differing consistency.

Table 6

Evaluation according to Kramer of the sensory test by scoring of cauliflower samples by a 10-member panel
(First test)

Number of samples	Taste	Order	Consistency	Order
1	19.0	1	19.5	2
2	19.5	2	15.0*	1
3	28.5	3	27.5	3
4	33.0	4	38.0**	4

Symbols: 1 = cauliflower heads cut in four
2 = heads separated into flowerets
3 = mashed with fork
4 = pulped
** highly significant at P = 99% probability level (15–35)
* significant at P = 95% probability level (17–33)

Table 7

Evaluation according to Kramer of the sensory test by scoring of cauliflower samples by a 10-member panel
(Second test)

Number of sample	Taste	Order	Consistency	Order
1	21.5	2	15.5**	1
2	19.0	1	20.0	2
3	25.0	3	29.5	3
4	34.5	4	35.0*	4

Symbols: 1 = cauliflower heads cut in four
2 = heads separated into flowerets
3 = mashed with fork
4 = pulped
** highly significant at P = 99% probability level (15–35)
* significant at P = 95% probability level (17–33)

In this experiment the heads of cauliflower were cut in four. The differences in consistency were achieved by boiling for different lengths of time and in taste by using salt solutions of different concentration for cooking. Thus, four samples were obtained of which two were identical in taste, two in consistency.

Cooking times of 15 and 25 min were applied. The samples where salt was applied, were cooked in a 1% solution. The following four samples were prepared:

- cauliflower boiled in 1% salt solution for 15 min,
- cauliflower boiled in plain water for 15 min,
- cauliflower boiled in 1% salt solution for 25 min,
- cauliflower boiled in plain water for 25 min.

The taste and consistency of the samples was scored, then the samples were pulped, thus the differences in consistency were eliminated. The pulped samples were judged only for taste. The results are summarized in Table 8.

The order of the samples for both taste and consistency was the same when samples cut in pieces were tasted. Cauliflower boiled in salt water for 15 min was found to be the best while the lowest score was given the sample boiled for 25 min in plain water. In relation to taste the differences were significant.

Of the pulped samples, where there was no difference in the consistency, the two samples boiled in salt solution were judged identically, thus difference was not observed between samples of the same taste. Of the samples cut in pieces boiled in salt water the one boiled for a longer time was ranked higher.

Significant difference was not found between the tastes of the pulped samples.

It is worthy of note that out of the cut and pulped samples, otherwise identical, it was always the pulped one which was ranked lower.

In order to further elucidate the interaction between taste and consistency in the course of hedonic evaluation linear regression was calculated on the basis of the average scores given the two properties and the correlation coefficients were determined. The results are summarized in Table 9.

Table 8

Evaluation according to Kramer of the sensory test by scoring of cauliflower samples differing both in taste and consistency
(Number of panel members: 10)

Number of sample	Rank sums		
	Taste	Consistency	Taste
	pieces		pulp
1	19.0	26.5	18.0
2	34.5*	32.0	32.5
3	15.0*	19.5	18.0
4	31.5	22.0	31.5

* Significant at P = 95% probability level (17–33)
Symbols: 1 = boiled for 15 min in salt solution
2 = boiled for 15 min in plain water
3 = boiled for 25 min in salt solution
4 = boiled for 25 min in plain water

Table 9

Correlation coefficients calculated on the basis of the average scores of 10-members panel in the sensory test of taste and consistency
(The number of repetition : 4)

Sample		r
String beans:	cut in pieces	0.775
	pulped	0.499
Raspberries:		0.976
Potato:	first test	0.296
	second test	0.317
Cauliflower:	first test	0.478
	second test	0.578

A close correlation was found only for raspberries for which consistency is a very important characteristic of quality. The statistical test of the r values has shown significance at the $P = 5\%$ level only for raspberries.

The correlation coefficients for potatoes which are consumed regularly in all four ways of preparation were rather low (0.296 and 0.317). It is worth noting that the interaction of taste and consistency is much higher with string beans in pieces than with their pulped parallels where differences in consistency are mostly eliminated.

3. Conclusions

On the basis of sensory analysis of four different foodstuffs the following conclusions may be drawn:

With foods, where consistency is an important quality parameter the order of taste and consistency of samples of identical taste as established by Kramer's method, was found similar. In some of the cases the differences between the samples were significant. In case of raspberries, where consistency is an extremely important quality characteristic, a close correlation ($r = 0.976$) was found between the average scores for taste and consistency. Since, however, this conclusions are drawn on the basis of the comparison of only four samples the results may be considered as tendencies only.

In case of potatoes which were served in ways of preparation habitually consumed, thus, non of the consistencies is unfamiliar, the above tendency was not observed.

The same tendency was not observed either with samples where the differences in consistency were eliminated by pulping (string beans, cauliflower).

It is shown by the study that the consistency of the foods investigated influences the evaluation of taste. This is mainly valid for foods where the

consistency is an important quality characteristic. The taste of foods the consistency of which differed from the familiar was scored lower by panel members. In foods which are consumed in different consistencies this effect was not observed.

On the basis of the experiment it may be suggested that when an exact judgement of the taste is required, the differences in consistency should be eliminated by intentional intervention, e.g. pulping.

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PRELIMINARY EXPERIMENTS FOR MEASURING MEAT COMPOSITION BY NEAR INFRARED REFLECTION TECHNIQUE

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The task is to develop a near infrared reflection method for the determination of the moisture, fat and protein content of the meat used in manufacturing sausages. A Neotec 6450 type Research Composition Analyzer (spectrocomputer) was used for the experiments. The log (1/R') spectra of the homogenized meat samples were measured through the glass window of the sample cell in the 1100–2 500 nm wavelength region.

The effect of some disturbing factors upon the optical characteristics measured was studied, and the error in spectrum measurement caused by the total disturbance was established. The effect of the thickness of the meat sample upon the spectrum of meat was measured. At a sample thickness of 10 mm the influence of this variable on the log (1/R') spectra can be kept below $3 \cdot 10^{-3}$. The effect of sample position on the log (1/R') spectra has also proved to have a maximum of $3 \cdot 10^{-3}$. A change in the temperature of the sample caused an almost parallel shift in the spectrum. One °C caused a shift of about $5 \cdot 10^{-3}$ in the log (1/R') value. Uneven chopping of the meat was found to cause the greatest disturbance estimated to be about several times 10^{-2}. The methodology of spectrum measurement has been specified on the basis of these observations. The reproducibility, which also contains the error derived from sample preparation, was determined and was characterized by the standard deviation spectrum (its value up to 2 300 nm is below $4 \cdot 10^{-2}$).

The error derived from the spectrophotometer: the repeatability also characterized by a standard deviation spectrum has a value of about $5 \cdot 10^{-3}$.

It was found that the error in spectrum measurement increases substantially over 2 300 nm. It is unlikely that wavelengths suitable for composition measurement will be found in this range. The effect of several sources of error appears as an almost parallel shift in the spectrum; thus, the majority of such errors can probably be compensated for by a suitable spectrum transformation.

Finally, to illustrate the dependence of the meat spectrum on composition, the spectra of mixtures of muscle and adipose tissues (in 20 mass % steps) are shown.

KÖRMENDY and co-workers (1979) studied the mathematical model of sausage production and found that knowing the composition of the raw materials just at hand serves as a precondition for producing any product at an optimal cost. The classical analytical methods are too time-consuming; thus, they are not suitable for this purpose. Therefore, the aim was to develop a rapid instrumental method for determining the moisture, protein and fat content of meat.

BEN–GERA and NORRIS (1968) established that the near infrared spectrophotometric method can be used to determine the moisture and fat content of meat emulsions. The linear equations created between the composition and the transmittance data, measured at two wavelengths with the special spectro-

photometer built by the authors (NORRIS et al., 1961), described the moisture content in the region of 45 to 75 mass% with an error of 1.4 mass% and the fat content in the region of 5 to 35 mass% with an error of 2.1 mass%. The main sources of error were found to be deviations in sample thickness and the low signal to noise ratio due to high absorption. By reflection measurements both errors can be reduced.

More recent papers contain solutions based on the measurement of reflection. ROSENTHAL (1973) and ANON (1973a, b) describe instruments developed for the determination of fat in ground meat. MASSIE (1976) built an instrument for a similar purpose operating with Ga As infrared emitters as radiation sources. The error of this instrument in the range of 18.3 to 33.3 mass % fat content appeared to be 2 mass %. Practical application was limited by the lack of an appropriate radiation source. It is mentioned by MASSIE that, because of temperature dependence, thermostation or temperature compensation would be required. HAUSER and WEBER (1978; 1980) carried out meat composition measurements with a Technicon Infra-Alyzer-Plus apparatus. Observing the temperature dependence of the optical characteristic measured, they constructed a thermostated sample holder to limit it.

The NIR technique to be applied here is based on the measurement of the diffuse reflectance spectra of the samples. These spectra are influenced not only by the composition of the samples but by various factors, such as errors deriving from the preparation of the sample, the geometry of the measurement in the spectrophotometer, etc. This paper contains the preliminary experiments, in which the effect of some individual disturbing factors is studied, the methodology of measurement is developed and the reliability of the measurement of meat spectra is established.

1. Materials and methods

1.1. Meat samples

The samples used in the experiments were obtained by courtesy of the National Meat Research Institute. Post-rigor, medium fat beef samples were used in the preliminary experiments. In order to demonstrate the dependence of the spectrum on composition, lean beef muscle tissue and adipose tissue were mixed in 20 mass %-steps.

1.2. Measurement of spectra

The log (1/R') spectra of the meat samples were measured with a Neotec 6450 type RCA (spectrocomputer) through the glass window of the sample cell in the wavelength range of 1100 to 2500 nm. Since the spectrum is also affected by the geometry of the measurement, a schematic diagram is given in Fig. 1.

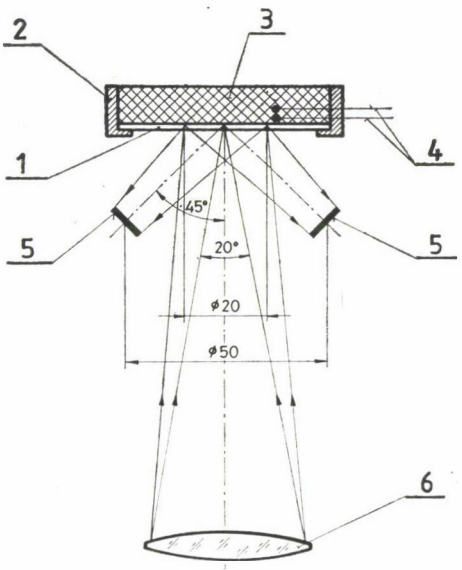

Fig. 1. Sketch of the geometry of meat spectrum measurement
1: glass window; 2: sample cell; 3: meat sample; 4: thermo-couples; 5: 4 10×10 mm PbS sensors; 6: last lens of the monochromator

A beam with an angle of approximately 20° and originating from a holographic grating monochromator with a band-width of 10 nm (LANDA, 1979) uniformly irradiated a 20 mm diameter spot of the sample. The ratio of the fluxes detected by the sensors scattered back diffusely by the sample on one hand and by a "white" ceramic reference standard on the other is R'; the reflectometer value (according to the IEC 1970 terminology). The spectrum measured by the instrument is the negative logarithm of R' as the function of the wavelength and the data of measurements performed in 2 nm steps stored on a floppy disc. Hereafter $V(\lambda)$ will be used instead of log (1/R') spectrum and will be called the basic spectrum. Namely:

$$V(\lambda) = -\log R'(\lambda)$$

The sensor system consists of four PbS detectors connected in parallel, each 10×10 mm in size and positioned on a circle 50 mm in diameter around a $\pm 45°$ cone (around the axis of the irradiating beam). The high rate monochromator scans the spectrum 2.5 times per secundum. In order to reduce the photometer noise the average of 20 scans was used.

1.3. Deliberately selected variation of some disturbing factors

To vary the sample thickness a sample cell was constructed with a cylindrical wall consisting of rings fitting into each other. By removing the top

rings one after the other (together with the sample portion they contained) the spectra were taken at different sample thicknesses.

By placing rings of different height under the sample cell the sample was shifted parallel to the direction of irradiation, and thus the effect of the positioning of the sample on the spectrum was determined.

The effect of comminution was studied by chopping the ground meat sample in a Moulinette chopper-blender to various degrees of fineness.

The effect of the temperature of the sample upon the spectrum was also studied. The measuring points of two thermo-couples were placed at 1 and 3 mm above the irradiated surface of the meat sample. The thin wires (0.15 mm Ø) of the thermo-couples were placed parallel to the surface of the sample, in expected isothermal planes to avoid the thermo-couples to disturb the temperature distribution within the sample. The control points were thermostated at 0 °C in thawing ice and the thermogram was recorded by a BD5 type Micrograph. The sample was pre-cooled to 1 °C in a refrigerator, then placed into the spectrophotometer. It was allowed to warm up slowly (0.8 °C per min) and the spectrum was recorded at various temperatures.

1.4. Calculations with the spectra

Some simple computer programmes were developed for carrying out operations with the spectra. They proved to be extremely helpful in the analysis and illustration of changes in the spectrum (as well as in characterizing and increasing their reliability). Between two spectra the four arithmetic operations may be carried out, while within one group of spectra the average spectrum, the standard deviation spectrum and the max–min range spectra may be determined.

2. Results and conclusions

2.1. The effect of sample thickness

Figure 2 shows the spectra of the same meat sample using different sample thicknesses. The spectra of meat samples of 4, 6 and 8 mm are practically identical, but, those of 2 and 1 mm thickness differ substantially. The value as well as the direction of the difference depends on the wavelength. More radiation reaches the sensors from the thinner samples between 1 325 and 1875 nm and less below 1325 and above 1875 nm. This may be explained as follows: if the thickness of the sample is small enough, some of the radiation passing through the sample reaches the farther (upper) surface of the sample; part of this radiation (with smaller incident angle) emerges from the material (and is thus lost for measurement), the other part is either normally or totally

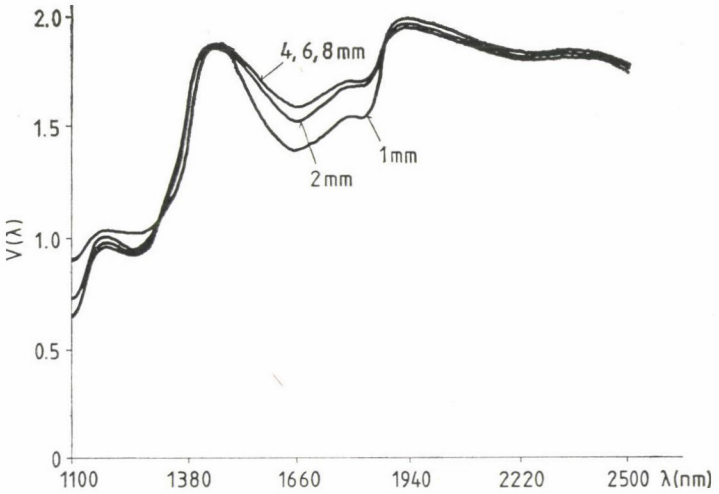

Fig. 2. Effect of sample thickness on the spectrum of meat

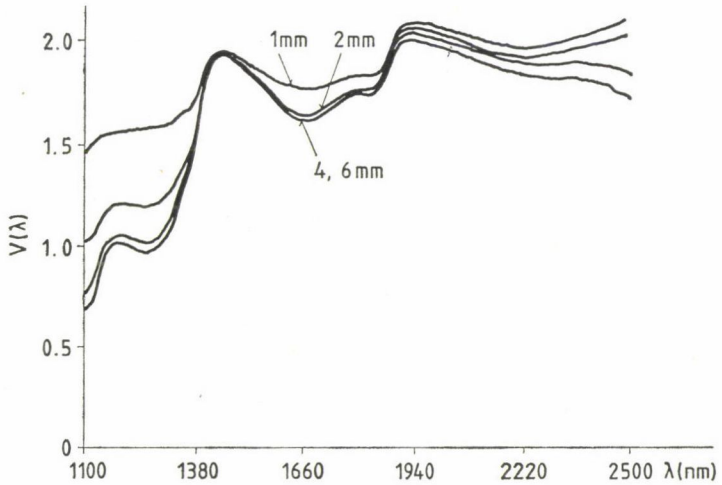

Fig. 3. Effect of sample thickness upon the meat spectrum when the upper surface was sprinkled with carbon black

reflected depending on the incident angle; some of it passes through the sample again and reaches the sensors. The ratio of the reflected and transmitted beam changes with the refractive index as a function of the wavelength. The change in sequence at different wavelengths in the spectra which can be seen in Fig. 2 was caused by these phenomena.

To prove this hypothesis the experiment was repeated with different thicknesses the farther (upper) surface of samples being sprinkled with carbon black. Thus, the increased absorption of the very thin blackened surface layer

practically eliminated the reflected portions. The spectra in Fig. 3 show that from 1–2 mm thick samples with blackened upper surface less radiation reaches the sensors than from unblackened ones.

Figure 4 shows the difference between the spectra from samples with thicknesses of 6 and 8 mm (see Fig. 2).

It can be seen that if the layer thickness is at least 8 mm, the change in $V(\lambda)$ in the wavelength range below 2 200 nm remains under 10^{-2} due to the effect of a 2 mm change in sample thickness.

In consequence of this experiment it was decided to use a sample cell with a thickness of 10 mm for the measurement of meat spectra. This thickness is reproducible within 0.5 mm and its effect on the spectrum seems to be below $3 \cdot 10^{-3}$.

2.2. Effect of the positioning of the sample

Figure 5 illustrates the spectra of a meat sample at different distances from the detector.

It was established that the shift in the spectrum was nearly parallel and proportional to the change in position. In practice, the maximum error of the position of the sample is about 0.2 mm (caused by the different thicknesses of the lower rim of the sample cell and by the error of its positioning).

The change in the spectrum caused by a 1 mm change in the position is shown in Fig. 6.

As can be seen from the curve, the error of positioning can be estimated to be $3 \cdot 10^{-3}$.

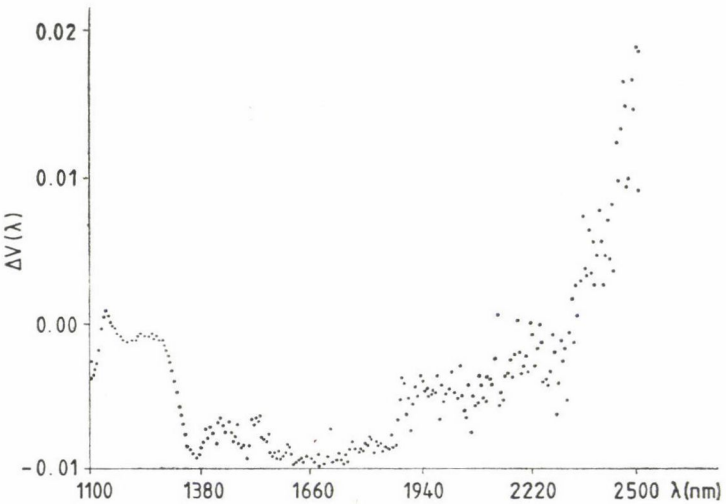

Fig. 4. Difference in the spectra of 6 and 8 mm thick meat samples

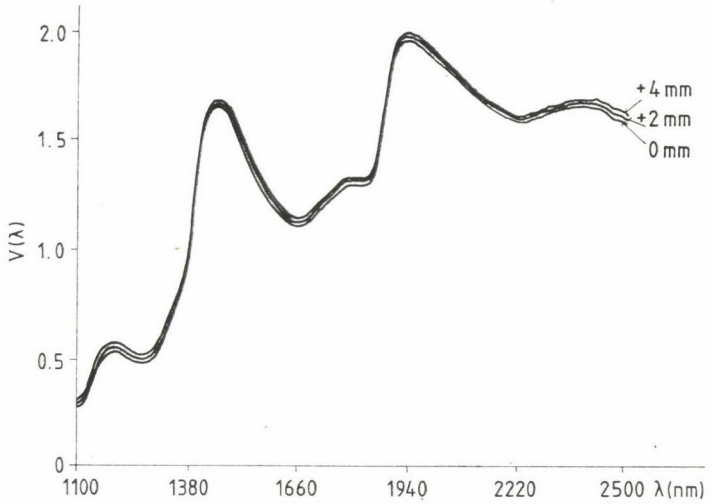

Fig. 5. Effect of the sample position on the spectrum

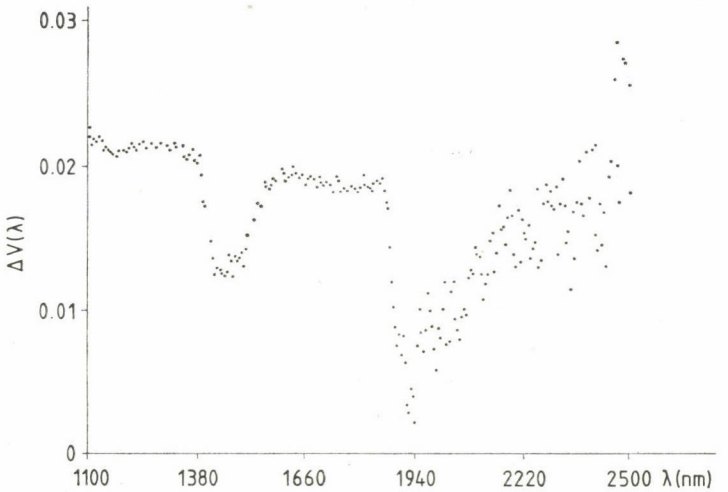

Fig. 6. Differences in the spectra caused by 1 mm shift in the sample position

2.3. Effect of sample temperature

The spectra of a meat sample taken at different temperatures are shown in Fig. 7.

The spectrum is shifted nearly parallel and proportionally with the temperature of the sample at a value of $5 \cdot 10^{-3}$ per °C. To eliminate this temperature dependence all the spectra were taken in practice at room temperature (waiting for the samples originally kept in a refrigerator to warm up). Thus, in practice the change in sample temperature may be estimated to cause an error of about 10^{-2} in the measurement of $V(\lambda)$.

2.4. Effect of chopping

The effect of the extent of chopping is shown in Fig. 8.

The substantial change in the spectra may be explained by the fact that the reflectometer value of meat is increased during chopping by the amount of air mixed in and by the fineness of particles. It is difficult to reduce the effect of these disturbing factors because the extent of chopping is difficult to measure. It is almost impossible to achieve identical size distribution, even by applying equal chopping time. The best method was to put the ground meat

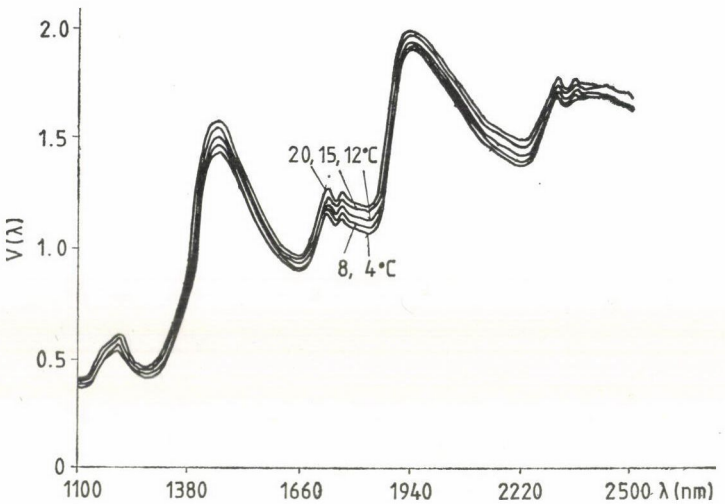

Fig. 7. Effect of sample temperature on the spectrum

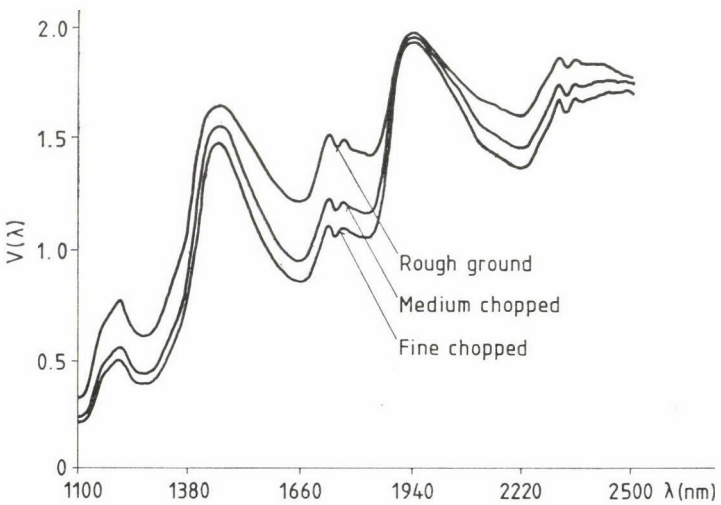

Fig. 8. Effect of the chopping extent on the spectrum

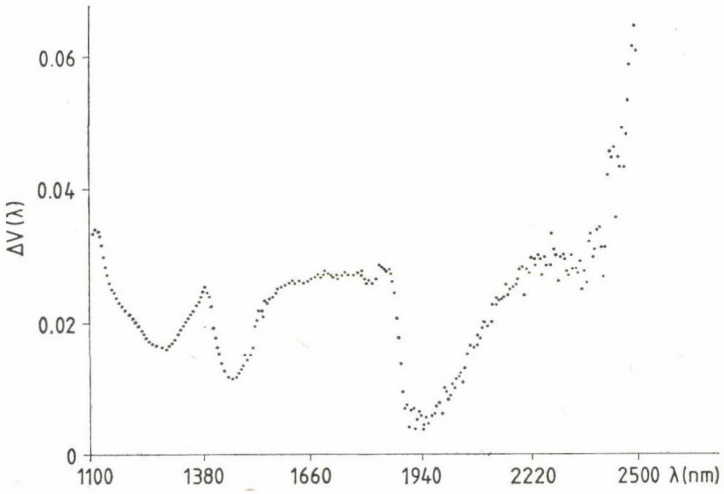

Fig. 9. Differences in the spectra caused by further chopping for 15 secundum

into the Moulinette apparatus and the chopping was continued until no further change was visible. Depending on the composition of the sample this took 20 to 40 secundum.

Figure 9 shows the change in the spectrum of a sample during a further 15 s chopping after it was estimated visibly to be stabilized.

Thus, the indefiniteness of chopping may cause an error in the $V(\lambda)$ spectrum of meat of the order of several times 10^{-2}.

The more thorough the chopping the better the homogeneity of the samples thus reducing the error of sampling. To illustrate this effect meat chopped to three very different degrees was placed in 10 sample cells each. The spectra were then taken. The calculated standard deviation spectra are shown in Fig. 10.

With the extent of chopping both the value of the standard deviation spectrum and its wavelength dependence decrease. The lower standard deviation found for fine chopped samples originates from different sources of errors and the higher standard deviation of samples chopped to a lesser degree can be explained by an increase in the sampling error due to higher inhomogeneity.

(Note: the effective amount of the sample from the viewpoint of measurement is not the total 15 g mass contained in the sample cell but only the amount actually giving the spectrum. The effective amount, estimated on the basis of an irradiated diameter of 20 mm and a penetration to a depth of about 4 mm, is increased by the effect of radiation being scattered over a more extended area and is reduced by the fact that the measurement gives less weight to the deeper layers. In the present case the effective amount of the sample can be estimated to be about 3 g.)

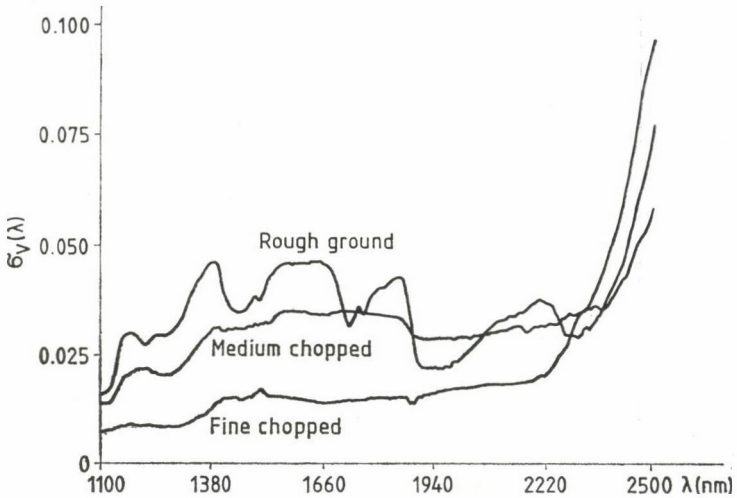

Fig. 10. Standard deviation spectrum of meat at various chopping periods (calculated from 10 spectra each)

2.5. Reproducibility and repeatability of spectrum measurement

Reproducibility is the criterion for the reliability of a spectrum measurement, as mentioned by CLARKE (1972) for example, in his discussion of the error in spectrophotometric measurements. The reproducibility of the spectrum may be characterized by the standard deviation spectrum incorporating all the disturbing factors which affect the spectrum (originating from the instrument, sampling and sample preparation). In order to determine this, the spectrum of a meat sample was measured thirty times using the methodology developed in the preliminary experiments. (The ground sample was homogenized in the Moulinette apparatus till the chopping appeared to be stabilized, then it was filled into sample cells 10 mm in depth and were measured at room temperature, etc.) The standard deviation spectrum characterizing the reproducibility, derived from 30 spectra, is shown in Fig. 11.

As can be seen from the figure, in the wavelength region below 2 200 nm the reproducibility of meat spectrum measurements is better than $4 \cdot 10^{-2}$. (It was assumed that in the spectrum range above 2 200 nm the increasing standard deviation is caused only by the low signal to noise ratio, since the signal of the detector at around 2 500 nm is much smaller than at around 1 100 nm. However, this assumption could not be proven.)

The repeatability characterizing the error originating only from the spectrophotometer instrument as observed in meat spectrum analysis was also determined. This, as the standard deviation spectrum calculated from repeated spectrum measurements on the same sample, is also shown in Fig. 11. This does

not contain the error resulting from the long-term instability of the instrument. In the greater part of the spectrum (up to 2300 nm) its value remained at less than $5 \cdot 10^{-3}$; thus, it is lower by nearly one order than the reproducibility. It is evident that the increase in standard deviation spectrum of the reproducibility in the range above 2300 nm cannot be explained purely by the low signal to noise ratio, because in this case the curve for repeatability would have a similar shape in this region. But it seems unlikely that a wavelength suitable for composition measurement will be found in this wavelength range.

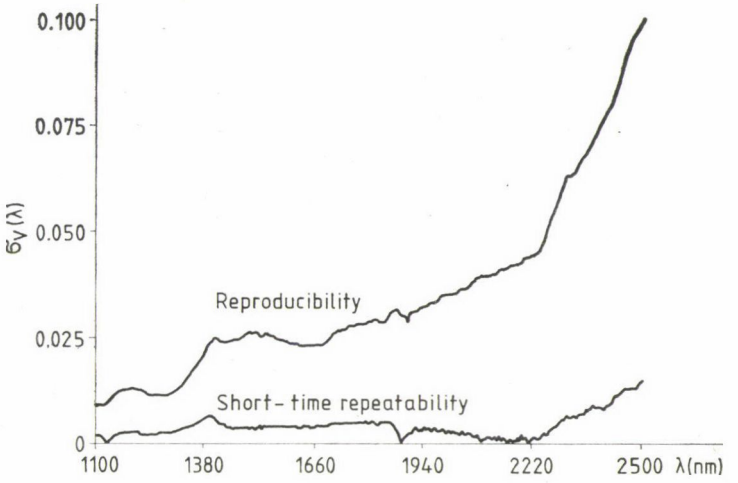

Fig. 11. Reproducibility and repeatability of meat spectra (characterized by standard deviation spectra)

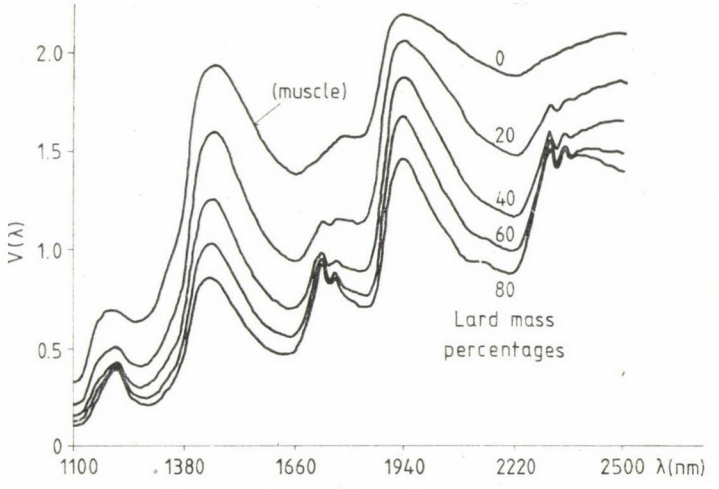

Fig. 12. Effect of meat composition on the spectrum (muscle tissue and lard mixtures)

An analysis of the disturbing factors affecting the meat spectrum shows that several of these cause a parallel shift in the spectra (e.g. positioning, sample temperature). By applying mathematical transformation invariable against shift to $V(\lambda)$ basic spectra these shift-causing errors may be compensated for. Such transformations are, for example:

$$V(\lambda) - V(\lambda_0) \text{ or } \frac{\partial V(\lambda)}{\partial \lambda} \text{ or } \frac{\partial^2 V(\lambda)}{\partial \lambda^2}, \text{ etc.}$$

2.6. Effect of the composition of the sample

The effect of the composition of meat samples on spectra (the measurement of which was the aim of the experiments) is shown in Fig. 12.

The two peaks characteristic of moisture are well visible at wavelengths 1 450 and 1 935 nm, as is the increase in amplitude parallel to an increase in fat content, for the double peaks characteristic of the fat content at wavelengths 1 725 and 1 765 or 2 305 and 2 435 nm.

*

The author is indebted to Ms. V. MIHÁLYI for providing the meat samples, to Dr J. GÖNCZY for developing the computer programmes and to Ms. K. ASCHENBRENNER for her conscientious assistance.

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SOME ASPECTS OF THE APPLICATION OF GLIADIN GEL ELECTROPHORETIC PATTERN AND PHENOL COLOUR REACTION IN THE IDENTIFICATION AND BREEDING OF WHEAT VARIETIES

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The gliadin pattern of wheat varieties generally cultivated in Hungary and of some other varieties was studied by horizontal starch gel electrophoresis. The phenol colour reaction was also carried out. These characteristics are of genetic significance.

It was established that a few of Hungarian wheat varieties are difficult to identify. Several varieties may be traced back to a common origin. It was observed that samples of the same gliadin pattern gave different phenol colour reactions. The same is true the other way round, too. The chromosomal allele coding of the gliadin pattern and that of the phenol colour reaction differs.

A basic problem of the identification of wheat varieties by biochemical methods may be the fact that plant breeders do not consider indicated the identification of the gliadin pattern and phenol colour reaction within variety.

Differences in gliadin patterns necessitate the thorough investigation of varieties. Samples of authentic varieties have to be tested grain by grain for gliadin pattern.

Biochemical methods are used in more and more countries to distinguish between wheat varieties. Of these methods the phenol colour reaction and the gel electrophoresis of gliadin proved valuable (FEHÉR, 1978; BOROS, 1980; NEHÉZ & NAGY, 1979).

Gliadins form the part of gluten dissolving in 70% ethanol. The gliadin pattern, that is the position of the bands obtained by electrophoresis, their intensity and number may be characteristic of the wheat variety.

The pattern of gliadin bands is genetically determined and independent of conditions of cultivation (LEE & RONALDS, 1967).

Several authors tried to relate gliadin bands, groups of bands (gliadin blocks) to parameters of baking and paste quality (BEBYAKIN & BALABOLINA, 1977; POPERELYA & SOZINOV, 1977; DAMIDAUX et al., 1978).

Both baking and paste quality are only slightly dependent on gliadin proteins and are only partially determined genetically. SZABÓ (1973) maintains that a good variety provides only a possibility for the good quality to develop.

The chromosomal allele relationships of gliadin bands become more and more known. Ordinary, that is aestivum varieties have genome A, B and D. chromosome group. Genome D is lacking in durum wheat varieties. The gliadin blocks are mainly coded by the chromosomes of group 1 and 6 (classic gliadins). In coding participate also chromosomes of group 2 (SHE-

PHERD, 1968; POPERELYA & SOZINOV, 1977). More and more chromosomal correlations are discovered by highly developed electrophoretic technique. The gliadins of low molecular mass (LMW) are coded by chromosomes 7 A, 4 B and 7 D (SALCEDO et al., 1979), other gliadins by chromosome 4 A (SASEK & KOSNER, 1977).

Glutenin electrophoresis also gives information on the genetic background of wheats (BIETZ et al., 1975).

The gliadin pattern of durum wheats is easily distinguished from that of the aestivum varieties because it does not contain the least motile bands (because of the lack of genome D). Durum wheat may be improved by the additioning of chromosome 1 D (WRIGLEY & SHEPHERD, 1970).

The viscoelasticity and cooking quality of pastes made of durum wheat semolina is affected by the quality of their gluten content and protein content. Durum wheats fall in two groups. Good varieties are characterized by the sharp gliadin band marked 45, while the weaker varieties by the sharp band marked 42 (DAMIDAUX et al., 1978).

Some authors consider a dark phenol colour reaction characteristic of not bad baking quality (TUZSON, 1933; FEHÉR, 1978). The phenol reaction is the discoloration of certain tissues of the pericarp of wheat grains by phenol vapour. The pericarp consists of maternal tissues. The discoloration in the phenol test is considered to be independent of conditions of cultivation. The phenol test was developed by PIEPER (1922).

At the Cereal Research Institute, Szeged, Hungary, these methods of analysis were used in breeding of durum wheat for paste production.

The cultivability of durum wheats in Hungary was first declared by SZÁNIEL (1976). Experiences gained in durum wheat cultivation in Hungary were reviewed by ERDEI (1976), BEKE (1980), BEKE and BARABÁS (1981), BEKE and SZEBELLÉDY (1981). The quality modifying effect of the location of growth and the use of fertilizers were discussed by SZÁNIEL and co-workers (1981), ERDEI and GYENES (1981). SZAUTER (1980) has shown that cultivation of durum varieties is necessary from the point of view of the paste industry, since the amount of aestivum varieties grown for this purpose is insufficient.

Partial results of this study were published in several papers (NEHÉZ, 1978; 1979; 1980a; b; 1981, and NEHÉZ et al., 1981).

1. Materials and methods

Certain relations of the gliadin pattern of wheat varieties in general cultivation and of some other varieties to the specific aspects of wheat breeding, are evolved.

Gel electrophoresis of gliadins was carried out earlier by the method of SOZINOV and POPERELYA (1978). Recently the method of AUTRAN and BOUR-

DET (1975) has been applied. Electrophoresis is carried out in a horizontal layer of potato starch, partially hydrolyzed, in aluminium lactate buffer (pH = **3.1**). Dyeing is carried out in a trichloroacetic acid containing aqueous nigrosine solution. Earlier the starch was hydrolyzed in this laboratory, at present a Connaught product (Canada) is used.

The phenol colour reaction is carried out according to the method of FEHÉR (1978).

2. Results

Figure 1 shows the gliadin bands obtained by the technique applied and related nomenclature. Some gliadin blocks representing good quality flour as described by POPERELYA and SOZINOV (1977) and transcribed according to the methodics used in this study, are illustrated. The figure after the symbol of the chromosome is the arbitrary mark for the allele block type as given by the above authors. It should be noted, however, that the transcription of gliadin blocks determined by different methods is not free from problems, it is

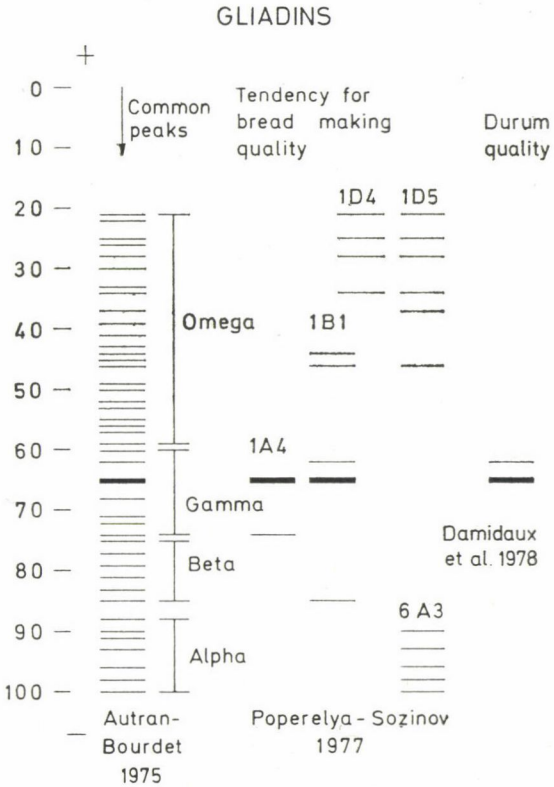

Fig. 1

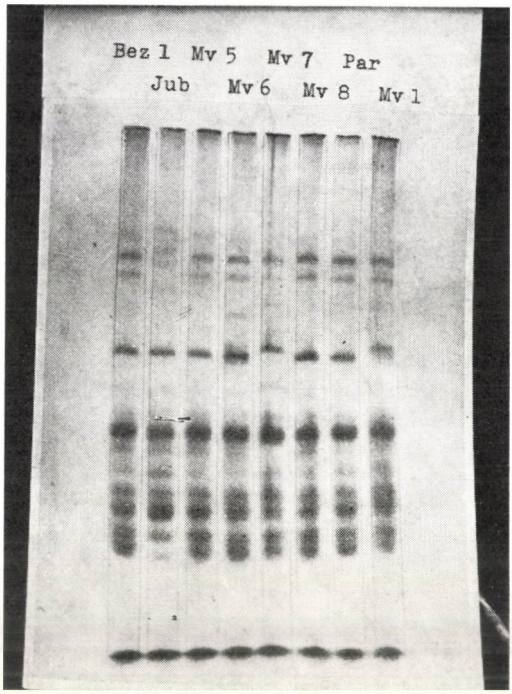

Fig. 2. Gliadin pattern of wheats

		Colour of phenol reaction
1. Bez	Bezoshtaya 1	Dark brown
2. Jub	Yubileinaya 50	Light brown-brown-dark brown
3. Mv 5	Martonvásári 5	Dark brown
4. Mv 6	Martonvásári 6	Dark brown
5. Mv 7	Martonvásári 7	Dark brown
6. Mv 8	Martonvásári 8	Dark brown
7. Par	Partizanka	Dark brown
8. Mv 1	Martonvásári 1	Brown-dark brown

even occasionally impossible. The band considered by DAMIDAUX and co-workers (1978) characteristic of good durum quality flour and another of poorer quality are shown, too (65 and 62, resp.).

In Figs. 2, 3, 4 and 5 the gliadin patterns of several wheat varieties and some samples of improved varieties, are represented. The results of phenol colour reactions are also shown. Five degrees of discoloration were taken into account.

Several varieties are of a common origin. In Table 1 the variety formulae (crossings) as declared by the breeders, are listed. The first mark in the formulae stands for the mother (in multiple crossing in parenthesis, too), those farther back for the father. In the ancestral lines Bezoshtaya 1 is frequently seen.

On the basis of the photos several varieties are easy to distinguish, while others are not.

One problem pertinent to the identification of varieties is that the improved varieties were not selected either by gliadin pattern or phenol reaction. For instance the phenol reactions of varieties Mv 1 and Yubileynaya 50 are mixed. Grains of Mv 1 discolored differently in the phenol test and had different gliadin patterns, while those of variety Yubileinaya 50 seemed identical. In the former the ratio of brown and dark brown grains was 42:8, while with the latter the ratio of light brown, brown and dark brown grains was 16:9:25 as an average of the samples tested.

The possibility of distinguishing by the phenol reaction is much lower than by gliadin pattern. Probably the colour reaction is coded by different chromosomes, alleles than the gliadins. In aestivum varieties the colour reaction is considered a two-step reaction. According to our present knowledge in the formation of colour 2 A (lighter colour), 2 D (ZEVEN, 1972), 5 B (modifying), 6 B (BHAT & GOUD, 1978) and 7 A (intermediary colour) and 5 D (BHO-

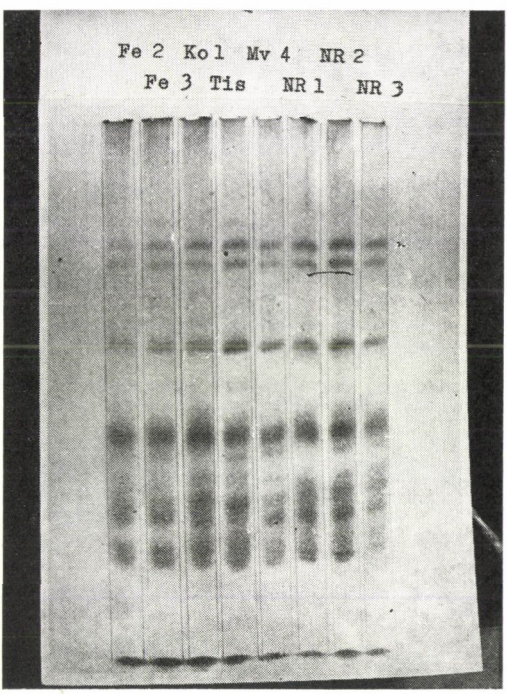

Fig. 3. Gliadin pattern of some wheat varieties

		Colour of phenol reaction
1. Fe 2	GK Fertődi 2	Dark brown
2. Fe 3	GK Fertődi 3	Dark brown
3. Ko 1	Kompolti 1	Dark brown
4. Tis	GK Tiszatáj	Dark brown
5. Mv 4	Martonvásári 4	Dark brown
6. NR 1	Novosadska Rana 1	Dark brown
7. NR 2	Novosadska Rana 2	Dark brown
8. NR 3	Novosadska Rana 3	Dark brown

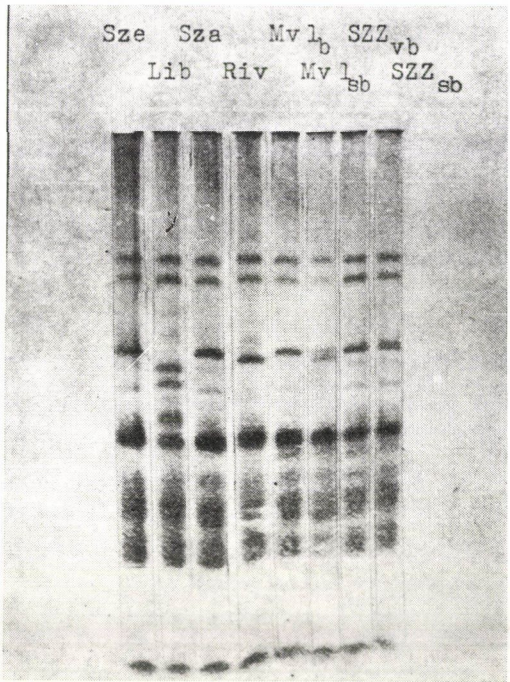

Fig. 4. Gliadin pattern of some wheat varieties

		Phenol colour reaction
1. Sze	GK Szeged	Light brown
2. Lib	Libellula	Light brown
3. Sza	Sava	Light brown
4. Riv	Rivoli	Dark brown
5. Mv 1_b	Martonvásári 1	Brown
6. Mv 1_{sb}	Martonvásári 1	Dark brown
7. SZ$_{vb}$	Super Zlatna	Light brown
8. SZ$_{sb}$	Super Zlatna	Dark brown

WAL & NARKHEDE, 1979) chromosomal alleles seem to participate. Darker colours descend dominantly.

Wheat varieties of identical gliadin pattern may give different phenol reactions, while this is valid the other way round, too.

Differently discolored grains of varieties which give mixed colour reaction were propagated and have been the subject of study for years. On several occasions the morphological pattern of the plants differed, too.

The durum varieties are easy to distinguish from the aestivum varieties because of their lack of the least motile bands (genome D). The presence or absence of band 65, characteristic of good quality, is also easy to judge.

In the cultivation of durum wheats in Hungary it is a basic requirement that they should be as frost-hardy as the common winter wheat varieties. The variety Novomichurinka proved to be sufficiently hardy. Its grains giving

a dark brown phenol reaction had an aestivum gliadin pattern. When they were sown the outgrowing plant was morphologically Yubileinaya variety.

Most of the durum varieties investigated by the author, gave a faint phenol reaction or non at all. Durum × aestivum hybrids have been tested for years.

The variety formulae given by breeders do not serve with exact information on the gliadin pattern. The formulae show the action of crossing and not its result. They select for other criteria.

Neither in improvement nor in cultivation practice is the uniform gliadin pattern or phenol reaction a criterion. The line of inheritence of gliadin blocks is usually co-dominant, the blocks descend independently from one another or split after crossing into different block combinations according to Mendel's rules. The varieties are obtained by selection throughout several

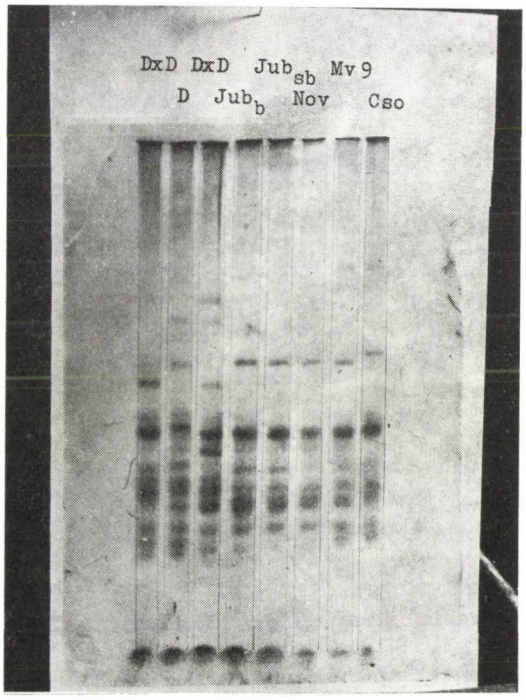

Fig. 5. Gliadin pattern of some wheat varieties

		Phenol colour reaction
1. D × D	Leukomelyan × Minaret	Pale
2. D	Solaris 650	(Pale)-dark brown
3. D × D	Leukomelyan × AAS...–DF	Pale
4. Jub$_{b}$	Yubileinaya 50	Brown
5. Jub$_{sb}$	Yubileinaya 50	Dark brown
6. Nov$_{sb}$	Novomichurinka	Dark brown
7. Mv 9	Martonvásári 9	Dark brown
8. Cso	GK Csongor	Light brown
D	durum	

Table 1

Lineage of wheat varieties according to breeders

Wheat varieties certificated for 1980

Bez	*Bezoshtaya 1* Lutescens 17 × Bezoshtaya 2
Jub	*Yubileinaya 50* Mironovskaya 808 × Bezoshtaya 1
Mv 5	*Martonvásári 5* [(Mironovskaya 808 – Bezoshtaya 1) × Bezoshtaya 1] × [(Bezoshtaya 1 – Produttore) × Bezoshtaya 1]
Mv 6	*Martonvásári 6* Bezoshtaya 1 × Moisson
Mv 7	*Martonvásári 7* Bezoshtaya 1 × Opal
Mv 8	*Martonvásári 8* Gamma radiation induced mutant of Bezoshtaya 1 × Ranka III
Par	*Partizanka* Bezoshtaya 1 × (Heines VII – Capmadore)
Mv 1	*Martonvásári 1* Bezoshtaya 1 × Mv 65–07
Fe 2	*GK Fertődi 2* (Population Fe Co 33 – Produttore) × Bezoshtaya 1
Fe 3	*GK Fertődi 3* (Population Fe Co 33 – Produttore) × Bezoshtaya 1
Ko 1	*Kompolti 1* Bezoshtaya 1 × Fertődi 293
Tis	*GK Tiszatáj* Bezoshtaya 1 × Fiorello
Mv 4	*Martonvásári 4* (Mironovskaya 808 – Bezoshtaya 1) × Bezoshtaya 1
NR 1	*Novosadska Rana 1* [(Bezoshtaya 1 – NS 262) × Mironovskaya 808] × Bezoshtaya 1
NR 2	*Novosadska Rana 2* [(Bezoshtaya 1 – NS 262) × Mironovskaya 808] × Bezoshtaya 1
NR 3	*Novosadska Rana 3* [(Bezoshtaya 1 – NS 262) × Mironovskaya 808] × Bezoshtaya 1
Sze	*GK Szeged* (Strampelli – Marco Michaelis) × Bezoshtaya 1
Lib	*Libellula* (Tovere – Guileri) + San Pastore
Sza	*Sava* Fortunato × Redcoat
Riv	*Rivoli* Champlaine × Reso

Newly certificated varieties

Mv 9	*Martonvásári 9* [(Mironovskaya 808 – Bezoshtaya 1) × Bezoshtaya 1] × [(Bezoshtaya 1 – Produttore) × Bezoshtaya 1
Cso	*GK Csongor* GT. 76.150 × Predgornaya 2

generations (mother stock selection). This selection is carried out by the eye or instrumentally. The individual grains belonging to the same variety are not necessarily of uniform gliadin pattern. The gliadin pattern may change in the course of variety maintenance.

Varieties may consist of several gliadin biotypes.

With varieties of varied gliadin biotypes and gliadin blocks it may be difficult to discover the type of the blocks. This applies also to varieties mixed mechanically.

The other side of the problem is that breeders try to produce varieties of high economic value. Their aim is to achieve high yields per hectare with possibly good baking or feeding quality. The coding of the gliadin pattern or that of the phenol test affects only a sphere of chromosomes and alleles. The varieties identical in these spheres may differ in several of their economically important properties, thus, for instance in their yields.

In identifying varieties the gliadin pattern or the phenol reaction may be useful, however, uncertain. Several methods together should be used to identify varieties. Further the morphology of grains, their colouring substances, hardness and other characteristics, can also be utilized.

The gel electrophoretic pattern and the phenol colour reaction may be useful in wheat improvement, particularly when used with other methods. Relations of the gliadin blocks to flour quality (good sedimentation 1 A 4, very poor 1 A 1, bread volume good, too 1 B 1; only some relation to their good quality 1 D 4, 1 D 5 and 6 A 3) are only tendencies and are advantageous in selection from a large sample population. Studies into heredity should be complemented by glutenin and enzyme gel electrophoresis and cytogenetical methods.

In addition to the refinement of electrophoretic methods it is necessary to investigate the gliadin pattern of authentic varieties grain by grain. This is the way in which the kinship between the gliadin patterns of wheat varieties can be judged.

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ALKYLRESORCINOLS IN DURUM WHEAT
II. ALKYLRESORCINOL CONTENT OF MILLING FRACTIONS AND MACARONI

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Seed samples of three durum wheat varieties were milled in a commercial mill. The bran, semolina and flour fractions, as well as the macaroni produced from the semolina, were examined for 5-alkylresorcinol (5–AR) content. Bran contained the highest amount of 5–AR (2 200 to 2 600 ppm on a dry weight basis), followed by flour (200 to 250 ppm), semolina (70 to 90 ppm), and macaroni ($\leq$ 50 ppm), respectively. The 5–AR content of the bran varied most, probably due to the joint effect of variety and milling. The very low 5–AR content of macaroni may be attributed to processing and prolonged wet drying. There was a negative correlation between pasta brownness and kernel 5–AR content, suggesting that aklylresorcinols may inhibit the browning reactions mediated by peroxidase and polyphenoloxidase. The yellow index and 5–AR content were not correlated with each other.

Although the 5-alkylresorcinol (5-AR) content of various cereal grains has been extensively studied (see Part I of this series), relevant data on milling fractions and cereal products are very sparse. VERDEAL and LORENZ (1977) found that the bran fractions of wheat, rye and triticale contained the highest amounts and the flour fractions the lowest amounts of alkylresorcinols, whereas the absolute losses during bread baking varied between 22 and 24%. As shown by WEIPERT and EL BAYA (1977), flours of both bread wheat and rye are relatively poor in 5-AR, and even wholemeal ryebreads do not contain more than 1 000 ppm 5-AR (initial content in the seeds: 1 300 to 1 800 ppm). However, corresponding data for the 5-AR levels in durum wheat milling fractions and pasta products are not available. In a previous paper (SÁGI & SÁGI, 1982) the 5-AR content of durum wheat varieties was reported in relation to various kernel characteristics. The purpose of this study was to determine the amounts of 5-AR in various milling fractions, as well as in macaroni produced from the semolina of three durum wheats. Further, the relationship between pasta colour and kernel 5-AR content was also examined.

1. Materials and methods

Seed samples from the 1978 harvest of GK 61–130, GK Basa and Raineri durum wheat varieties were tempered and milled in a commercial bread wheat mill modified for durum wheat milling, and equipped with a semolina classifier

and purifier line. In this study, the bran, flour and semolina fractions were used. Macaroni was processed and dried in the Pasta Factory of the Békéscsaba Canning Plant with the usual large-scale procedure. The 5-AR content of the samples was determined as described by WEIPERT and EL BAYA (1977) (see also SÁGI & SÁGI, 1982) except that the reagent was prepared with acetone instead of water for determining 5-AR in milling fractions and ground macaroni. For the pasta colour measurements semolina was produced from seed samples of twelve durum wheat varieties using a QC-109 type Labor MIM laboratory mill equipped with a 700 μm rotating drum screen to separate the bran. The flour was sifted out with a 150 μm screen and the semolina was passed through a laboratory purifier three times.

Colour measurements were performed on pressed pasta discs of uniform thickness and water content by means of a Momcolor (Hungary) tristimulus colorimeter. Yellow and brown indexes were calculated according to the ISO standard (O. I. N., ISO/TC 34, 1974). The moisture content of the samples was determined by drying to constant weight at 100 °C, and ash percentage by incineration in a muffle furnace.

All measurements were repeated at least five times. The results are presented as average values with the related standard errors on the graphs. Correlation coefficients between 5-AR contents and colour indexes were calculated with a Canon programmable desk calculator, and the significance of the correlations was determined using the r-test.

2. Results

Alkylresorcinol data (Figure 1) showed that, independently of the variety, durum bran fractions contained by far the greatest amount of 5-AR. Flours had a 5-AR content an order of magnitude lower, while semolina contained only small amounts, and macaroni only traces ($\leq$ 5 mg per 100 g dry weight) of alkylresorcinols. Varietal effects on the 5-AR level in durum wheat milling fractions were not significant, but the 5-AR in the bran fluctuated more than in the other fractions.

Semolina yields – although relatively low – were fairly constant (Table 1). The bran percentage of GK 61–130 was somewhat lower than that of the other two durum wheats, which had similar bran yields, whereas in the case of flour extraction, the opposite was observed. The ash content of the flours was variety-dependent, but the flours always contained more ash than the semolina fractions.

Considering the original 5-AR content of the semolina (7.3–8.7 mg per 100 g dry weight) and that of the macaroni ($\leq$ 5 mg per 100 g dry weight), end product losses of alkylresorcinols may be as high as 32–43 %.

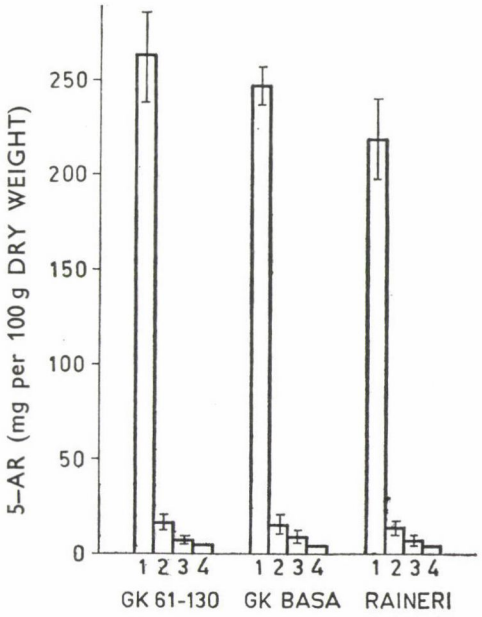

Fig. 1. Alkylresorcinol content of durum wheat milling fractions and macaroni produced from semolina. Determination in acetone extracts by spectrophotometry at 435 nm according to WEIPERT and EL BAYA (1977). 1: bran, 2: flour, 3: semolina, 4: macaroni

Table 1

Milling fraction percentages and ash content in semolina and flour of durum wheats

Variety	Milling fraction (%)[a]			Ash % dry weight	
	Bran	Semolina	Flour	Semolina	Flour
GK 61–130	46.2	45.1	8.7	0.82	1.22
GK Basa	49.3	45.3	5.4	0.93	1.31
Raineri	49.2	45.2	5.6	0.81	0.99

[a] On the basis of total product

There was a non-significant, positive correlation between the yellow index (YI) of the pasta and the kernel 5-AR content. Brown index (BI) and 5-AR content correlated non significantly and negatively (Figure 2).

3. Conclusions

Among the milling fractions of bread wheat, triticale and rye, the bran invariably contains the highest amounts of alkylresorcinols (VERDEAL & LORENZ, 1977; WEIPERT & EL BAYA, 1977). This is to be expected, since these

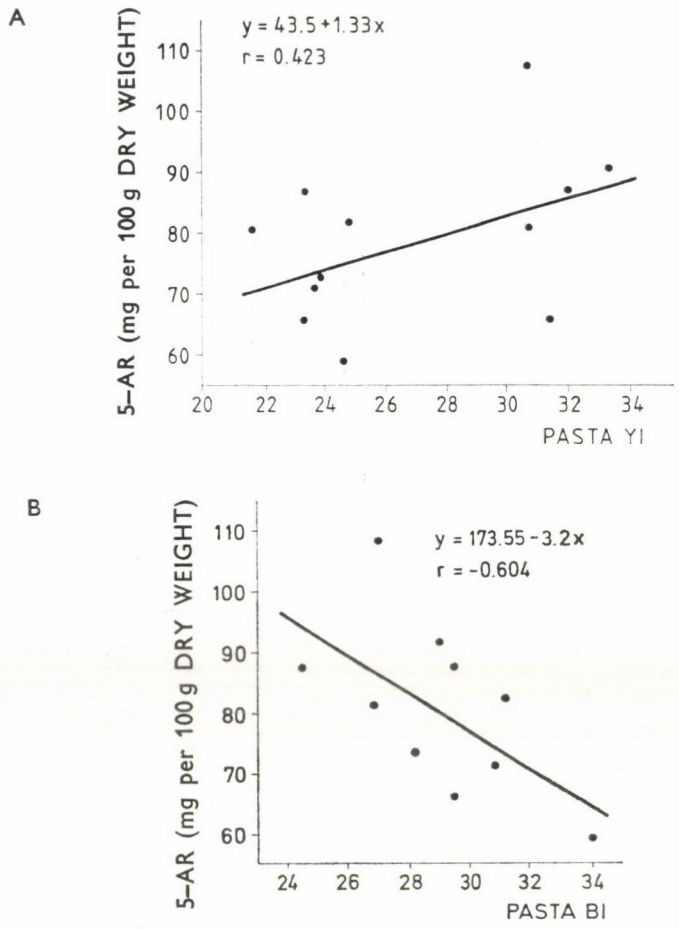

Fig. 2. Correlations between the yellow (YI, A) and brown (BI, B) indexes of the pasta and the 5–AR content in the kernels of several durum wheats. Pasta colour indexes were measured with a Momcolor tristimulus colorimeter and calculated from the colour coordinates. 5–AR contents were determined by the diazotized p-nitraniline method of WEIPERT and EL BAYA (1977)

phenolic compounds are concentrated in kernel pericarps (WENCKERT et al., 1964; WIERINGA, 1967), the main potential components of bran. Starting from the bran, there is a decreasing 5-AR gradient in the shorts and flour (VERDEAL & LORENZ, 1977). The present results indicate a very similar distribution of 5-AR in durum wheat milling fractions, as alkylresorcinols predominate in the bran and occur in very small quantities in both flour and semolina (Figure 1).

The low semolina yield (45%) and higher ash percentage of the flours (Table 1) suggest that the durum flour fractions contained some bran residues. This could result in an elevated 5-AR level of the flours as compared to that of the semolina, which is much less contaminated by bran particles (Figure 1).

The quantity of 5-AR varied to a greater extent in durum wheat bran fractions than in the other milling fractions. This can be explained by the genotypically controlled 5-AR content of the kernels, however, at least in the case of GK 61–130, the higher bran 5-AR content may also have been due to a reduced dilution by endosperm (flour), because of its comparatively lower bran and higher flour yields (Table 1). Unfortunately, at the time of this study no authentic seed samples were yet available for investigation.

The differences in 5-AR level in the bran of durum wheats cannot be attributed to a modifying effect of storage (STUCZYNSKI et al., 1974), since all the milling fractions were stored for the same period under identical conditions.

In wholemeal breads, absolute 5-AR losses during baking amounted to 22% for rye, 24% for triticale and 23.5% for wheat. For wholemeal ryebread the residual 5-AR content was about 0.1%, and for triticale and wheat breads the corresponding figures were 0.05% and 0.03%, respectively (VERDEAL & LORENZ, 1977). It is speculated that 5-AR losses during breadmaking may be brought about by fermentation rather than thermal degradation (VERDEAL & LORENZ, 1977), but heat effects and oxidation during the baking process may also decompose 5-AR (WEIPERT & EL BAYA, 1977). In the present experiments, macaroni made from semolina retained hardly any 5-AR in excess of the lower determination limit of the method used, and based upon the 5-AR content of the semolina, alkylresorcinol losses approached 40%. This is a greater loss than that observed during breadmaking by the authors mentioned above. Obviously, macaroni processing, especially the prolonged drying, may contribute to the high losses of alkylresorcinols. Nevertheless, some structural or other factors in the bran may partly protect its 5-AR complement during bread baking (wholemeal breads), while these protective factors may be absent from the semolina. Home cooking probably reduces the 5-AR level of macaroni still further. Thus, cooked macaroni can be regarded as practically free of alkylresorcinols.

The negative correlation found between pasta brownness and kernel 5-AR content in ten durum wheats suggests that alkylresorcinols may play a role in the biochemical processes leading to the browning of pasta products. Since macaroni or spaghetti browning is thought to be evoked by the concerted action of peroxidase and polyphenoloxidase (KOBREHEL et al., 1972; KOBREHEL et al., 1974), it can be postulated that alkylresorcinols inhibit the activity of both enzymes. On the other hand, the weak correlation between yellowness and the 5-AR level reflects the fact that alkylresorcinols do not modify the lipoxygenase activity.

*

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BOOK REVIEW

Studies of food microstructure

Scanning Electron Microscopy, Inc. (P. O. Box 66507.
AMF. O'Hare, I11., 60666) 1981; 342 pages

For the more exact understanding of structural properties, stability and consistency of the products in the food industry and to determine the method and the time of the effective control, the scanning electron microscopy, this new method of great promise has increasingly gained ground. The relevant results of experiments in this field are summarized in this book.

The book is divided into 4 parts. In part 1, the preliminary operations necessary to the examination by electron microscopy of foods and the general information on the technique are described. In part 2, results of the microstructure of meat and those of investigations into the connections of fine structure with the stability are to be found. In part 3, the results of experiments on microstructure of gels and dairy products are discussed. In part 4, informations on the microstructure of foods of plant origin, such as soy applied products, wheat and flour produced therefrom, are given.

Results gained in the above field can be studied in the book consisting of 342 pages. Several pictures made by electron microscopy are included, which show the different structures. Considering that the material of the book is based on the subject-matters from 1979 to 1981 of journal Scanning Electron Microscopy and follows the editing principles of journals, several references and abstracts are given in addition to the scientific achievements.

E. Kovács

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PRODUCTION OF PROTEIN ISOLATES FROM EXTRACTED SUNFLOWER GRITS

M. DEMECZKY, ZS. SZIGETI and K. VÁSÁRHELYI-PERÉDI

(Received: 13 June 1981; revision received: 21 October 1981; accepted: 25 March 1982)

The aim of our work was to produce protein isolates free of fibre from sunflower seed grits, a by-product of sunflower oil manufacture, while the residual grits remain suitable for use as fodder. To achieve this six protein isolation technologies were developed. The products of these technologies are of different properties and can be utilized in the food industry. The basic technology (T 1) involves alkaline extraction of proteins, their precipitation by acid, centrifuging and drying of the precipitate. The total protein content of the product is 84%. If the protein precipitate after decantation is exposed to enzymic hydrolysis (T 2) prior to centrifuging, the water-soluble part of the isolate increases to 80%.

The direct enzymic extraction of sunflower seed grits was attempted, too (T 3). The extract was precipitated by acid and dried. The total protein content of the product was 66%, while the water-soluble protein amounted to 37%.

The protein content as obtained by the basic technology (T 1) could be increased to 88% by washing the protein precipitate with ethyl alcohol (T 4). The latter treatment caused a substantial improvement of colour and of the microbiological condition.

A substantial improvement in colour may be achieved by using a 5% solution of $MgCl_2$ for extraction (T 5). The product of this technology is of 75% protein content, white in colour.

If the solution of 2.4% protein content, obtained by alkaline extraction, is neutralized and concentrated four-fold by a membrane separation technique (T 6) and spray-dried, the product contains 67% protein and is easily soluble in water.

The material and protein balance of the various technologies was exactly determined and the products were evaluated. Thus, it was established that by all six technologies about 30–40% of the protein content of the sunflower grits could be isolated. Losses were lowest in the membrane separation technique. The residual fibrous by-product contained about 28–34% protein.

By alkaline dissolution of the protein content of the extracted grits, and by spray-drying the neutralized product, a protein isolate of good quality, free of fibres and suitable for animal feed, was obtained.

Production of high quality feed and of protein isolate for human consumption by the basic technology was attempted on pilot plant scale, too. The results of this work are also given below.

In recent years world population has increased enormously. This increase makes it more and more difficult to provide food for the population. In view of solving this problem the exploration of new protein sources is imperative.

The production of protein of animal origin is aggravated by the circumstance of its costs being 4–10 times higher than that of plant protein.

The grits gained from extracted seeds are poor in oil. The extracted grits are important as feeding stuffs (MARINOV & ANGELOVA, 1973). However, since their protein content is high it is worth while to study the possibility of producing from them protein isolates suitable for human consumption or high grade, fibre-free animal feed.

In various countries of the world the problem of converting oil seeds directly into protein for human consumption, is resolved. Since the first soya protein concentration plant has been put into operation (1949) a great mass of information has been collected on the technology, (DEMECZKY & SZIGETI, 1974; DEMECZKY & SZIGETI, 1975) the conditions of industrial processing and on the consumer acceptance of the product (BALLA, 1977; BESANCON, 1977; BOUQUIN, 1977; FLEMING & SOSULKI, 1977; FRENCH, 1977; JOHNSON & SNYDER, 1978; RIEDEL, 1977; SAVI, 1978; STARON, 1977; WOLF, 1977). Future trends in the utilization of the protein content of oil seeds may be determined on the basis of the works of the authors mentioned above.

The success of the utilization of soya protein in various food items stimulated the interest in the utilization of other oil seed proteins for human consumption. The sources studied among others were: sesame, coconut meal, peanut (BALASUBRAMANIAM & SIHOTANG, 1979; CHEN et al., 1979; DENDY & GRIMWOOD, 1973). Cotton seed became recently one of the raw materials studied (HENSLEY & LAWHON, 1977; THOMPSON, 1978). Gossipol was removed from cotton seeds by extraction or hydrolysis. Lately rape seed meal has aroused interest (AMAN & GILLBERG, 1977; GILL & TUNG, 1978; GIRAULT, 1973; KODAGODA et al., 1973; PASZKOWSKI, 1977) as well as the utilization of sunflower seed protein (BAU & DERBY, 1980; BAUDET & MOSSE, 1977; RHAMA & RAO, 1979; SHCHERBAKOV, 1977; SOSULKI & FLEMING, 1977; SHTOISHAVLIEVICH et al., 1977; VIRICH et al., 1977). These proteins, due to the high percentage of their sulfur containing amino acids, are of high interest for the food industry. Sunflower seed is free of every kind of substance unwholesome to human health.

1. Materials and methods

The raw material used in the experiments was the benzene-extracted sunflower seed meal. First class meal ($42.3 \pm 0.5\%$ protein content) was used in the laboratory experiments. In industrial experiments a mixture of grade I and II extracted sunflower seed (protein content $39.2 \pm 0.6\%$) meal was used.

The main point of protein isolation is the extraction of protein from the sunflower seed meal and the precipitation of the fibre-free protein from the solution.

1.1. Methods used for protein isolation in the laboratory

In each of the six technologies 1200 g of extracted sunflower seed meal was used. To set the pH, 5 *N* NaOH solution or 4 *N* HCl solution was used. To hinder oxidative polymerization of the polyphenols present in sunflower seed, in some of the technologies a 0.25% Na_2SO_3 solution was used as antioxidant. The protein extract was separated from the fibrous by-product by centrifuging and the latter dried at 105 °C. Each technological process was repeated seven times.

Basic technology (T 1): The sunflower seed meal was extracted at room temperature for 1 h with a tenfold amount of a solution containing antioxidant and set at 10.5 pH. The slurry was centrifuged and filtered. The protein extract thus obtained was precipitated at its isoelectric point at pH 3.8. After sedimentation the sediment was separated from the acid effluent by decantation and centrifuging and finally dried by lyophilization.

Basic technology with enzymic hydrolysis (T 2): This technology is identical with the basic technology up to the point of sedimentation of the protein precipitate. Hereafter the sediment was separated from the liquid by decanting and the pH was set at 7.5. Pronaz, a proteolytic enzyme (CHINOIN, Budapest) was added to obtain 8–9 degrees Anson. Hydrolysis was carried out at 40 °C for 1 h. The enzyme was inactivated at 90 °C and the product was dried by lyophilization.

Direct enzymic extraction of the meal (T 3): The meal was extracted in tenfold amount of water containing antioxidant and Pronaz for 1 h at 40 °C and pH 7.5. Then the suspension was heated to 90 °C to inactivate the enzyme. After centrifuging and filtering the protein was precipitated at pH 3.1 and sedimented. The sediment was then decanted and dried by lyophilization.

Basic technology combined with ethyl alcohol treatment (T 4): In this technology the centrifuged protein precipitate was washed in 300 cm^3 96% ethyl alcohol at room temperature for 20 min under blending. The centrifuged alcohol was regenerated while the protein was dried by lyophilization.

Extraction of the meal with $MgCl_2$ solution (T 5): The meal was extracted with a 5% solution of $MgCl_2$ for 1 h at room temperature and pH 7.0. After centrifuging and filtration the protein content was precipitated at pH 2.0. The sediment was settled, decanted, centrifuged and dried by lyophilization.

Membrane separation concentration of the alkaline extracts (T 6): The alkline protein extract was neutralized and centrifuged. The clear protein solution was applied to a flat-membrane apparatus of 0.1 m^2 permeable surface at a pressure of 10 bar. After washing the concentrate with water the previously centrifuged precipitate was re-added and the mixture was spray-dried.

1.2. Method applied in industrial-scale edible protein isolation

In a tank of 2500 dm^3 capacity fitted with blender 200 kg extracted meal were mixed with 1800 dm^3 of an 0.25% Na_2SO_3 solution. The pH of the mixture was set at 10.5 with about 10 dm^3 40% NaOH and after 1 h separated in a NOGS-325 type decanter at 2600 rpm. The by-product of the procedure is a dense suspension and it was dried on an alfalfa drier. The decanted liquid phase was filtered through a basket water-filter and was pumped into a tank of 2500 dm^3 capacity and its pH was set with about 16 kg concentrated HCl solution at 3.8.

The precipitated protein was separated on an Alfa-Laval separator. The liquid phase was drained, while the dense phase was collected. The dense phase was neutralized in a tank fitted with a blender with about 3 kg of a 40% NaOH solution. The neutral suspension was then spray-dried. The block scheme of the industrial process is shown in Fig. 1.

1.3. Method applied in the production of fodder protein on industrial scale

An amount of 1.6 tons of extracted sunflower seed meal of 39% protein content was processed by the following method. In a tank fitted with mixer and of 2000 dm^3 capacity 1800 dm^3 of water was introduced. To this 5.0 kg anhydrous sodium sulfite were added under mixing. Then 200 kg of extracted sunflower meal were added and the pH was set exactly at 10.5 with about 11 dm^3 of a 40% NaOH solution. After a 1-h extraction period the slurry

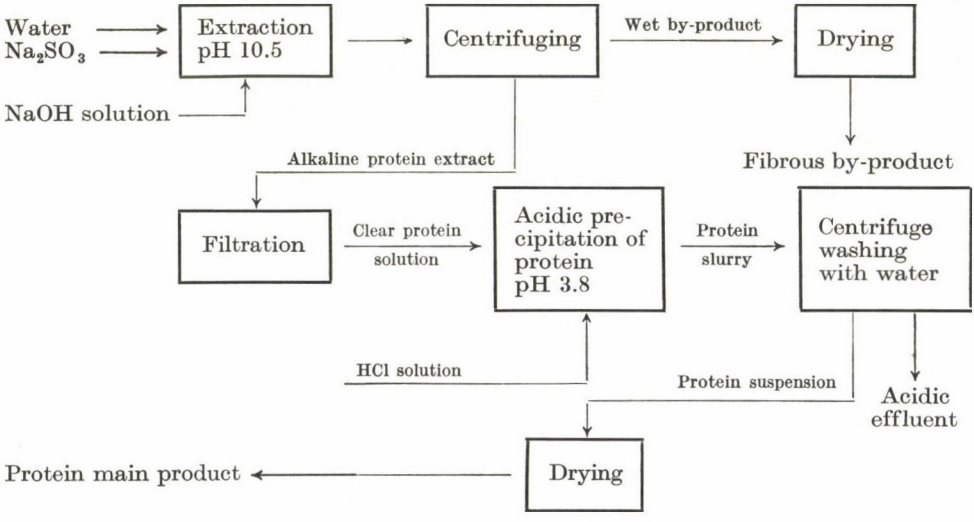

Fig. 1. Block scheme of the pilot plant production of sunflower seed protein for human consumption

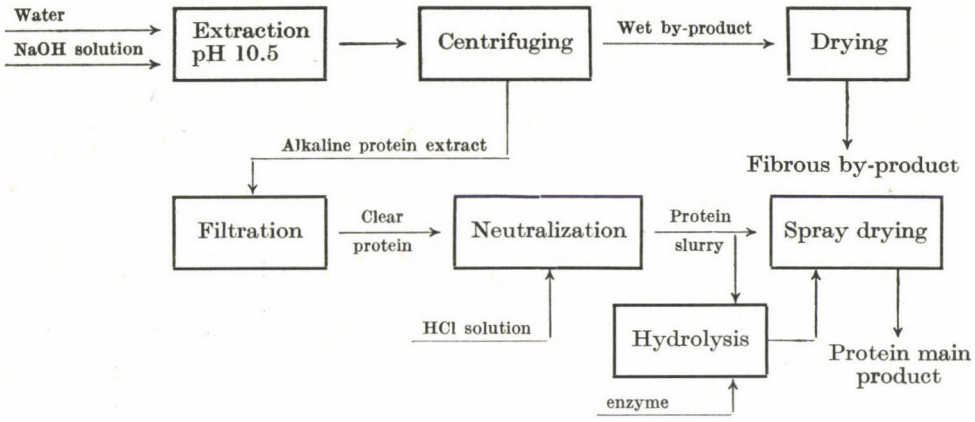

Fig. 2. Block scheme of the industrial production of sunflower seed protein for animal feed

was separated on a NOGS-325 type decanter. The alkaline extract was strained into a mixing tank of 3 m^3 capacity and its pH was set at 7.5 with a concentrated HCl solution.

Half of the neutral protein suspension thus obtained was dried directly on a spray-drier. The other half of the suspension was hydrolyzed with enzyme in the following way: the suspension was conducted into Fryma containers of 2000 dm^3 capacity. To each container 1.8 kg Pronaz enzyme was added, the temperature was raised to 40 °C. After 30 min hydrolysis the container was heated to 80 °C and kept at this temperature for 15 min to inactivate the enzyme. The liquid was spray-dried on an Anhydro equipment.

The residual sunflower seed meal as a by-product of decanting, was dried on an alfalfa drier. The block scheme of the process is shown in Fig. 2.

1.4. Methods used in analysing the products of the various technologies

Determination of the solids content. Direct drying at 105 ± 2 °C was applied according to the pertinent HUNGARIAN STANDARD (1977).

Determination of protein. The method of Kjeldahl was used (LÁSZTITY, 1968) and a Kjel-Foss automatic analyzer.

Determination of the soluble solids. Five g of the air-dried sample were extracted with a predetermined amount of distilled water for 1 h on a shaking apparatus at room temperature. The amount of distilled water was calculated to provide 200 cm^3 at 5 g solids content. After extraction the extract was centrifuged and the liquid filtered. To the second extraction half of the original volume of water was used. The centrifuged and filtered liquids were united, measured and their solids content was determined.

Table 1

Comparison of the characteristics

Sign of technology	Technology	Protein extraction		Production of the protein from the extract		
		mode	efficiency (%)		mode	efficiency (%)
T 1	Basic technology	Alkaline pH=10.5	48±2	Acidic protein precipitation	pH=3.8	72±2
T 2	Basic technology combined with enzymic hydrolysis	Alkaline pH=10.5	48±2		pH=3.8	72±2
T 3	Direct enzymic extraction	Enzymic pH=7.5	50±2		pH=3.1	63±2
T 4	Basic technology and alcoholic treatment	Alkaline pH=10.5	48±2		pH=3.8	72±2
T 5	Extraction with $MgCl_2$ solution	Salty pH=7.0	49±2		pH=2.0	66±2
T 6	Concentration of the alkaline extract by membrane separation technique	Alkaline pH=10.5	48±2	Membrane separation pH=7.0		86±5

[a] without spraying and extraction loss
[b] percentage of meal protein

Table 2

Properties of the products obtained

Sign of technology	Technology	Dry matter (%)	Protein content (%)[a]
T 1	Basic technology	95±3	89±2
T 2	Basic technology with enzymic hydrolysis	89±3	67±2
T 3	Direct enzymic extraction of the meal	94±2	70±3
T 4	Basic technology with alcoholic treatment	97±2	91±2
T 5	Extraction of the meal with $MgCl_2$ solution	96±1	78±1
T 6	Membrane separation concentration of the alkaline extract	93±4	72±5

[a] percentage of solids
[b] percentage of protein content
[c] scatter below 1%

of laboratory technologies

Drying method	Protein in the main product as percentage of protein in the meal	Protein content of the main product	Protein content of the by-product	Total loss during production	Protein obtained in the main and in by-product
		%			
Lyophilization	33±3	84±4	34±1	9	80[b]
	38±2	60±2	34±1	10	85[b]
	33±3	66±3	31±1	9	74[b]
	30±2	88±2	34±1	11	77[b]
	33±3	75±1	28±1	10	78[b]
Spray drying	42±6	67±5	34±1	10[a]	78[b]

by laboratory technologies

Soluble solids (%)[a]	Protein soluble in water (%)[b]	Sulfurous acid content (%)[a]	(Cl^-) content expressed in NaCl (%)[a]	Colour characteristics	
				Brightness (%)[d]	Wavelength (mμ)[e]
9±2	4±2[c]	0.06±0.02	1.3±0.2	76	379
82±2	80±2	0.30±0.01	3.9±0.6	66	582
40±5	37±1	0.34±0.01	5.2±0.3	22	578
8±4	2±2[c]	0.15±0.10	0.88±0.2	79	576
22±1	6±1	0.04±0.01	13.5±0.6	81	578
71±4	67±5	0.42±0.05	—	68	579

[d] deviation of measurements: ± 6
[e] deviation of measurements: ± 2

Determination of the soluble protein. This process differs from the previous one only in calculating the amount of water to 5 g protein (200 cm^3 per 5 g) and the protein content of the combined extracts was determined.

Determination of the sodium chloride content. The potassium thiocyanate method according to HUNGARIAN STANDARD (1972) was applied. Results give the total chloride content expressed as sodium chloride.

Determination of the sulfurous acid content. The rapid iodometric method in accordance with HUNGARIAN STANDARD (1972) was applied. The sulfurous acid content is equal to the potassium iodide solution used up.

Measurement of colour. The colour co-ordinates were measured on a Lovibond Tintometer. From these the wavelength, brightness factor and visual density were calculated.

Determination of the in vitro *digestibility.* The enzymes: hydrochloric acidic pepsin and pancreatin were used according to the method of BRAD (1967).

2. Results

2.1. Comparison of laboratory techniques

The characteristics of the protein isolating technologies are compared in Table 1. In Table 2 the properties of the isolates as obtained by the different technologies are shown.

In the course of the in vitro digestibility tests it became apparent that the digestible raw protein content of the isolates, except that obtained by membrane separation, did not differ significantly from their total protein content. Thus, the protein content of the protein isolates may be completely hydrolyzed with the enzymes pepsin and pancreatin. The protein content of the isolate obtained by membrane separation technique is digestible to $91 \pm 1\%$.

2.2. Results of industrial-scale processes

By processing 200 kg extracted sunflower seed meal of 43.8% protein content, 48.5 kg isolate of 75.3% protein content was obtained. In the course of the process 74.4 kg by-product of 25.5% protein content was obtained. The properties of the protein isolate are summed up in Table 3.

The in vitro digestible protein content of the product is $82.3 \pm 0.1\%$.

2.3. Results of industrial-scale isolation processes of protein for animal feed

The properties of the products are contained in Table 3. In the course of analyses it was established that no significant difference was found between the in vitro digestible raw protein and the total protein content, therefore the total raw protein content is digestible to 100%.

Table 3

Properties of protein products obtained in industrial experiments

Product	Solids content (%)	Protein content (%)	Soluble solids (%)[a]
Protein isolate for human consumption	93.7±0.8	75.3±0.5	67.2±0.1
Protein isolate free of fibre for animal feed	95.0±0.2	49.8±0.1	55.2±1.9
Hydrolyzed protein for animal feed	93.8±0.6	49.0±5.1	64.4±5.1

Product	Protein soluble in water (%)[b]	Sulfurous acid content (%)[c]	(Cl^-) content (%)[d]
Protein isolate for human consumption	67.8±0.1	0.11±0.02	2.8±0.6
Protein isolate free of fibre for animal feed	57.2±3.0	0.99±0.02	7.8±0.7
Hydrolyzed protein for animal feed	85.5±0.8	0.84±0.01	5.6±0.4

[a] % of solids content
[b] % of total protein content
[c] calculated as SO_2
[d] calculated as NaCl

3. Conclusions

3.1. Comparison of laboratory technologies for producing protein isolates of different functions

Important differences in the efficiencies of the individual technologies were not found, but losses were lowest in the membrane separation technique. The efficiency of protein extraction was the highest with the direct enzymic process. The protein recovery from the protein extracts was highest with the membrane separation technology.

The most important factor in judging the technologies is the properties of the product. The isolate of highest protein content was obtained from the protein suspension produced by the basic technology and treated with ethyl alcohol. With technologies utilizing enzymic hydrolysis the protein content of the main product is lower. As regards solubility in water of the product, differences were significant. The main product of highest solubility was obtained by enzymic hydrolysis and membrane separation. The colour of the products varied from light brown to white. The isolate obtained by extraction with $MgCl_2$ had the lightest colour. The odour and flavour of the products is pleasant and reminds one of salted sunflower seed and their digestibility is satisfactory.

3.2. Conclusions drawn from the experiences of industrial-scale production

The experiences hitherto gained prove that the extraction of sunflower seed protein may be carried out on simple equipment economically. The two crucial and at the same time mechanically most difficult points of the technology are the separation of the protein suspension and the drying of the dense protein slurry. The experiments have shown the Alfa-Laval BRPX 213-355 separator and the Anhydro spray drier to meet the requirements.

The utilization of sunflower seed meal as a raw material of vegetable protein is important because it is available continuously in large quantities as an industrial by-product and at the same time it is the only oil seed which does not contain any unwholesome or toxic agent.

*

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EXPERIMENTS ON THE PHYSICOCHEMICAL TREATMENT OF DAIRY WASTEWATERS

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The results of physicochemical experiments conducted over several years with batch and continuous processes are described.

As an introduction the quality characteristics of wastewaters from the dairy products industry are reviewed, and the conclusions of general validity are presented in seven paragraphs.

Of the continuous experiments based on the batch results only those considered representative are described, together with the equipment and method used. The results obtained are analysed in detail mainly for their potential practical implications. In wastewater treatment practice three methods were found practicable for destabilizing the lyophilic colloidal protein solution. The proteins thus coagulated viz. the chemical precipitate obtained can be separated by flotation and sedimentation. Operational parameters revealed flotation to be the superior one of the two. The choice between the two processes is governed by the method of coagulation adopted, further by uses of the resulting sludge and by the size of the plant. In general, phase separation by flotation in combination with coagulation at the isoelectric point (with metal ion or LNS as the coagulant) seems preferable at larger plants, whereas at minor plants lime precipitation (accelerating the process by metal ions) followed by sedimentation appears advisable.

Milk is one of the most important staple foods of man and this explains the rapid development of the group of industries processing it. In Hungary, steadily growing volumes have been handled by about 80 plants ranging in capacity from 10 to 250 thousand litre per day, producing varying volumes of wastes under a correspondingly wide spectrum of site and recipient conditions. No uniform methods of treatment could thus be developed, explaining to a certain extent also the fact that the wastes management problems are still largely unresolved.

In Hungary, any wastewater to be acceptable for being discharged into a recipient stream is required by law to meet to following criteria:

COD	50–150 mg l^{-1}
fats	2– 10 mg l^{-1}
suspended solids	100–500 mg l^{-1}

the actual values depending on the severity of pollution in a particular area. In the case of public sewers, the corresponding limit values are 400, 60 and 75 mg per litre, resp., beyond which the plant producing the effluent is obliged

to pay a pollution fine, or a progressively rising sewer rate (ORDER OF THE COUNCIL OF MINISTERS 1969; ORDER OF THE NATIONAL WATER AUTHORITY 1970; ORDER OF THE COUNCIL OF MINISTERS 1975; ORDER OF THE COUNCIL OF MINISTERS, 1978).

Of the total volume of dairy wastes amounting to 8 million m^3 per day, round 80% (6.4 million m^3 per day) are discharged into a communal sewer system, while the remaining 20% (1.6 million m^3 per day) into a recipient stream. The heavy pollution caused in the public sewers lends priority to the development of a technology by which the efficiency of primary treatment can be improved substantially over the normal level. The importance of the problem is underlined by the fact that very few sewer networks terminate in a plant providing full biological treatment, and the existing plants are often overloaded. Intensive pretreatment at the processing site is thus warranted also in order to relieve the load on the public sewage treatment facilities.

A research project was launched accordingly to explore the basic phenomena by detailed laboratory experiments based on the familiar principles of physicochemical wastewater treatment, and starting from the results obtained to develop by pilot plant experiments the technically–economically most efficient design.

1. Wastewater parameters

The main parameters of the wastewaters to be treated are shown in Table 1 indicating the extreme and – where available – the mean values as well (GOU et al., 1978; SHABI, 1974; TOÓKOS, 1981; WARTBURTON et al., 1979) As will be perceived from the tabulated data, the average concentration of the wastewaters from the different types of processing plant is almost identical. Presuming modern manufacturing methods, the wastewaters from cheese making are slightly stronger, while those produced when making powdered milk somewhat weaker than the normal dairy effluents. The relative proportions of the water components are also virtually identical, the wastewaters containing largely the same amounts of the pollutants that can be retained on filter paper and which are thus mainly colloidal in character. It will be concluded also that the composition of dairy wastes in Hungary does not exceed the range of values mentioned in the international literature.

The majority of pollutants is present in colloidal form. The purpose of physicochemical treatment is to neutralize the forces which tend to stabilize the colloids and to separate the aggregates formed. In the wastewater, just as in the milk proper, several disperse systems are present simultaneously, which interact with each other. As regards wastewater technology, the two

main systems of interest are the complex system of protein colloids and the fat emulsions. These latter are stabilized by the proteins. This is why the fats can be separated, once the proteins are separated (LÁSZTITY, 1968; WOLFRAM, 1977).

2. Review of the literature

Chemical treatment of dairy wastes has found no widespread application, since the bulk of the polluting substances is present in dissolved form, so that but moderate removal rates could be attained. Moreover, the natural colloid solutions are difficult to break down, the wastes are not detrimental to sewers, and consequently the sewer rates, or pollution fines were up to recent times less costly than the construction of expensive treatment facilities. From the sparse literature on the subject it is inferred that iron or aluminium salts applied alone, or in combination proved here again most effective, occasionally supplemented by the addition of some flocculant. Adjustment to the proper pH is evidently essential. Chemical treatment is normally followed by air- or electroflotation. Sporadic attempts using electrocoagulation have been reported on. In laboratory experiments an aluminium anode was used following coagulation with iron(III) chloride (50–100 mg l^{-1}) and sedimentation. This treatment resulted in COD, BOD_5 and fat removal rates of 78, 84 and 95%, respectively (TOÓKOS, 1978).

With reference to existing facilities, the manufacturers of treatment equipment claim reductions of 70–85% in BOD_5, 90–95% in fats and 80–85% in suspended solids, further complete protein removal. The main unit operations of the treatment process realized in one of the dairies in Hungary according to a licence by ICI (England) are as follows: neutralization (hydrochloric acid and hydrated lime), addition of aluminium sulphate (120 mg l^{-1}) and lime (40 mg l^{-1}) and finally electroflotation. Direct current (D. C.) of 38 V voltage is used for the electrolysis of water. The cathode is stainless steel, while the anode is made of titanium coated with lead dioxide. The service life of the electrode grid is one year on the average.

Another method accomplishes two objectives, namely recovery of valuable substances coupled with a substantial reduction of concentration, evidently by a considerably more complicated process. In the first stage the proteins are precipitated with digestible organic sulphate esthers, such as glucose trisulphate. The precipitate and other insoluble substances are separated by flotation or sedimentation. In the second stage, the dissolved phase is passed through a cation exchanger, where the amino acids, polypeptides are recovered. Hereafter the carbohydrates are removed from the effluent by anion exchange (JØRGENSEN, 1970; MATOV, 1967; SHERMAN, 1979; SOLYMOS, 1978; STRÖM, 1971).

Table 1

The quality

	COD (g l^{-1})	BOD_5 (g l^{-1})	Fats (solvent extract) (g l^{-1})
Dairies catering for the population	0.9–3.2 / 2.4	0.6–2.5 / 1.6	0.3–0.7 / 0.4
Cheese manufacturing	1.4–5.0 / 3.7	0.7–3.3 / 2.1	0.2–0.7 / 0.32
Powdered milk manufacturing	0.7–2.8 / 2.2	0.6–2.1 / 1.27	0.15–0.36 / 0.23
Mixed products	0.5–6.6	0.4–6.0 / 1.9	

(Summary of data published in the literature)

Although not related directly to the subject under consideration, it should be noted that the dairies are often interested in the removal of fats only. This is why flotation is anticipated to play a relatively important role in this group of the food industry. The correctness of this assumption is reflected by the growing number of methods registered with the patent office (FENYVES, 1978).

The methods described in the foregoing are expensive.

3. Experiments

3.1. Laboratory experiments. The primary aim of the laboratory experiments was to study the chemical parameters of flocculation. The main parameters controlling the process are the type(s) of chemical, the necessary feed rate, the optimal pH of the medium and the length of the reaction period.

These experiments were performed in jars of 1 l volume. The contents (raw wastewater + precipitant) were stirred first for 1 min at 84 rpm., subsequently for 5 to 10 minutes at the reduced rate of 60 rpm. Stirring was followed by a settling period of 30 to 60 minutes.

The wastewater used in these experiments was obtained from a dairy catering for the population of the capital.

The efficiency of treatment was determined by chemical analyses performed according to the specifications detailed in COMECON Standard Methods of Water Analysis (VIZGAZDÁLKODÁSI KUTATÓ INTÉZET, 1975).

3.1.1. Results of laboratory experiments. The chemicals listed in Table 2 were used alone, or in different combinations at different feed rates in the laboratory experiments. Each alternative was tested on the same wastewater

of dairy effluents

N by Kjeldahl method ($g\ l^{-1}$)	Lactose ($g\ l^{-1}$)	Total dry residue ($g\ l^{-1}$)	Total organic subst. ($g\ l^{-1}$)	pH	Sedimented solids in Imhoff jar in 60′ ($cm^3\ l^{-1}$)
0.015–0.06 / 0.04	0.3–0.7 / 0.4	1.1–2.7 / 2.2	0.4–1.7 / 1.3	6.8–7.0 / 6.9	–
0.01–0.05 / 0.04	0.3–1.0 / 0.63	–	–	4–8 / 6.3	9.9
0.01–0.05 / 0.025	0.2–0.55 / 0.36	–	–	–	–
0.02–0.08					

sample by 5 experiments, the results of which agreed to within ± 5 per cent under identical conditions. The mean value of these data is mentioned hereafter as a single result.

The main coagulants (iron and aluminium) proved successful, especially in combination with hydrated lime. The precipitates obtained with iron salts were of superior stability, but higher amounts of iron lent colour to the effluent.

It is known from the literature on the treatment of surface water and domestic sewage that at very high colloid contents the ranges of flocculation and coagulation coincide (STUMM, 1977). For the food industry in general a coagulant feed rate of round 60 mg l^{-1} is mentioned as the value where coagulation begins. Rapid coagulation occurs at feed rates from 100 to 600 mg l^{-1}, while the optimal range is between 200 and 400 mg l^{-1} (ANDERSON, 1971). Since no details have been published, we have determined the lowest feed rates for aluminium and iron salts as coagulants at which polyhydroxide compounds developed in the optimal pH range corresponding to the isoelectric point, as well as in the case of precipitation with lime at pH = 11. In this latter case the efficiency was improved by adding a metal ion. The range of chemical concentrations indicated may be regarded optimal, since although higher feed rates result in higher removal rates, the improvement is in no proportion with the additional expenditure. To attain a pH of 10–11, the feed rate of $Ca(OH)_2$ must be from 0.5 to 1.0 g l^{-1}. The experiments were made with wastewater having a concentration (strength) normally encountered in this branch of industry. The results showed a 60–70% reduction in COD after 60 minutes settling in the beaker. The general trend of the relationship between chemical feed rate and COD removal is illustrated in Fig. 1.

Table 2

Chemicals tested in the experiments

Primary coagulants	Polyelectrolytes	Bentonites
$Al_2(SO_4)_3 \cdot 18H_2O$	PEI	Yugoslavian No. 1
$FeCl_3 \cdot 6H_2O$	PAA	Yugoslavian No. 2
$FeSO_4 \cdot 7H_2O$	D 444 K	*Istenmező* activated with sodium carbonate
	P 411 K	
	P 2935/a	*Istenmező* with 25% sodium carbonate
	P 2830/74	
	P 423 K	*Mád* bentonite
	Floconit	*Istenmező*, activated 290
	Sedipur A	Wine clarifier
	Sedipur T-1	Kaoline
	Sedipur TF-5	
	Sedosan	
	Perfectamyl A-5114/2	
	Perfectamyl A-5114/3	
	Perfectamyl A-5114/4	
	Starch ether	
	Separan N-10	
	Praestol 444/K	

Others: $Ca(OH)_2$ as single precipitant and for pH adjustment
L-Sulphonic acid (for protein precipitation)
Herkoflock (for sludge concentration)
for pH adjustment: H_2SO_4, NaOH and $Ca(OH)_2$

The flocculating effect of the different anion and cation active polyelectrolytes was studied in special detail, but the experiments, in which the flocculants were used alone produced negative results. The polyelectrolyte Praestol 444/K was the only one showing promising effects. After charge reversal with the main coagulant, results similar to those mentioned in the foregoing were obtained. Bentonites could not be used alone, either.

After the foregoing general review and evaluation, the results considered typical and used subsequently will be presented in detail.

The experimental results have been plotted in Fig. 2, demonstrating the correctness of the chemical feed rates indicated as optimal in Fig. 1. The experimental results were on the average identical at both pH values, as regards treatment efficiency and precipitated COD alike. Nevertheless, it may

be concluded that the results obtained at basic, higher pH values were more consistent. In the acidic range, at lower pH values the treatment efficiency obtained with both iron chloride and aluminium sulphate varied by 20 to 25%, while the COD of the raw wastewater ranged from 800 to 4800 mg l^{-1}. At higher pH values the variation in efficiency was 12 to 15%, while the strength of the raw wastewater varied between 1800 and 4 800 mg l^{-1}. When using calcium as the precipitating agent, the addition of a metal ion is essential for improving the settling properties of the precipitate and thus the quality of the effluent. The feed rate is higher than in the case of precipitation in the acidic range at the isoelectric point. It should be noted here that the pH range from 10 to 11 is not positively superior, since similarly good results have been obtained at a pH of 7 and 8 as well (Fig. 2).

The polyelectrolytes used in combination with the basic coagulants aluminium and lime were PEI (0.32 mg l^{-1}), PAA (0.05 mg l^{-1}), P 444 (3.2 mg l^{-1}), P411 K (9–10 mg l^{-1}), P 2935 A (0.68 mg l^{-1}), P 2830/74 (0.6 mg l^{-1}), P423 K (4 mg l^{-1}), Floconit (2 mg l^{-1}), Sedipur A (3 mg l^{-1}) and Herkoflock (2 mg l^{-1}). The concentrations indicated were found to produce optimum results. In the case of some polyelectrolytes higher feed rates failed to improve the settling behaviour of the flocs. The improvement in removal rate over that attained in the reference experiment was no more than 10 to 15%.

In combination with aluminium as the primary coagulant we conducted experiments with the product Deriton (40 mg l^{-1}) used normally for clarifying wine, Yugoslavian bentonite No. 2 (10 mg l^{-1}), Istenmező bentonite activated with 25% sodium carbonate (80 mg l^{-1}), Yugoslavian bentonite No. 1

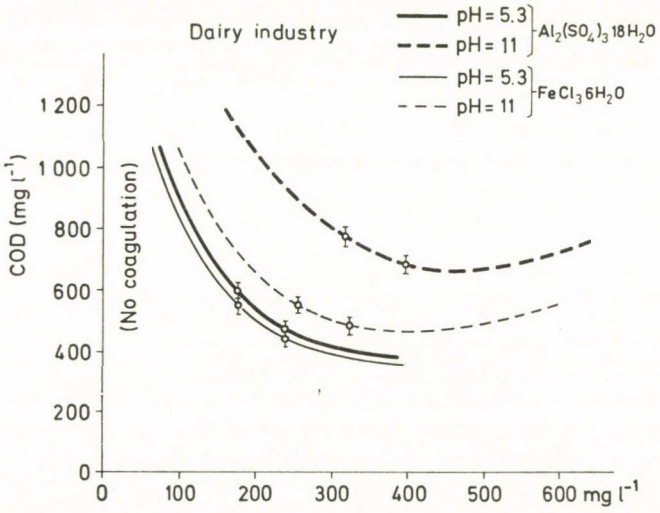

Fig. 1. The chemical feed rate vs. COD removal relationship (dairy industry)

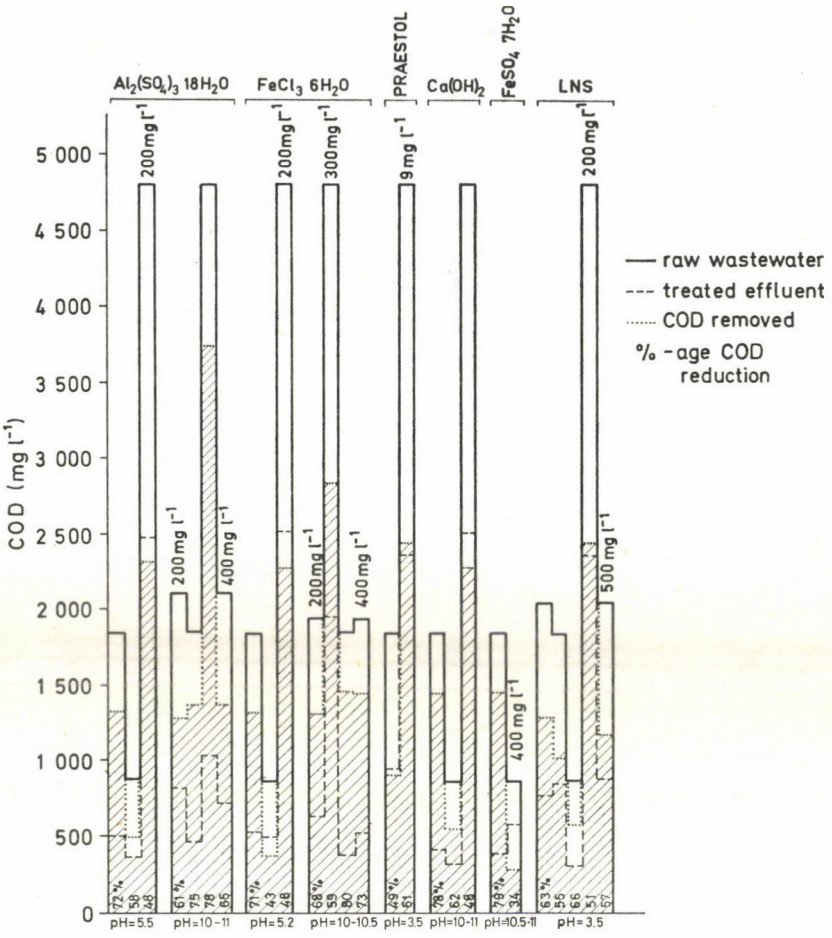

Fig. 2. Results of experiments with dairy wastewater

(60 mg l^{-1}) and Istenmező sodium carbonate bentonite (160 mg l^{-1}) and all produced satisfactory results. The two latter proved especially effective, reducing the COD by 80–85%. The settling behaviour of the precipitate could be improved effectively by adding small amounts of the polyelectrolyte Herkoflock.

The improvement obtained with flocculation promoting additives over the treatment using aluminium, or iron + calcium hydrate is thus insufficient to warrant the increased cost.

Sodium lignine sulphonate of high molecular weight is effective in precipitating the proteins around the isoelectric point only. In the medium maintained accurately at this pH value, intensive flocculation was observable, but the small flocs formed were impossible to separate by sedimentation. The

commercial grade sulphite liquor was highly contaminated with carbohydrates. This is the reason why at feed rates higher than 300 mg l^{-1} the reduction of proteins and fat content was not accompanied by a substantial reduction of the COD.

The quality of the treated effluent fluctuated between wide limits and the moderate treatment efficiency was considered unsatisfactory. The COD of the raw wastewater ranged from 880 to 4800 mg l^{-1}, while that of the treated effluent from 300 to 2300 mg l^{-1}.

The experimental results obtained with the combination of chemicals producing the highest COD removal efficiency were plotted graphically (Fig. 3) and assuming the relationship to be a linear one, the regression equations were determined. The lower and upper validity limits of the equation were specified. Hereafter we have calculated also the correlation coefficient for each regression equation. The correlation will be seen to be a very close one. In this way we have succeeded in verifying the correctness of linear regression, further the close correlation between the COD of the raw wastewater and the amount of COD removed from the treated effluent (Fig. 3).

The experiments made with wastewaters from a cheese factory yielded very poor results. The combinations of chemicals found most effective were as follows: aluminium sulphate + lime, or sodium hydroxide together with

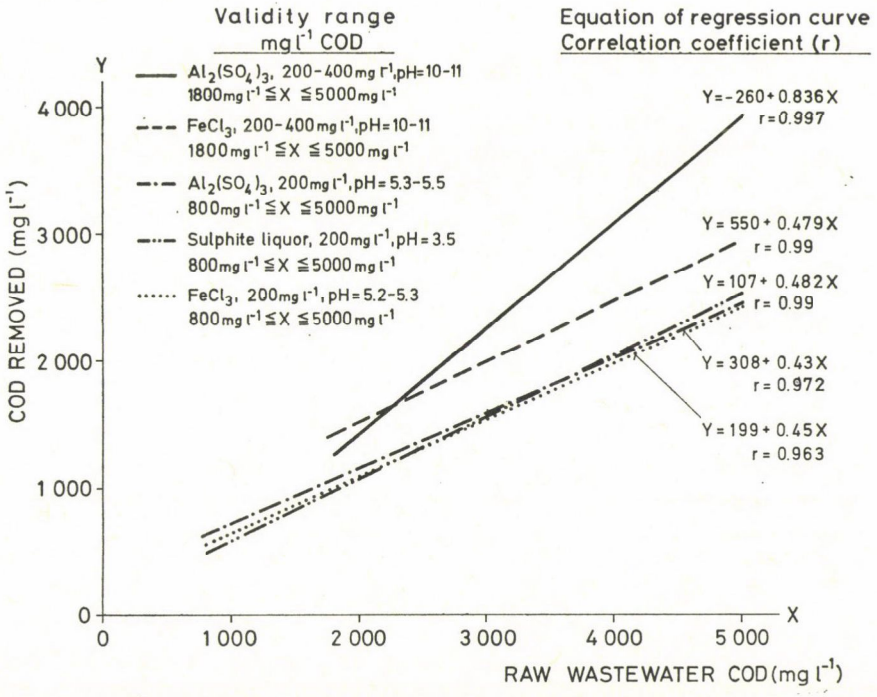

Fig. 3. Linear regression equations for dairy wastewaters

P 44 K (3.2 mg l^{-1}) and Herkoflock (0.2 mg l^{-1}) produced a COD removal of 45%, while Istenmező activated – 290 – bentonite (80 mg l^{-1}) + Herkoflock (0.2 mg l^{-1}) removed 55% of the initial COD. (For the sake of clarity and to facilitate applications in practice the measurement data plotting on the regression lines have been omitted. It is proposed to deal with these separately within the more theoretical comparative treatise on the effluents from the poultry- and meat industries.)

3.1.2. Conclusions from the laboratory experiments. Having analysed the results of the laboratory experiments, the following conclusions have been arrived at:

1. The efficiency of the coagulation–flocculation process is hardly affected by the choice between media with acidic, or basic pH values. It should be noted, however, that the results obtained in basic media, i.e., in the range of higher pH values, are slightly better and at the same time more consistent. Thus it will be appreciated that the pH has a dominant effect on the physical properties of the flocs, since it influences their formation and aggregation, further their size, stability and settling behaviour.

2. The optimal chemical feed rate was found to be specific for the type (composition) of the wastewater to be treated, but to remain unchanged between wide limits (500–600%) of raw water strength. In other words, when using a particular coagulant at a constant feed rate, the treatment efficiency is hardly influenced by the strength of the raw wastewater.

Highest COD removal rates can be attained with several combinations of chemicals.

3. The amount of pollutants removed was found to increase in linear proportion with the strength of the wastewater treated. In other words, a relatively constant treatment efficiency can be expected. Evidently, the same close correlation exists between the raw wastewater and the treated effluent as well.

4. At the optimal attainable removal efficiency, aluminium sulphate and iron chloride coagulants are suited to most widespread use.

5. The bentonites and most polyelectrolytes are alone ineffective for precipitating the colloidal proteins. The polyelectrolyte Praestol 444/K was alone found to produce satisfactory results.

6. Owing to its carbohydrate content, sulphonic acid performed slightly poorer than expected. The precipitate formed was highly unstable and impossible to remove by sedimentation.

7. The foregoing conclusions may be regarded to be of general validity. The experiments with wastewaters from the poultry and meat industry yielded similar results. Examined together with the results on dairy wastes it is of interest to note that major fluctuations in treatment efficiency (COD removal) are due mainly to differences in the type (composition) of the wastewater,

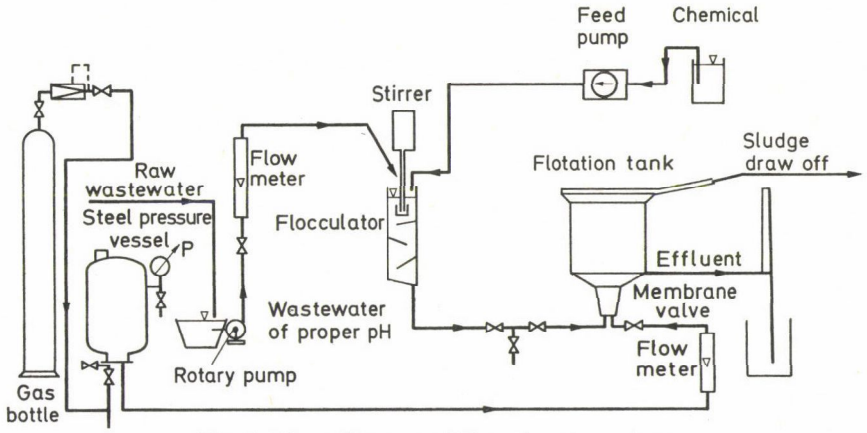

Fig. 4. Flow diagram of flotation treatment

rather than to the strength thereof, or to the choice of chemical found successful, or of the pH.

3.2. Pilot-plant experiments. The immediate objective of the pilot-plant experiments was to substantiate the results obtained at laboratory scale and to find the most effective method of separating the precipitate from water. The experiments were controlled by the results of chemical analyses performed immediately on the site.

After lengthy experimental measurements and several modifications of the design, two models were developed eventually. The flow diagram of flotation treatment is shown in Fig. 4.

The main data of the flotation cell are as follows:

Net volume:	23	litres
Effective flotation surface:	7.08	dm^2
Side length (without diffuser extension, or bottom):	0.3	m

The continuously arriving material was rapidly mixed for 1.5 to 8 minutes over the upper section of the flocculator, with inflow rates varying from 20 to 100 litres per hour. The stirrer speed found optimal for this purpose was found to be 150 rpm. The time available for the formation of flocs in the lower section ranged from 7 to 35 minutes.

The operating parameters were not adjusted to variations in wastewater strength. The specific operating parameters, together with the hydraulic load rates, have been compiled in Table 3.

Owing to conditions particular to this experiment tap water was saturated with air in the saturation tank, instead of the effluent commonly used in industrial practice. Consequently, the effluent characteristics to be mentioned

Table 3

Specific operating parameters and hydraulic load rates

	Flotation water ($l\ h^{-1}$)	Sewage inflow ($l\ h^{-1}$)				
		20	40	60	80	100
Recirculation ratio	10	33	20	14.3	11	9.1
	20	50	33	25.0	20	16.7
Retention time	10	0.77	0.46	0.33	0.29	0.21
(hours)	20	0.57	0.38	0.29	0.23	0.19
Hydraulic loading rate	10	0.42	0.71	0.99	1.27	1.55
($m^3\ m^{-2}\ h^{-1}$)	20	0.56	0.85	1.13	1.41	1.69

Table 4

Correction factors to parameters and load rates

Sewage inflow ($l\ h^{-1}$)	0	20	40	60	80	100
Flotation water ($l\ h^{-1}$)	10	1.5	1.24	1.17	1.12	1.1
Flotation water ($l\ h^{-1}$)	20	2.0	1.50	1.33	1.25	1.2

subsequently must be converted to actual cases using the correction factors given in Table 4.

The relationship between the hydraulic data of the flotation device and the suspended solids content of the inflow are expressed in terms of the ratios A/S (air: suspended solids), giving at the same time a fair picture about the unit operating costs of flotation. Using flotation water of checked saturation and a pressure maintained constant at 4.5 ± 0.5 bars during the experiments, the dissolved air content was found to be 1.26 g l^{-1}. The A/S ratios made dimensionless by using identical mass units were obtained using this figure and were entered into the tables to be mentioned later.

During the experiments the equipment was regarded to start operating under steady conditions, reached in a time after starting up twice as long as the retention times given in Table 3.

The flow diagram of sedimentation treatment is shown in Fig. 5.

The main data of the sedimentation equipment are as follows:

volume of the conical part: 10 litres,

volume of the cylindrical part: 17 litres.

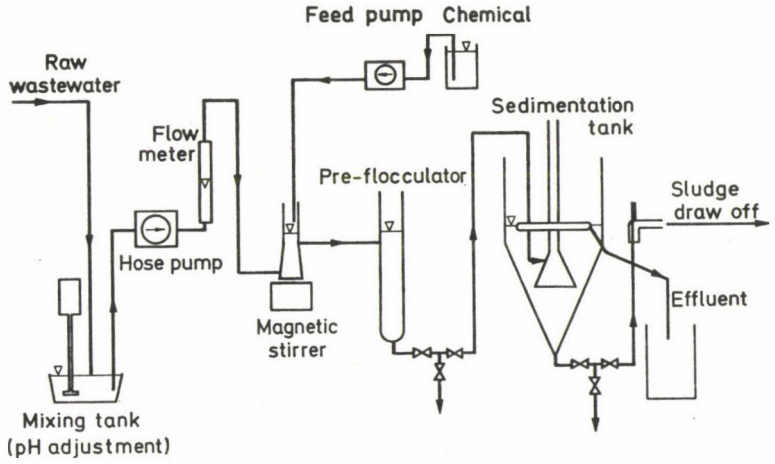

Fig. 5. Flow diagram of sedimentation treatment

Hydraulic retention time calculated for the conical part: 2 hours, at a wastewater flow of 5 l h^{-1} adopted as the design discharge. Under these conditions the surface loading rate is 0.059 m^3 m^{-2} h^{-1}. The circular pipe draining the effluent has been adjusted to the net volume of the sedimentation tank of v = 12 litres.

The main hydraulic parameters of the sedimentation tank in operation are shown in Table 5.

In order to maintain the pH accurately at the desired value, automatic pH control devices were installed into both (flotation and sedimentation) equipments. The transducer was mounted in the central part of the flocculator.

3.2.1. Results of the pilot-plant experiments. A representative sample of the experimental results obtained will only be presented here, together with the related flotation and sedimentation characteristics.

The wastewater used originated from the same plant as in the case of the laboratory experiments.

Parallel to the continuous plant experiments of extremely high water demand, the separation behaviour of the precipitate was investigated at reduced scale. Susceptibility to flotation treatment was found to be highest if the particles carried a positive electrokinetic charge of specific magnitude.

Table 5

Hydraulic characteristics of the clarifier equipment

Inflow (l h^{-1})	6.8	13.2
Retention time (h)	1.8	0.92
Surface loading rate (m^3 m^{-2} h^{-1})	0.08	0.155

Table 6

Treatment of dairy

Chemical used	Feed rate (mg l^{-1})	$\bar{S}_{in}$ (mg l^{-1})	Treatment (pH)	COD_{in} (mg l^{-1})	S_{in} (mg l^{-1})
Flotation without treatment	—	153	8.8	2050	153
$FeSO_4 \cdot 7H_2O$	200	153	9.9–10.1	2050	648
	400	153	9.7– 9.9	2050	750
	500	153	10.2–10.4	2050	820
	600	153	9.8–10	2050	815
LNS	100	120	3.2– 3.3	2120	421
	250	120	3.0– 3.2	2120	576
	350	120	3.2– 3.3	2120	525
$Al_2(SO_4)_3 \cdot 18H_2O$	200	153	9.9–10	2110	670
	300	170	5.5	2200	660
	400	153	9.8–10.1	2110	750
	600	153	9.7–10	2110	750
	800	153	9.8–10.2	2110	920
$FeCl_3 \cdot 6H_2O$	200	130	9.7–10	1950	645
	300	130	9.8–10.1	1950	710
	300	170	5.2	2200	760
	400	130	9.7– 9.9	1950	730
Praestol	10	165	4.2	1786	275

Symbols: see Table 7

The hydrogen ion concentration, which influences the magnitude of the zeta-potential, is thus a factor that can be optimized. The optimum pH was obtained for aluminium, iron(III) and Praestol 444/K as 5.0–5.5, 4.8–5.5 and 4.2, respectively. The settling behaviour of the precipitate is, evidently, much poorer at these pH values than in the range of higher hydrogen ion concentrations.

In the flotation treatment experiments summarized in Table 6, the rate of flotation water flow was invariably constant at 10 l h^{-1}. The wastewater inflow is characterized by the following data: flotation without chemical additive, treatment with L-sulphonic acid, addition of Al(III) and Fe(III): 40 l h^{-1}, treatment with Fe(II): at 20 and 40 l h^{-1}. Surprisingly good fat

wastes by flotation

Fat_{in} (mg l^{-1})	A/S	Loading rate (kg m^{-2} h^{-1})	COD_{eff} (mg l^{-1})	S_{eff} (mg l^{-1})	Fat_{eff} (mg l^{-1})	Dried sludge (%)	COD removal (%)
165	0.21	0.09	920	22	17	—	55
165	0.10	0.18	1220	245	21	—	40
165	0.04	0.42	930	16	17	8.3	55
165	0.04	—	440	120	—	7.2	78
165	0.04	0.46	480	38	13	—	77
225	0.075	0.24	980	11	—	—	54
225	0.055	0.32	1040	12	—	—	51
225	0.060	0.30	1080	13	—	—	49
235	0.047	0.38	630	28	—	—	70
—	0.0378	0.466	760	27	—	7.1	65
235	0.042	0.42	580	39	—	4.0	73
235	0.042	0.42	580	39	—	4.0	73
235	0.034	0.52	440	86	—	4.1	79
193	0.049	0.36	485	113	—	5.1	75
193	0.044	0.40	445	75	—	—	77
—	0.0328	0.536	720	30	—	6.2	67
193	0.043	0.41	420	90	13	5.4	78
—	0.0908	0.193	620	5	—	7.2	65

removal figures were obtained (89%) by flotation treatment without any chemical additive (COD removal: 55%). These experiments were performed with the objective of obtaining a better understanding of the phenomena, so that the results may be regarded as promising only. Flotation treatment in combination with some chemical is likely to remove 70 to 75% of the COD, except for L-sulphonic acid, where the COD removal rate was 50 to 60%. As in the case of the laboratory experiments higher aluminium and iron feed rates were necessary in the basic pH range (lime treatment), but the results were more consistent, better and the precipitate was more stable.

The experiments with sedimentation treatment were performed with substantially the same wastewater loading rates. In the experiments sum-

Table 7

Treatment of dairy

Chemical used	Feed rate (mg l^{-1})	$\bar{S}_{in}$ (mg l^{-1})	Treatment (pH)	COD_{in} (mg l^{-1})	S_{in} (mg l^{-1})
$FeCl_3 \cdot 6H_2O$	150	182	6.7	1650	520
	200	165	9.8–9.9	2260	911
	300	182	7.2	1650	575
	300	182	7.1	1650	575
	400	165	9.8–9.9	2260	1060
$FeSO_4 \cdot 7H_2O$	600	165	9.7	2260	1110
$Al_2(SO_4)_3 \cdot 18H_2O$	300	182	7.2	1650	421
	300	182	7.1	1650	400
	800	165	10.1	2260	885

Symbols used in Tables 6 and 7:

$\bar{S}_{in}$ = Suspended solids in the raw waste
COD_{in} = COD of the raw waste
S_{in} = Suspended solids in sample taken from the bottom tap of the flocculator
Fat_{in} = Fat content (solvent extract) from the raw waste
A/S = Ratio of air to suspended solids (S_{in})
COD_{eff} = COD of effluent
S_{eff} = Suspended solids in effluent
Fat_{eff} = Fat content (solvent extract) of the effluent
Loading rate = Amount of suspended solids kilogrammes per 1 m^2 surface and per hour

marized in Table 7 the wastewater inflow rate was varied from 6.8 to 32.5 l h^{-1}. At these flow rates and with the wastewater quality parameters indicated in the Table, the results of treatment were comparable to those obtained by flotation treatment.

3.2.2. Conclusions from the pilot plant experiments. Among the food industry wastewaters those produced at dairies represent a typical group. The main pollutants are present in emulsified and colloidal forms. The effectiveness of physicochemical treatment depends primarily on the success at which the proteins stabilizing also the fat emulsion can be precipitated. The lyophilic colloidal solutions of the proteins can be destabilized in several different ways, but in wastewater treatment only those tested by us are of practical interest. These can be classified into three main groups:

1. Flocculation around the isoelectric point of the proteins (e.g. precipitation with sulphonic acid).

2. Coagulation with the conventional primary coagulants [$Al^{(III)}$, $Fe^{(III)}$, $Fe^{(II)}$], in the optimal pH range for the formation of polyhydroxide compounds (sweep coagulation).

wastes by sedimentation

Fat_{in} (mg l^{-1})	Loading rate (kg $m^{-2} h^{-1}$)	COD_{eff} (mg l^{-1})	S_{eff} (mg l^{-1})	Fat_{eff} (mg l^{-1})	Dried sludge (%)	COD removal (%)
—	0.143	500	10	—	1.9	69
248	0.149	390	95	18	—	81
—	0.161	445	22	—	1.9	73
—	0.229	405	9	—	—	75
248	0.089	270	22	5	1.6	88
248	0.094	450	24	13	1.8	80
—	0.112	360	5	—	2.3	78
—	0.154	420	25	—	2.0	74
248	0.072	530	29	15	1.0	76

3. Precipitation with lime, at pH above 10.

Precipitation with lime does not guarantee alone the coagulation of caseine. For this reason the primary coagulant must be added not only in order to separate the precipitate, but because the denaturation of the proteins is substantially accomplished in this stage. The effectiveness of coagulation–flocculation, as well as the value of the operational parameters depend strongly on the pH. This can be traced back to the electrokinetic charge carried by the submicroscopic and microscopic particles. The fundamental prerequisite of coagulation–flocculation at the isoelectric point is to create within the protein structure the amino acid form which is neutral towards its surroundings. In the case of dairy wastes this takes place in the 4.3–4.7 pH range.

Lignine sulphonic acid acts on the one hand as precipitant, on the other as polyelectrolyte. In performance it was in no way superior to Praestol 444/K.

Although for reasons of economy no additive was used to accelerate settling, it should be noted that the addition of polyelectrolytes in small quantities is warranted by the fact that the flocs precipitating at the pH corresponding to the isoelectric point are very small and highly dispersive. These properties make them difficult to separate. The optimal pH level is determined by the anionic, kationic, or neutral character of the polyelectrolyte applied. When using a flocculant carrying a negative charge, the charge on the protein must be reversed slightly (from negative to positive) by the proper adjustment of the pH. In the case of lignine sulphonic acid this means that the pH must be lowered by about 1 unit below the pH corresponding to the

isoelectric point. The extent of lowering depends slightly on the concentration of the lignine sulphonic acid as well. E.g. when using L-sulphonic acid at the feed rate of 100 mg l^{-1} the optimal pH is 3.8, whereas at 200 mg l^{-1} it is 3.2.

"Sweep coagulation" which forms the second group of methods is affected by the electrokinetic potential mainly through the separation susceptibility of the precipitate, as well as through the important physicochemical influences.

The flotating micro-bubbles enter into the closest contact with the precipitate to be separated if the coagulate carries a weak positive charge. The optimal pH of flotation is between about 5.4 and 5.6 for both aluminium and iron hydroxide. This agrees well with the familiar zeta-potential relationship described in the literature (GRUTSCH & MALLATT, 1976). At this pH separation by precipitation–sedimentation is substantially more difficult, since the precipitate tends to rise to the surface. Settling behaviour is improved by raising the pH.

Confirming the results of the laboratory experiments, the pilotplant experiments with continuous flow have also demonstrated that the highest COD removal efficiency can be attained by several different combinations of chemicals and that the chemical concentrations vs. efficiency function resembles a saturation curve. (Owing to secondary contaminations the L-sulphonic acid is an exception to this.) The correlation between optimal hydrogen ion concentration, optimal chemical concentration and treatment efficiency – which controls the effectiveness of coagulants – is shown in Table 8.

Transferring the data of Table 8 to local conditions it is concluded from our experimental data that for treating more highly concentrated, stronger wastewaters lime precipitation, while for developing a technology aimed at the recovery of protein feed, some kind of flocculation at the isoelectric point is the potential solution.

Table 8

Correlation between treatment efficiency and chemical feed rate for dairy wastewaters

Chemical	pH	Optimal chemical feed rate (mg l^{-1})	Efficiency (%)
L-Sulphonic acid	3.4–3.6	150–200	60–65
Praestol 444/K	4.5	8– 10	62–65
$FeCl_3 \cdot 6H_2O$	5.2–5.4	200–250	63–65
	10–11	250–350	75–78
$Al_2(SO_4)_3 \cdot 18H_2O$	5.2–5.6	200–250	62–77
	10–11	400–500	72–73
$FeSO_4 \cdot 7H_2O$	7.5–11	400–500	60–70

3.3. Operational assessment of coagulate separation

Concerning precipitate separation the methods examined can be classified into two categories:

1. The main mass of the precipitate is formed by the coagulated–flocculated pollutants.

2. Besides the coagulants the precipitate contains important amounts of foreign matter which strongly affect the separation properties. These include $Al(OH)_3$, $Fe(OH)_3$, lime. The first group comprises the methods, which are based on coagulation at the isoelectric point. The aggregates formed in this process contain proteins and fats which have specific gravities lower than water. In the majority of cases these can be separated by flotation alone. The precipitates formed in "sweep coagulation" and lime precipitation belong to the second group, since these contain substantial amounts of coagulant and can, therefore, be separated besides flotation by sedimentation as well.

By comparing the operational parameters of continuous air-bubble flotation and the Graever clarifier, the following conclusions may be arrived at:

– There is a significant difference between the retention times needed to attain the same suspended matter concentration in the effluents from the two facilities. Consequent therefrom, the loading rate of flotation may be higher, normally up to five time as high as of the clarifier.

– Under identical coagulation conditions, the quality of the effluents from both kinds of treatment unit is also identical.

– Flotation is substantially more stable in operation.

The flotation susceptibility of precipitates formed during the different coagulation processes is described by the ratio of flotation air to the sludge formed, i.e., by the A/S ratio. In terms of this ratio we have found that the hydroxide precipitate obtained with aluminium sulphate and iron(III) chloride (in the 2.5–5.4 pH range) floats most readily and that this process is the most stable one. Adopting 30 mg l^{-1} as the still acceptable concentration of sus-

Table 9

Flotation behaviour of the precipitates from dairy wastewaters

Coagulant	pH	A/S	Loading rate (kg m^{-2} h^{-1})
$FeCl_3 \cdot 6H_2O$	5.2– 5.4	0.0218	0.80
LNS	3.2– 3.5	0.030	0.6
$Al_2(SO_4)_3 \cdot 18H_2O$	10–10.2	0.031	0.54
Praestol 444/K	4.5– 5.2	0.06	0.30
$FeSO_4$	10–10.1	0.05	0.37

pended solids in the effluent, the A/S ratios for precipitates resulting with different coagulants have been compiled in Table 9.

It should be noted that very good settling efficiencies have been attained in the Graever clarifier at retention times as low as 20–30 minutes. Their inclusion into the treatment process appears to be preferable to the horizontal, or vertical flow clarifiers normally applied.

When comparing clarification and flotation, substantial differences were experienced in the dry residue of the sludge formed. With proper sludge removal the lowest concentration observed was 5%, but values as high as 12 and even 15% were also measured.

In contrast to these figures, the dry substance in the sludge from clarifiers was 1 to 3% only. The comparison of these operational parameters of air-bubble flotation and the Graever clarifier indicates thus positively the superiority of the flotation equipment.

The choice between the proper method of precipitate separation should also be governed by the consideration that the recovery of the valuable protein and fat pollutants precipitated from the wastewater, makes them suited – after proper stabilization – to reduce the costs of the process, which is impossible in the case of the conventional treatment methods. Beneficial use of the sludge formed is possible with the coagulation processes at the isoelectric point alone, since these products contain no substances which would be harmful in animal feed. In such cases the precipitate can be separated by flotation only. Owing however, to the high costs of installation and to the complicated technology involved, these methods can be suggested for treating larger wastewater volumes only, where the by-products can be processed further without undue difficulty.

At minor plants the method based on lime precipitation appears to be practicable for wastewater treatment. The large volume of useless sludge is undesirable, but this is offset by the fact that owing to the high hydroxide-ion concentration the effluent is of better bacterial quality. Moreover, the process is more simple and safe to control.

4. Conclusions

The results of physicochemical experiments conducted with batch and continuous processes over several years are described.

As an introduction the quality characteristics of wastewaters from the dairy industry are reviewed, and the major results published in the literature on the subject are summarized.

Following the presentation of the results of batch-type bottle experiments, the conclusions of presumably general validity are presented in seven paragraphs.

Of the continuous experiments based on the batch results only those considered representative are described, together with the equipment and method used. The results obtained are analysed in detail mainly for their potential practical implications. In wastewater treatment practice three methods were found practicable for destabilizing the lyopihlic colloidal protein solutions. The protein thus coagulated, viz., the chemical precipitate obtained can be separated by flotation and sedimentation. The operational parameters revealed flotation to be superior of the two. The choice between the two methods is governed by the method of coagulation adopted, further by uses of the sludge resulting and the size of the plant. Phase separation by flotation in combination with coagulation at the isoelectric point (with LNS, or metal ion as the coagulant) seems preferable generally at larger plants, whereas at minor plants lime precipitation (accelerating the process by metal ions) followed by sedimentation appears advisable.

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ENDO-POLYGALACTURONASE IN TOMATO PROCESSING

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In the course of tomato processing by the warm technology of enzyme treatment the effect of temperature (30, 40, 45 °C), the period of incubation (30, 60 min), and the concentration of endo-PG (0.05, 0.1, 0.2%) were investigated on the juice yield, the dry matter content and viscosity of the tomato juice, the straining loss, the duration of the concentration and the dry matter content and the yield of the final product.

No significant differences were found in the investigated parameters when the period of incubation was prolonged to 60 min.

The increase of the endo-PG concentration from 0.05% to 0.1% improved all the investigated parameters, but a further increase of the enzyme concentration (0.2%) did not prove to be advantageous in the course of tomato processing.

The treatment of crushed tomatoes with 0.1% endo-PG decreased very highly significantly (by 11–26%) the straining loss, and the period of concentration (by 12–55%) when compared to those of the untreated samples at all the three investigated temperatures, for 30 min incubation. The dry matter yield of the final product (tomato paste), obtained from a unit quantity of fresh tomato, was very highly significantly (by 13–16%) and significantly higher than those of the control at 30, 40 and 45 °C, resp.

The particle size distribution of the tomato paste produced by the use of endo-PG on industrial scale was also determined. It was found that endo-PG treatment resulted in an increase in the particles in the range of smaller particles (10–20, 10–30 μm) at the expense of the larger ones (30–70, 40–90 μm).

To facilitate the concentration of tomato juice of the tomato varieties bred for mechanical harvesting, crushed tomatoes were treated with endo-PG.

In the laboratory experiments the enzyme treatment resulted in a lot of beneficial effects (ZETELAKI-HORVÁTH & GÁTAI, 1978) which were successfully reproduced on industrial scale, too (CZUKOR et al., 1977).-

On industrial scale the enzyme was fed together with the crushed tomatoes into the tank cars at the field stations of the canning factories, where it developed its effect on the tomato substrate during transportation. This incubation period varied from 1 to 3 hours depending on the distance between the pre-processing station and the factory. The temperature of tomato in the tank car varies between 12–20 °C according to season.

In canning factories, having no field stations, the entire tomato processing operation must be carried out in the plant. In this case the period of enzyme treatment must be minimized in order to not delay much the process-

ing rate. The period of enzyme treatment can be shortened by the increase in enzyme concentration and the temperature of incubation.

The aim of this work was to determine the suitable parameters of endo-PG treatment in the case of pre-heated crushed tomato substrate.

1. Materials and methods

1.1. Enzyme preparation

An enzyme preparation, obtained from *Aspergillus awamori* at the Central Food Research Institute, Budapest and sterilized by gamma radiation (ZETELAKI-HORVÁTH & KISS, 1978) was used. The activity of the preparation was adjusted to 300 l h^{-1} g^{-1}, and determined by viscosimetry (ZETELAKI-HORVÁTH & VAS, 1972).

1.2. Tomato variety

Tomatoes of Peto Mec variety were used as substrate.

1.3. Parameters of the enzyme treatment

1.3.1. Warm technology. Temperature: 30, 40 and 45 °C. Endo-PG concentration: 0, 0.05, 0.1, 0.2%. Duration of the enzyme treatment: 0, 30 and 60 min.

1.3.2. Cold technology. Crushed tomatoes were incubated with 0.1% endo-PG at 15 °C for 120 min.

1.4. Method of enzyme treatment

Tomatoes were ground in a meat grinder and portioned into 10 liter glass dishes. Samples were incubated at 30, 40 and 45 °C in a water bath and mixed continuously at an agitation speed of 200 rpm. At the end of the incubation periods, the enzyme was inactivated by heating in a water bath at 80 °C for 15 minutes. After inactivating the enzyme, the crushed tomatoes were strained through a 0.5 mm screen. The obtained tomato juice was concentrated in a Rotadest (at 60 °C, and 80 kPa) apparatus.

1.5. Evaluation of the enzyme treatment of tomatoes

1.5.1. Determinations in the strained juice.

1.5.1.1. Juice yield. – The juice yield was determined and given in cm^3.

1.5.1.2. Dry matter (DM). – DM content was determined and its quantity given in per cent, while the total DM obtainable from a certain amount of tomato was given in g.

1.5.1.3. Viscosity. – Specific viscosity was determined in an Ostwald type viscosimeter. The calculation was carried out by the following equation:

$$\eta_{spec} = \frac{t_{sample}}{t_{water}} - 1$$

where:

t_{sample} = flow time (s) of the fruit samples,
t_{water} = flow time (s) of the water.

1.5.1.4. Straining loss. – Dry weight of the residue on the screen was determined and expressed as per cent of the treated crushed tomatoes (w/w).

1.5.2. Measurements from the tomato concentrate

1.5.2.1. Dry matter – obtained from a certain amount of tomato juice was given in g.

1.5.2.2. Consistency. – The consistency of the tomato concentrates was tested by spreading of a certain amount of sample in an Adams type consistometer (VAS & FÁBRI, 1958), as given in our previous work (ZETELAKI-HORVÁTH & GÁTAI, 1977).

1.5.2.3. Concentration period. – Duration of the concentration of the tomato juice in a vacuum evaporator, necessary for obtaining total concentration of the juice (concentration to the limit of condensability), was determined after different enzyme treatments and was given in minute.

1.5.2.4. Particle size determination of the tomato concentrate. – The size of the particles in the tomato concentrate was determined by sedimentation technique as given in our previous papers (ZETELAKI-HORVÁTH & URBÁNYI 1978; ZETELAKI-HORVÁTH, 1980).

2. Results

2.1. The effect of endo-PG treatment on the straining loss

With the exception of the lowest enzyme concentration at 30 °C, the treatment of crushed tomatoes with endo-PG resulted in a significant, highly significant and very highly significant decrease in the straining loss, when compared to the untreated samples (Fig. 1).

The lowest straining loss was obtained after an enzyme treatment at 40 °C with 0.1% endo-PG for 60 min. Nearly the same straining loss was attained, when 0.2% endo-PG was used for 60 min at 45 °C.

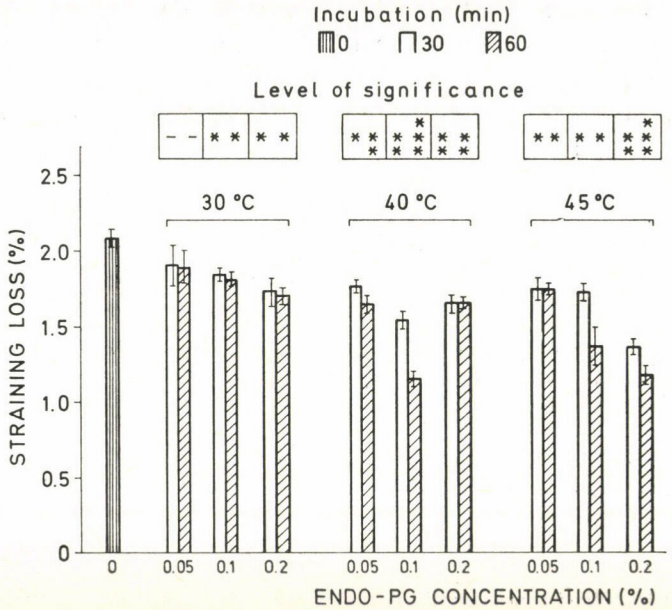

Fig. 1. The effect of enzyme treatment on the straining loss as a function of the endo-PG concentration and the incubation period. Significance of differences between the straining loss obtained with and without enzyme treatment

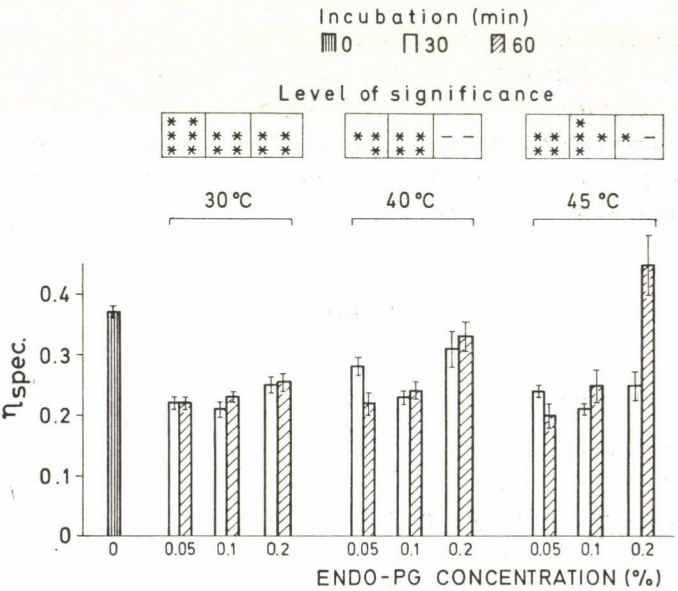

Fig. 2. The effect of enzyme treatment on the specific viscosity of the tomato juice as a function of the endo-PG concentration and incubation period. Significance of differences between the specific viscosities obtained with and without enzyme treatment

2.2. The effect of endo-PG treatment on the viscosity of the juice

The specific viscosity of the tomato juice decreased as a result of the enzyme treatment at every temperature when 0.05 and 0.1% endo-PG were used in the case of both incubation periods (Fig. 2).

Increase in the enzyme concentration, the temperature and the incubation period equally decreased the viscosity of the tomato juice. The use of 0.05% endo-PG for 60 min at 45 °C resulted in the lowest specific viscosity of the tomato juice. The use of 0.2% endo-PG at 40 and 45 °C caused an increase in the specific viscosity in comparison to the other enzyme treated samples, while at 45 °C when treated with 0.2% enzyme for 60 min, the specific viscosity of the juice increased so much that it exceeded that of the control.

2.3. The effect of endo-PG treatment on the dry matter content of the juice

Endo-PG treatment of the crushed tomatoes resulted in the highest DM content of the juice when 0.1% endo-PG and 40 °C temperature were used with an 30 min incubation period (Fig. 3). Similar DM contents of the juice were obtained with the use of 60 min incubation period and 0.2% endo-PG.

No significant differences were found however, when DM contents of the juice of the untreated samples were compared with those of the endo-PG treated ones.

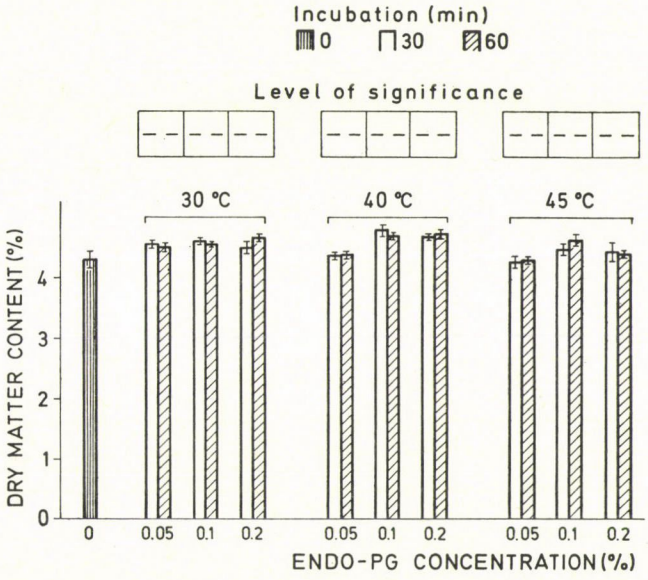

Fig. 3. The effect of enzyme treatment on the dry matter content of the juice as a function of the endo-PG concentration and incubation period. Significance of differences between the DM content of juices obtained with and without enzyme treatment

2.4. The effect of endo-PG treatment on the juice yield

The juice yields after endo-PG treatment at 30 and 40 °C increased highly significantly (in the case of both incubation periods and every enzyme concentration) when compared to the untreated samples. When samples were incubated for 60 min with 0.1 and 0.2% endo-PG at a temperature of 45 °C, the juice yields were only significantly higher than those of the untreated samples (Fig. 4).

The juice yield obtained when a 40 °C temperature, 60 min incubation period and 0.1% endo-PG concentration was used, proved to be significantly higher than the other good yields obtained at 30 and 45 °C temperature and with 0.2% enzyme concentration. There was no significant difference between the juice yields obtained at 40 °C with 0.1 and 0.2% endo-PG, respectively.

2.5. The effect of endo-PG treatment on the amount of DM obtained in the juice

In comparison to the untreated control samples the quantities of DM obtained in the juice increased significantly when crushed tomato samples were incubated at 30 and 40 °C with 0.1 and 0.2% endo-PG for 30 min. Very

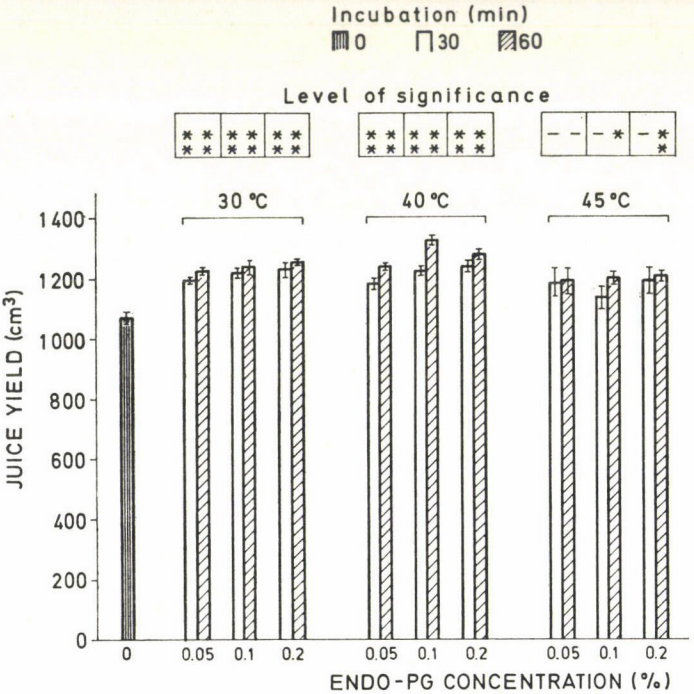

Fig. 4. The effect of enzyme treatment on the juice yield as function of the endo-PG concentration and incubation period. Significance of differences between the juice yields obtained with and without enzyme treatment

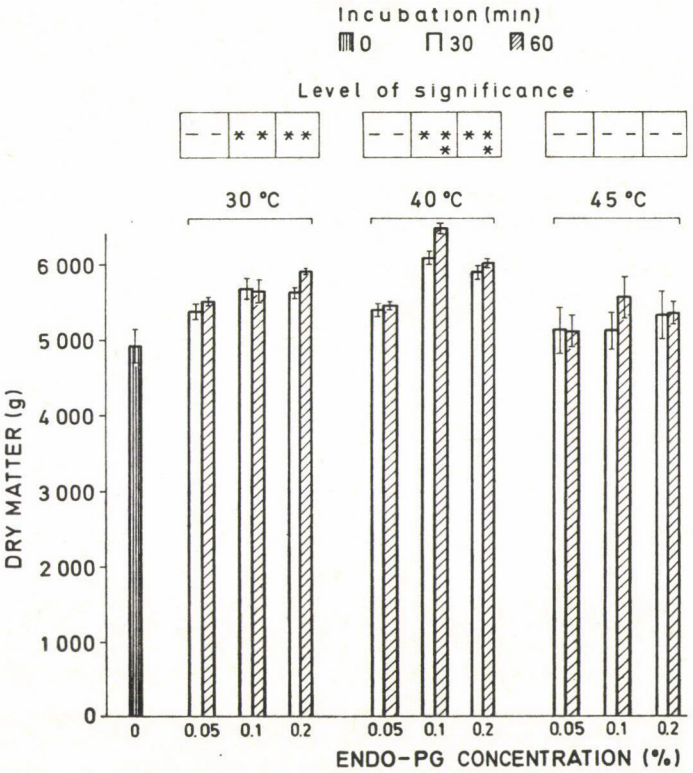

Fig. 5. The effect of enzyme treatment on the amount of dry matter, recoverable in the juice, as a function of the endo-PG concentration and incubation period. Significance of differences between the amounts of dry matter obtained with and without enzyme treatment

highly significant differences were found between DM quantities obtained without enzyme treatment and with 0.1 and 0.2% endo-PG during 60 min incubation periods (Fig. 5).

No significant difference was found between the amount of DM obtained at 40 °C with 0.1 and 0.2% endo-PG treatment for 60 min, while the amount of DM obtained at 30 °C with 0.2% endo-PG treatment for 60 min proved to be significantly lower.

2.6. The effect of endo-PG treatment on the length of the concentration period

It was found in the laboratory experiments, that the enzyme treatment decreased considerably the length of the concentration period, necessary to attain a final limit of condensation. At 30 °C the incubation with 0.1 and 0.2% endo-PG for 60 min resulted only in a very highly significant and highly significant decrease in the concentration period related to those of the un-

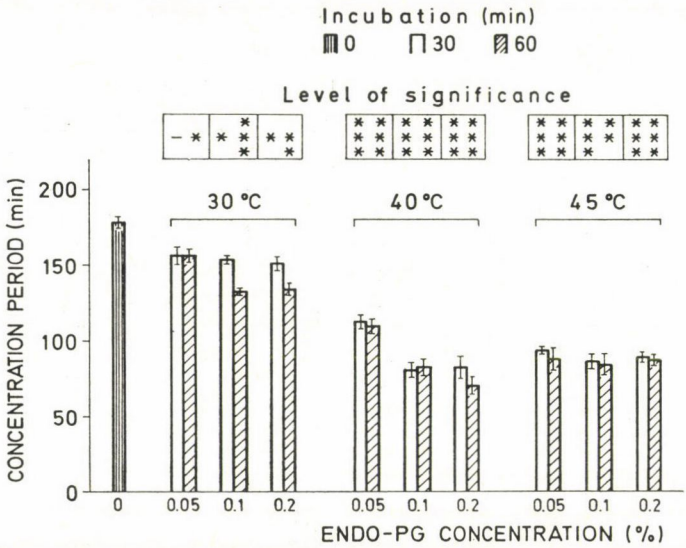

Fig. 6. The effect of enzyme treatment on the duration of the concentration period as a function of endo-PG concentration and time of incubation. Significance of differences between concentration periods measured with and without enzyme treatment

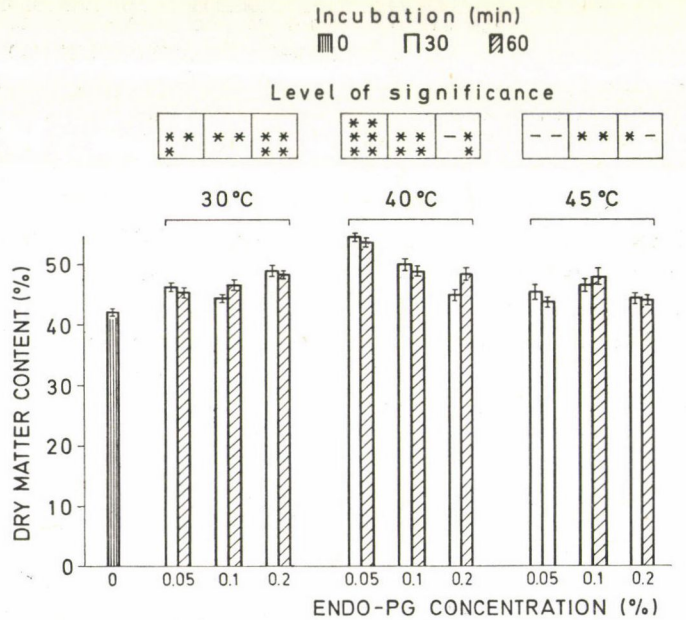

Fig. 7. The effect of the enzyme treatment on the dry matter content of the tomato concentrate as a function of the endo-PG concentration and the time of incubation. Significance of differences between the DM contents obtained with and without enzyme treatment

treated samples, while incubation of the samples at 40 and 45 °C, decreased very highly significantly the concentration period with every enzyme concentration and both incubation periods used (Fig. 6).

The shortening of the concentration period is of great importance in industrial scale production from the point of view of economy and the increase of processing capacity at the peak of the season.

2.7. The effect of endo-PG treatment on the DM content of tomato concentrate

Samples of the untreated and endo-PG treated tomato juice were totally concentrated in Rotadest vacuum evaporators. It was found in the course of our work that the enzyme treated samples could be concentrated better than those of the control (Fig. 7).

The DM content of the tomato concentrate could be increased with the enzyme treatment at every temperature used.

Of the investigated temperatures, the incubation of the crushed tomatoes at 45 °C with endo-PG, resulted in a tomato concentrate of the lowest DM content.

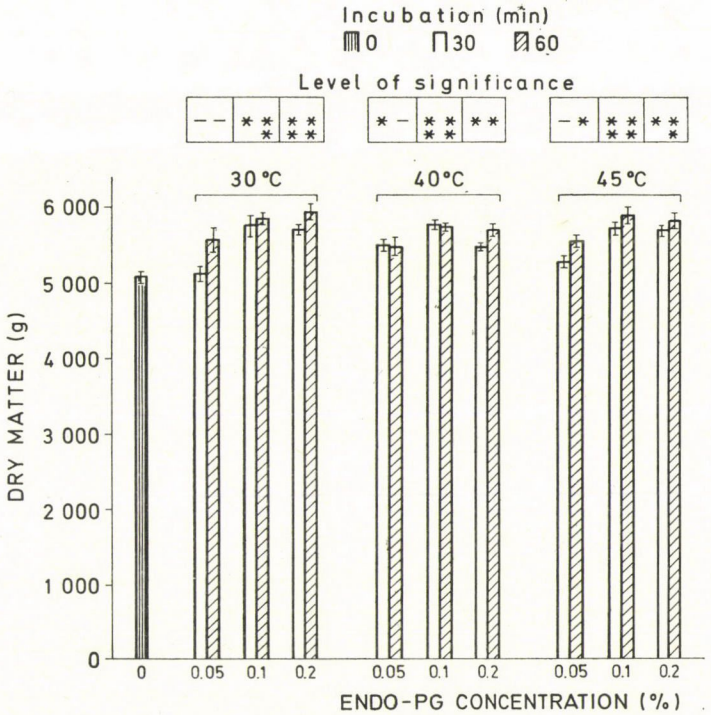

Fig. 8. The effect of the enzyme treatment on the amount of dry matter obtained from a certain amount of tomato as a function of the endo-PG concentration and the time of incubation. Significance of differences between the dry matter contents obtained with and without enzyme treatment

2.8. The effect of endo-PG treatment on the amount of DM obtained in the concentrate

In comparison to the untreated samples, the total DM, obtained from a certain amount of tomato, increased significantly and very highly significantly with the use of 0.1 and 0.2% endo-PG for both 30 and 60 min at all the three temperatures used (Fig. 8).

Related to the untreated samples, the enzyme treatment (with 0.1% endo-PG for 30 min at 30, 40 and 45 °C) resulted in a 13–16% higher amount of DM. The increase of the incubation period to 60 min in most cases increased further the quantity of DM.

2.9. The effect of endo-PG treatment on the consistency of the tomato paste

The consistency of the samples was investigated by the spreading test of the tomato concentrates after diluting them to the same DM content.

It was found that tomato concentrates prepared by enzyme treatment spread significantly, highly significantly and very highly significantly less than those of the untreated ones (Fig. 9).

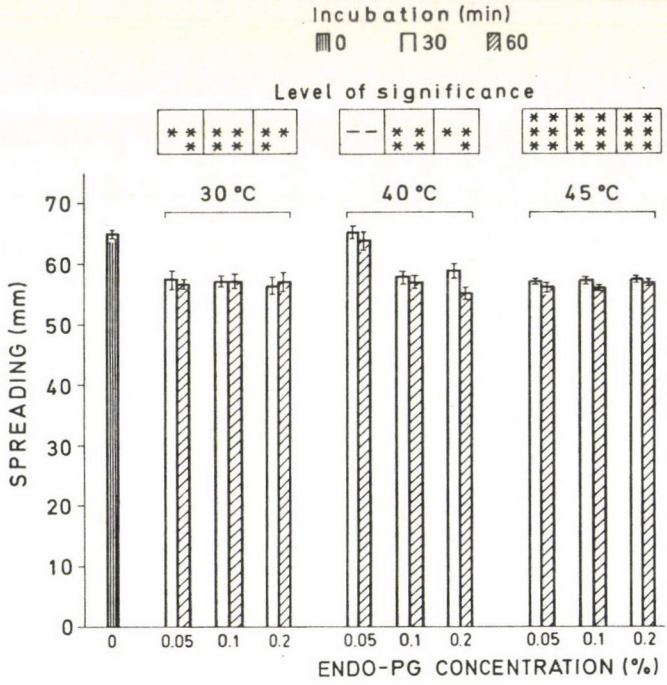

Fig. 9. The effect of enzyme treatment on the consistency of tomato concentrate as a function of the endo-PG concentration and the time of incubation. Significance of differences between the DM contents obtained with and without enzyme treatment

The consistency of the tomato concentrates obtained by different enzyme treatments, did not differ significantly from each other.

2.10. The effect of endo-PG treatment on the particle size of the tomato paste

Tomato concentrates prepared on industrial scale in different parts of the country (Mosonmagyaróvár and Szeged) were tested by sedimentation technique to determine their particle size distribution. Both series of samples originated from a mixture of different tomato varieties.

Samples originated from Mosonmagyaróvár were prepared by warm technology, when crushed tomatoes were treated with 0.1% endo-PG for 30 min at 45 °C (Fig. 10).

After determining the particle size distribution of the different tomato concentrates, it was found that the enzyme treatment increased the frequency of the particles in the range of small sizes (10–40 μm) at the expense of the particles belonging to a range of larger particles (50–100 μm).

The frequency of the particles in the case of warm technology was the highest at a size of 40 μm for both the enzyme treated and the untreated samples. The frequency of the small particles belonging to sizes of 20, 40 and 30 μm, increased significantly and very highly significantly, respectively, compared to those of the untreated samples.

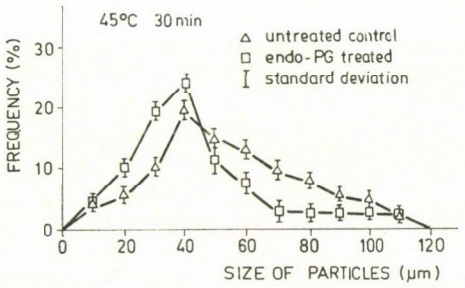

Fig. 10. The effect of enzyme treatment on the particle size distribution of tomato concentrates obtained without and with enzyme treatment (endo-PG 0.1%, 45 °C for 30 min)

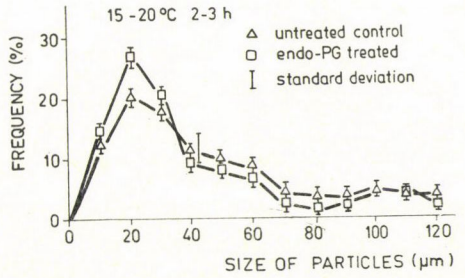

Fig. 11. The effect of enzyme treatment on the particle size distribution of tomato concentrates obtained without and with enzyme treatment (endo-PG 0.1%, 15–20 °C for 2–3 h)

Samples from Szeged were prepared by cold technology, when the enzyme treatment was carried out in tank cars during the transport period of the crushed tomatoes from the field station to the factory (with 0.1% endo-PG for 2–3 h at 15 °C).

When the particle size distribution of the final product (tomato concentrate) was investigated, in the enzyme treated samples the frequency of particles in the range of 10–30 μm was higher (Fig. 11) while in the range of 40–90 μm lower.

The frequency of the 10 and 20 μm particles was significantly and highly significantly higher in the enzyme treated samples than in the untreated ones.

3. Conclusions

It was found in our previous work that the endo-PG treatment of tomatoes (a traditional variety [K3] and a variety bred for mechanical harvesting [Peto Mec]) at the temperatures of 15, 20 and 25 °C had a beneficial effect on tomato processing.

When compared to the control samples the enzyme treatment increased the amount of DM obtainable from a unit quantity of tomato (1–3%), decreased the straining loss (1–3%) and the length of the concentration period (15–25%) as well (ZETELAKI-HORVÁTH & GÁTAI, 1978).

As a result of endo-PG treatment of the crushed tomatoes, the stability of the tomato juice could also be improved (ZETELAKI-HORVÁTH & GÁTAI, 1977). The enzyme treated tomato juice was stable longer than five months while in the untreated juice fibrous particles sedimented in several days.

The improved stability of the tomato juice obtained previously is in agreement with the results of other authors (SULČ & CIRIČ, 1968; PILNIK, 1969; GRAMPP, 1969) and with the results of our sedimentation experiment obtained in this work. Namely the particle size distribution of the enzyme treated samples changed so, that the frequency of the small particles increased considerably at the expense of the larger ones, both at environmental (15 °C) and higher (45 °C) temperatures as well.

In this work the enzyme treatment was carried out at temperatures of 30, 40 and 45 °C in order to minimize the time of incubation. It was found that the increase in the duration of incubation from 30 to 60 minutes caused no significant differences in the values of the investigated parameters in the majority of the experiments.

The increase of the endo-PG concentration from 0.05 to 0.1% decreased significantly the specific viscosity of the juice, while increased highly significantly the juice yield and the DM content of the juice and very highly significantly the amount of DM obtainable in the final product.

Further increase of the endo-PG concentration (from 0.1 to 0.2%) did not prove to be advantageous.

Increase in the temperature of incubation from 30 to 40 °C showed positive effect on the most important parameters, while with a further increase of the temperature to 45 °C no better results could be obtained.

Endo-PG treatment with 0.1% endo-PG at 30–40 °C for 30 min decreased the straining loss by 11–26% and the time of concentration by 12–55%. The shortening of the period of concentration means an increase in the processing capacity and a decrease in the energy consumption, necessary for the production of a unit amount of tomato concentrate.

As a result of the decreased straining loss by endo-PG treatment the amount of DM obtained in the produced tomato paste increased highly significantly (at 40 °C) and significantly (at 30 and 45 °C).

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STUDY OF DIETARY FIBRE CONTENT AND FIBRE COMPONENTS OF CARROTS

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The dietary fibre content of carrot samples was examined using a modification of the Hellendoorn enzymatic method, whereby the pectin content dissolved out in the course of the analysis was determined as the difference between the pectin contents of the original sample and the water-insoluble dietary fibre, and this value was used to correct the value of water-insoluble dietary fibre. Fractionated dissolution and the carbazol colour reaction, followed by extinction measurements were used to determine the pectin content. For the purposes of comparison, the crude fibre content was also determined using the Weende method. The water-insoluble dietary fibre was decomposed into crude hemicellulose and crude lignocellulose components by fractionated decomposition. The quantity of water-insoluble dietary fibre, dietary fibre corrected for dissolved pectin content, and the components were studied in carrots divided into three groups according to mass. The distribution of these components within the carrot samples was also examined.

The mean value of water-insoluble dietary fibre content obtained for carrots of the variety Gonsenheim on a dry matter basis was $24.0 \pm 0.2\%$, the dietary fibre content $30.9 \pm 0.5\%$, the crude hemicellulose content $10.5 \pm 0.1\%$, the crude lignocellulose content $14.5 \pm 0.6\%$ and the pectin content expressed as galacturonic acid $11.0 \pm 0.2\%$.

No correlation could be found between the mass of the carrot samples and the value of dietary fibre just as between the mass of the samples and the quantity of components of the water-insoluble dietary fibre.

A significant difference at the $P = 5\%$ level of probability was found between both the water-insoluble dietary fibre and the corrected dietary fibre values and that of crude hemicellulose when studying the xylem and the tissues of the cortex and phloem separately.

The carrot (*Daucus carota*) is one of the most important vegetables in Hungary. Large quantities of carrots are used both directly in the households and after processing by the preserving and refrigeration industries. It is of particular importance in infant nutrition and hospital diets. DOBSZAY and SÁRKÁNY (1974) recommended carrots for the dietotherapeutic treatment of infants suffering from diarrhoea. EASTWOOD (1978) considers carrots to be the most effective vegetable for the dietary treatment of diverticulosis.

A significant proportion of the dry matter content of carrots is made up of fibre, according to the new conception, dietary fibre, which is the part of the plant which cannot be hydrolysed by the human digestive enzymes, and which consists mainly of the cellulose, hemicellulose, lignin and pectin compounds found in the cell walls. These undergo partial decomposition by

the bacteria in the colon, but during their sojourn in the digestive tract they exert an effect on intestinal activity, and consequently on the metabolism, due to their various physicochemical properties. The most important of these is the water-binding capacity of the hydrophilic polysaccharides, the cation exchange ability of certain acidic polysaccharides and the bile salt adsorption ability of lignin and the pectins (SPILLER et al., 1975). In consequence, the reduction in the transit time is held to be a proven advantage of dietary fibre, resulting in a curative effect on a number of illnesses (constipation, diverticulosis). The beneficial effect of dietary fibre is suspected, though not proved for a number of other diseases (e.g. cancer of the colon, diabetes, haemorrhoida, etc.) (INSTITUTE OF FOOD TECHNOLOGISTS, 1979).

The role of carrots in dietotherapy is associated with the favourable composition of its dietary fibre content, a large proportion of which is pectin, giving it excellent water-binding capacity: a value of 23.7 g water per g fibre was found by ROBERTSON and co-workers (1980a). This is in agreement with the statement by EASTWOOD (1978), who reported that carrot fibre was capable of binding 20–30 times its own mass of water. Carrot fibre also has excellent bile salt adsorption ability, which ROBERTSON and co-workers (1980b) found to depend on the degree of maturity. These authors also found an increase in the ion exchange capacity of carrot fibre parallel with an increasing degree of maturity (ROBERTSON et al., 1980a). Nevertheless, the dietary fibre content of carrot does not depend either on the variety or on the degree of maturity, nor is there any difference in the contents of the main components, cellulose and lignin (ROBERTSON et al., 1979).

The purpose of the present research was to determine whether the dietary fibre content of carrots depends on the size of the carrot, and to discover whether the dietary fibre content of the xylem and phloem differs within the carrot.

1. Materials and methods

1.1. Experimental material

Carrots of the Gonsenheim variety grown at Vecsés were chosen as the model for the experiment, and samples were taken every two weeks in October and November 1980. Some of the examinations were also carried out on carrots of unknown variety bought from retail traders in December 1980.

1.2. Preparation of samples

On each occasion of sampling half of the sample was divided into three groups according to mass (30–50 g, 80–100 g, 150–200 g), while the other half was separated manually into two parts, so that the xylem and the phloem,

including the cortex, could be studied separately. The separated groups of samples were prepared for analysis by homogenisation for 5 minutes in a Blender-type mixer.

The dry matter content of the samples was determined by drying to constant weight at 105 ± 2 °C.

For the purposes of comparison, the crude fibre content of the samples was also determined using the Weende method.

1.3. Determination of dietary fibre and its components

The enzymatic method elaborated by HELLENDOORN and co-workers (1975) was employed for the determination of dietary fibre. However, since the water-soluble compounds are dissolved out in the course of this treatment, the quantity of water-insoluble dietary fibre ("indigestible residue") obtained as the result of enzymatic decomposition was corrected with the amount of pectin dissolved out during the determination (HORVÁTH-MOSONYI et al., 1981).

The water-insoluble dietary fibre was separated into crude hemicellulose and crude lignocellulose fractions using the method proposed by ELCHAZLY and THOMAS (1976), taking all the polysaccharides which can be hydrolysed by 5% (v/v) H_2SO_4 as crude hemicellulose. The remaining crude lignocellulose contained cellulose, lignin and insoluble mineral substances. Further separation of this fraction was not carried out, since relevant literature, without exception, gives the lignin content of carrot as "trace" (max. 2% on a dry matter basis), in addition to which the determination of lignin is considered to be the least reliable and least accurate step in the analysis (ROBERTSON et al., 1979; SOUTHGATE, 1978; THEANDER & AMAN, 1979; VAN SOEST, 1963). Our measurements showed that the acid-insoluble mineral content of the crude lignocellulose fraction did not exceed 0.4% of dry matter, so this also was ignored in later stages.

The dietary fibre content of carrot was compared to the crude fibre content.

1.3.1. Determination of water-insoluble dietary fibre ("indigestible residue"). 50 g homogenized sample was mixed with 100 mg pepsin dissolved in 50 cm^3 0.2 mol per dm^3 HCl (activity: 2500 units per mg according to Anson) and the mixture was incubated at 40 °C for 18 hours, then neutralised with 4 mol per dm^3 NaOH. 50 cm^3 pH 6.8 phosphate buffer, in which 100 mg pancreatin (with a decomposing strength 4 times that of trypsin and 6 times that of amylase) and 300 mg sodium dodecyl-sulphate had previously been dissolved, was then added and the mixture was kept at 40 °C for an hour, after which the pH was adjusted to 4.5 with 4 mol per dm^3 HCl. It was then centrifuged at 3000 rpm for 30 min. The residue was washed with water and acetone, then suspended in 100 cm^3 acetone, filtered through a glass filter and

washed with ethanol. The ethanol was evaporated at room temperature and the water-insoluble indigestible residue was measured gravimetrically after drying at 105±2 °C for 5 hours and cooling.

1.3.2. Determination of the components of water-insoluble dietary fibre. The indigestible residue was suspended in 10 cm^3 5% (v/v) H_2SO_4, then kept over a boiling water bath for 2.5 hours after the addition of a further 10 cm^3 5% (v/v) H_2SO_4. The water evaporated was replaced several times. After cooling to room temperature it was mixed with 30 cm^3 ethanol and left to stand for 10 minutes before filtering through a glass filter. The residue was washed free of acid with 50% ethanol, then washed in acetone and ether, and left to stand until the ether had evaporated. It was then dried in a 100 °C drying cabinet for 2 hours, left to cool and weighed. The weight loss represented the crude hemicellulose content and the residue the crude lignocellulose content.

The course of the analysis is illustrated in Fig. 1.

1.3.3. Determination of the quantity of pectin dissolved out. In order to determine the amount of pectin compounds dissolved out in the course of enzymatic digestion, the pectin content of the original material and that of the indigestible residue remaining after the enzymatic decomposition were determined, after which the water-insoluble dietary fibre value was corrected with the difference in the two results.

The soluble sugars which would disturb the evaluation were removed from the experimental material by extraction with 85% ethanol, after which

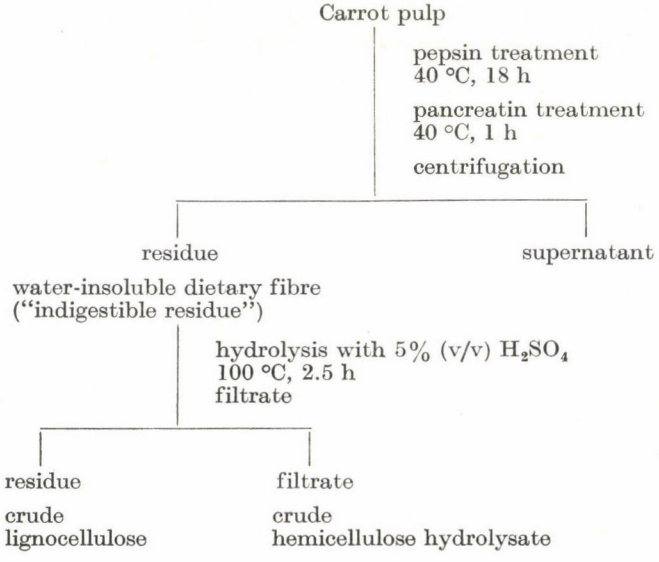

Fig. 1. Course of determination of water-insoluble dietary fibre content and separation of the components

fractions containing water-soluble pectin compounds, oxalate-soluble pectates and pectinates, and alkali-soluble protopectins were prepared by fractionated dissolution (ROUSE & ATKINS, 1955; CENTRAL FOOD RESEARCH INSTITUTE, 1973). The determinations were carried out on these fractions using the carbazol colour reaction and extinction measurements at 530 nm after McCOMB and McCREADY (1952).

The determination of pectin was carried out as follows: 10 g experimental material was boiled for 20 minutes with 85% ethanol under a reflux condenser, and then it was cooled and filtered. The extraction was then repeated. The alcohol-insoluble residue was dried in the air and ground to a powder. 0.5 g of the air-dry powder was shaken for 30 minutes in 40 cm^3 water, then centrifuged at 3000 rpm for 20 minutes. The supernatant contained the water-soluble pectin compounds. The residue was shaken with 20 cm^3 0.75% ammonium oxalate solution for 30 min and centrifuged. The supernatant contained the pectates and pectinates. The supernatant obtained after shaking the residue with 20 cm^3 0.1 mol per dm^3 NaOH for 30 minutes and centrifugation contained the protopectins.

One cm^3 of each fraction was added to 6 cm^3 portions of cooled cc. H_2SO_4, boiled for 10 min on a water bath, cooled and brought into reaction with 0.5 cm^3 0.15% carbazol solution (dissolved in 95% ethanol). After standing for 15 min the extinction of the solution was measured at 530 nm.

The calibration curve was prepared from galacturonic acid monohydrate in the manner described above.

2. Results

The results of analyses on carrot samples divided according to mass and those separated into two parts (xylem and cortex + phloem) are illustrated in the following tables and figures. All the values are given as a % of dry matter, together with the standard deviations.

A total of 6 analyses were carried out on carrots separated into two parts.

Samples of Gonsenheim carrots taken every two weeks in October and November 1980 are marked 1, 2, 3 and 4, while samples of carrots of unidentified varieties bought from retail traders in December 1980 are marked 5 and 6.

For the examinations according to mass, samples were taken from Gonsenheim carrots on a total of four occasions, twice in October and November 1980.

The mean values, each obtained from an $n = 5$ series for the analysis of the dietary fibre content, dietary fibre components and crude fibre con-

tent of carrot samples of the Gonsenheim variety, taken at the same time, are contained in Table 1, together with the standard deviations.

Mean values from 3 parallel measurements on each of 4 samples of different ages for the dietary fibre corrected with the quantity of dissolved

Table 1

Analytical data of dietary fibre and crude fibre content of carrots
Mean and standard deviation values of five parallel measurements as a % of dry matter

Component analysed	%
Water-insoluble dietary fibre	24.0±0.2
Crude hemicellulose	10.5±0.1
Crude lignocellulose	14.5±0.6
Pectin content of the sample	11.1±0.2
Pectin content of the water-insoluble dietary fibre	5.5±0.2
Pectin dissolved out	5.6±0.2
Corrected dietary fibre content (indigestible residue + dissolved pectin)	30.9±0.3
Crude fibre	9.4±0.2

Table 2

Analytical data of fibre in Gonsenheim carrots of different weights
Mean and standard deviation values of three parallel analyses carried out on samples taken every two weeks on a total of four occasions, as a % of dry matter

Mass of sample (g)	Water-insoluble dietary fibre (%)	Pectin content of sample (%)	Pectin content of water-insoluble dietary fibre (%)	Pectin dissolved out (%)	Corrected dietary fibre (%)	Crude fibre (%)
30– 50	25.3±0.4	10.8±0.5	5.3±0.2	5.5±0.5	30.8±0.8	10.0±0.6
80–100	25.0±0.3	11.6±0.4	5.5±0.2	6.1±0.5	31.1±0.6	8.9±0.4
180–200	25.3±0.4	10.7±0.4	5.2±0.2	5.5±0.4	30.8±0.6	8.5±0.4

Table 3

Analytical data for samples from various parts of Gonsenheim carrots
Mean and standard deviation values for three parallel analyses on samples taken every two weeks on a total of four occasions, as a % of dry matter
A = cortex + phloem, B = xylem

Sample	Water-insoluble dietary fibre (%)	Pectin content of sample (%)	Pectin content of water-insoluble dietary fibre (%)	Pectin dissolved out (%)	Corrected dietary fibre (%)	Crude fibre (%)
A	25.0±0.7	11.3±0.2	5.3±0.2	6.0±0.3	31.0±0.7	9.4±0.3
B	27.8±0.3	12.4±0.6	5.9±0.2	6.5±0.6	34.3±0.6	9.2±0.1

pectin, and for the crude fibre content of Gonsenheim carrot samples divided into three groups according to mass are given in Table 2.

Mean values for the dietary fibre content corrected with the amount of dissolved pectin, and for the crude fibre content of samples taken from the xylem and the cortex + phloem of Gonsenheim carrots are contained in Table 3.

The crude fibre, water-insoluble dietary fibre, crude hemicellulose and crude lignocellulose contents of carrot samples taken at different times and separated according to mass are summarized in Table 4.

The crude fibre, water-insoluble dietary fibre, crude hemicellulose and crude lignocellulose contents of carrot samples of the variety Gonsenheim and of an unidentified variety separated into two parts and examined at different times are presented in Table 5.

The data given in Tables 4 and 5 are each the means of 3 parallel analyses, together with the relevant standard deviations.

The data in Table 4 showed no definable tendency, but from those in Table 5 it could be observed that the data obtained from the analysis of the xylem (B) were always greater in value than those obtained from the analysis of the cortex + phloem (A) taken from the same sample. Consequently, these

Table 4

Water-insoluble dietary fibre components and crude fibre content of Gonsenheim carrots of various masses

Mean and standard deviation values for three parallel analyses as a % of dry matter

	Mass of sample (g)	Crude fibre (%)	Water-insoluble dietary fibre (%)	Crude hemicellulose (%)	Crude lignocellulose (%)
1	30– 50	8.3±0.4	25.2±0.4	9.2±0.2	16.0±0.6
	80–100	7.6±0.2	25.8±0.5	11.0±0.1	14.8±0.8
	180–200	7.0±0.6	23.8±0.3	9.5±0.2	14.3±0.8
2	30– 50	13.0±0.3	25.7±0.6	10.9±0.3	14.8±0.5
	80–100	13.6±0.3	25.5±0.2	9.8±0.2	15.7±0.7
	180–200	14.9±0.4	25.1±0.6	10.0±0.2	15.1±0.5
3	30– 50	9.2±0.4	26.5±0.2	9.9±0.1	16.6±0.8
	80–100	8.7±0.3	23.6±0.1	8.3±0.1	15.3±0.6
	180–200	8.6±0.1	27.0±0.3	11.9±0.2	15.1±0.5
4	30– 50	9.4±0.5	23.6±0.2	11.1±0.3	12.5±0.7
	80–100	10.5±0.2	25.1±0.1	12.3±0.3	12.8±0.6
	180–200	10.0±0.3	25.3±0.2	12.3±0.3	13.0±0.6

Table 5

Water-insoluble dietary fibre components and crude fibre content of samples prepared from various parts of the carrot
Samples 1—4 were taken from Gonsenheim carrots at various times, while samples 5 and 6 are from an unknown variety.
A = cortex + phloem, B = xylem
Mean and standard deviation values of three parallel analyses as a % of dry matter

Sample		Crude fibre (%)	Water-insoluble dietary fibre (%)	Crude hemicellulose (%)	Crude lignocellulose (%)
1	A	5.9±0.2	22.2±0.3	9.6±0.2	12.6±0.6
	B	9.7±0.2	31.8±0.4	12.8±0.2	19.0±0.5
2	A	6.1±0.3	22.3±0.3	8.4±0.1	13.9±0.5
	B	9.3±0.2	24.0±0.1	9.9±0.2	14.1±0.5
3	A	10.4±0.4	27.2±0.2	8.0±0.1	19.2±0.7
	B	12.7±0.3	28.8±0.3	8.8±0.1	20.0±0.6
4	A	8.3±0.3	26.7±0.2	13.1±0.1	13.6±0.8
	B	9.2±0.3	28.1±0.2	13.8±0.1	14.3±0.7
5	A	8.8±0.2	43.5±0.4	24.9±0.3	18.6±0.5
	B	9.7±0.1	50.6±0.5	30.6±0.2	20.0±0.9
6	A	14.8±0.3	41.0±0.2	28.1±0.1	12.9±0.6
	B	16.1±0.1	50.9±0.3	34.8±0.2	16.1±0.2

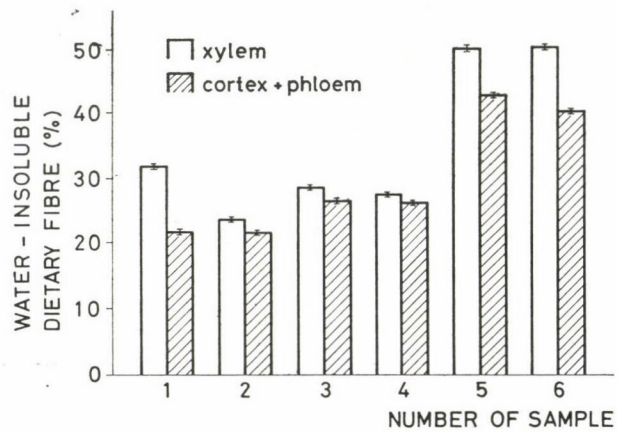

Fig. 2. Water-insoluble dietary fibre content of the xylem and the cortex + phloem of four carrot samples of known variety and two of unknown variety, as a % of dry matter, together with the standard deviation. Each column represents the mean of three parallel analyses

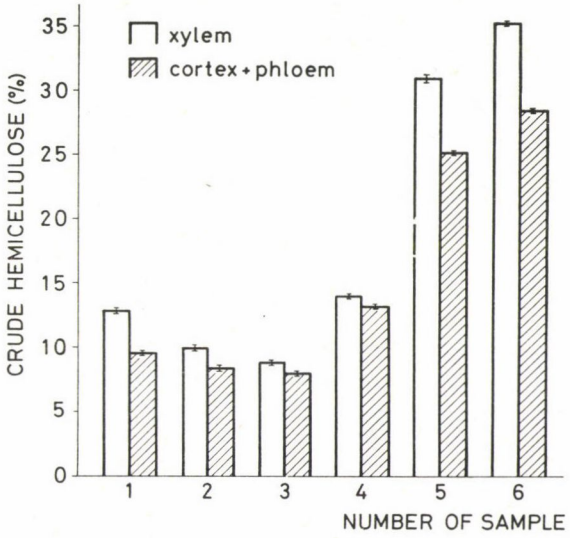

Fig. 3. Crude hemicellulose content of the xylem and cortex + phloem of four carrot samples of known variety and two of unknown variety, as a % of dry matter, together with the standard deviation. Each column represents the mean of three parallel analyses

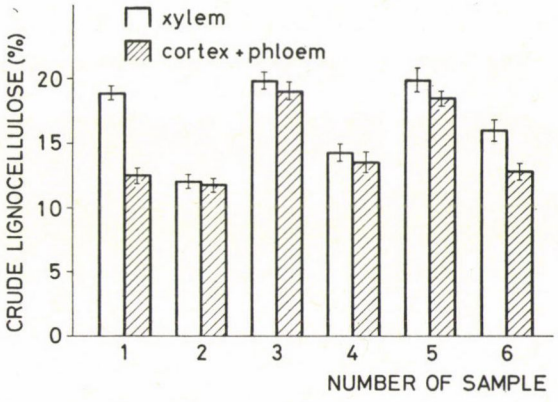

Fig. 4. Crude lignocellulose content of the xylem and cortex + phloem of four carrot samples of known variety and two of unknown variety, as a % of dry matter, together with the standard deviation. Each column represents the mean of three parallel analyses

values were subjected to separate analysis of significance and illustrated in column diagrams.

The water-insoluble dietary fibre content, the crude hemicellulose content and the crude lignocellulose content of the xylem and cortex + phloem of carrot samples 1, 2, 3, 4, 5 and 6 are depicted in Figs. 2, 3 and 4, respectively.

The pectin content of the xylem and cortex + phloem of carrot samples 1, 2, 3, 4, 5 and 6 is illustrated in Fig. 5, expressed as galacturonic acid.

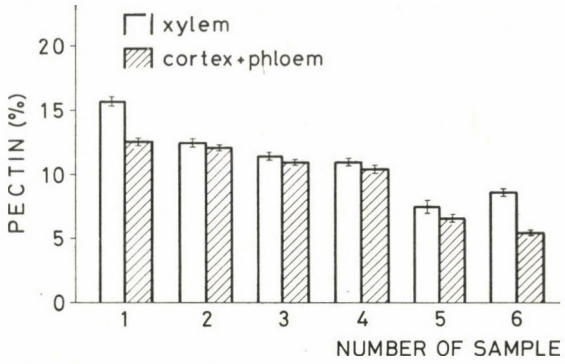

Fig. 5. Total pectin content of the xylem and cortex + phloem of four carrot samples of known variety and two of unknown variety, expressed as galacturonic acid, as a % of dry matter, together with the standard deviation. Each column represents the mean of three parallel analyses

3. Conclusions

It was found from the analyses that the dietary fibre content of carrot was always several times greater than the crude fibre content, and that the water-insoluble dietary fibre content of samples prepared from the cortex + phloem of the carrots was lower at the P = 5% level of probability than that in samples prepared from the xylem. When the water-insoluble dietary fibre of each sample was decomposed into its crude hemicellulose and crude lignocellulose components, higher values were obtained in all cases for the xylem, though the difference was only significant, in all samples at the P = 5% level of probability, for the crude hemicellulose content. Significant differences for the crude lignocellulose and pectin contents were only found in two cases, but the tendency was observable here, too. The data of the t-test carried out to determine the significance are summarized in Table 6.

Table 6

Student's t-test for the comparison of different parts of the carrot
The difference between the amount in the cortex + phloem and that in the xylem formed the basis for comparison
($n = 3$, $t_{tabl.} = 4.3$)

Component analysed	$t_{calc.}$					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Water-insoluble dietary fibre	19.2	5.4	4.4	5.0	11.1	27.4
Crude hemicellulose	11.3	6.7	5.7	5.0	15.8	29.9
Crude lignocellulose	8.2	0.3	0.9	0.7	1.0	5.0
Pectin	10.6	1.4	1.8	2.1	3.4	11.1

Thus, the distribution of dietary fibre was not found to be uniform in the carrot samples examined; the xylem contains significantly more dietary fibre than the rest of the carrot. This difference was probably the consequence of differences in the hemicellulose content. In all cases there was found to be a tendency for all the dietary fibre components studied to be present in larger quantities in the xylem. Examinations of a larger number of samples from different varieties of carrots are needed to determine unambiguously whether the difference is also significant for the pectin and crude lignocellulose values.

In carrot samples of the same variety, stored for the same length of time, but of different mass or size, no difference could be demonstrated in the content of dietary fibre or of its components.

The value obtained for the water-insoluble dietary content of carrots are in good agreement with the values published by SOUTHGATE (1978), SCHWEIZER and WÜRSCH (1979) and ANDERSON and CLYDESDALE (1980) for raw carrots.

It seems probable on the basis of these examinations that the xylem and the cortex + phloem tissue of carrots are not entirely equivalent from a nutritional point of view, since the data show the xylem to contain less β-carotene, but more dietary fibre. For carrots of the same variety and age, the mass or size of the carrot does not influence the dietary fibre content.

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EFFICIENCY OF DIFFERENT MUTAGENIC TREATMENTS AND THE PROTOPLAST FUSION TECHNIQUE IN PRODUCING METHIONINE-RICH MUTANTS FROM YEASTS

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Four industrially important yeast strains (*Candida guilliermondii*, *Saccharomyces lactis*, *Kluyveromyces lactis*, *Kluyveromyces fragilis* and *Kluyveromyces marxianus*) were treated with UV light, gamma radiation and nitrous oxide, aiming at producing methionine rich mutants. UV treatment proved to be highly efficient in this regard and stable mutants with methionine content of up to 80% higher than that of the parent strain, *Kluyveromyces fragilis* 1068, were produced. Of the strains tested *Kluyveromyces fragilis* 1068 gave the best results with UV treatment.

The protoplast fusion of auxotrophic mutants of *Saccharomyces lactis* and *Kluyveromyces lactis* were also tried for the same purpose.

Hybrids of *Saccharomyces lactis* with significantly higher methionine content than that of the parent strains were also obtained. *Kluyveromyces lactis* hybrids proved better than *Saccharomyces lactis*, as in addition to the high methionine content, their protein content also increased. With *Saccharomyces lactis*, increases in methionine content were accompanied by decrease in protein content.

Among the novel food sources presently being developed and studied, single-cell protein from yeasts holds a prominent role (CHEM & PEPPLER, 1977). In fact yeasts are highly efficient producers of protein from different carbon sources, they show an elevated level of protein content ranging from 38.0 to 70% of dry weight (FAO, 1970) and have a reasonably high lysine content as well as sufficient amounts of threonine and tryptophane. But a common characteristic of the most important yeast proteins is their deficiency in methionine and cysteine (ANN & MILLER, 1979).

The importance of methionine rests with its role in the transamination reaction as a CH_3-donor and sulphur source in protein synthesis.

Methionine deficiency also causes the presence of B_6 antivitamin (CORNICELLI, 1978). Therefore an adequate methionine quantity *in vivo* is necessary for the conversion in the complex of vitamins B. Although the addition of synthetic amino acids, missing from a protein, improves the biological value of the latter (WAWRZYCKA, 1979), but it was found (TREVIS, 1979) that in monogastric animals (i.e. pigs) nutrients enriched with yeast protein gave better results than nutrients fortified with synthetic free amino acids. Therefore it is believed that the need exists for proteins rich in limiting amino acids (like methionine) in a bound form in the protein molecule rather than in their free forms.

In this work, it was attempted to produce yeast with higher methionine content. The methionine content was measured as its ratio in the total dried yeast biomass. Protein and nucleic acid contents were taken into consideration, too.

1. Materials and methods

1.1. Yeast strains used

Candida guilliermondii 812	Bratislava CCY 51-1-2	From the Czechoslovakian collection
Kluyveromyces fragilis 732	Bratislava CCY 21-40-1	
Kluyveromyces marxianus 857		
Kluyveromyces fragilis 1068		
Saccharomyces lactis NCYC 671		
Saccharomyces lactis NIACY 290		
Kluyveromyces lactis 1223 VM37	His.-mutant	From the collection of Szeged
Kluyveromyces lactis 1224 Cap 2	His.-mutant	
Kluyveromyces lactis 1225 K9	Met.-mutant	
Kluyveromyces lactis 1226 K9-01	Met.-mutant	

1.2. Culture media

Components:	Concentration (g dm^{-3})
1.2.1. Glucose yeast extract medium	
Glucose	10
Yeast extract	5
Agar	20
1.2.2. Sulphur-rich medium	
$MgSO_4$	0.10
KH_2PO_4	0.14
$Na_2HPO_4 \cdot 12H_2O$	0.80
NaCl	1.00
$(NH_4)_2SO_4$	4.00
Glucose	20.00
Yeast extract	5.00
Agar	30.00
1.2.3. Sulphur-deficient medium	
Urea	1.93
$MgSO_4$	0.0025

KH_2PO_4	0.14
$Na_2HPO_4 \cdot 12H_2O$	0.80
NaCl	1.00
Glucose	20.00
Agar	30.00

1.2.4. Selective minimal medium

$(NH_4)_2SO_4$	5.00
KH_2PO_4	1.00
$MgSO_4$	0.5
Glucose	10.00
Agar	30.00
Wickerham's vitamin sol.	1.00

1.2.5. Osmotically stabilized mineral

Medium OSM

$(NH_4)_2SO_4$	5.00
KH_2PO_4	1.00
$MgSO_4 \cdot 7H_2O$	0.5
Glucose	10.00
Wickerham's vitamin sol.	1.00
Agar	15.00

1.2.6. Osmotically stabilized yeast extract

Medium OSY (Oxoid)

Glucose	10.0
KCl	0.6 mol
Agar	20.0

1.2.7. Osmotically stabilized agar

Medium OSA

Agar	1.5
$CaCl_2$	0.3 mol

1.2.8. Synthetic broth

as in 1.2.2. except that no agar was added

1.2.9. Pretreatment solution (A)

Hydroxy-methylaminomethane (IRIS)	0.1 mol
Mercaptoethanol	1.0
EDTA	5.0 mmol

1.2.10. Medium for Leuconostoc mesenteroides

after ANTOMISSKI

1.3. Mutagenic methods

1.3.1. UV treatment. A suspension from a 26-h culture of the strain to be tested was prepared. Cell concentration was determined with a haemacytometer. 0.1 cm^3 quantities of the dilution containing 10^2, 10^3, 10^4, 10^5 and 10^6 cells per cm^3, respectively, were spread over the surfaces of pre-poured, solidified yeast extract media in Petri dishes.

The inoculated Petri dishes were then exposed to UV light (from a Tungsram, Germicide type lamp of 15 W) at 30 cm distance, for 0–300 s at 30-s intervals so that Petri dishes with higher cell concentrations were exposed to UV for longer periods. The Petri dishes were immediately placed in the dark until transferred to incubators at 30 °C for 72 h. After the incubation period, the colonies were counted, checked by replica plate method on minimal, sulphur-rich and sulphur-deficient media. Colonies which failed to grow on the minimal medium were isolated as auxotrophic mutants and were further tested for the missing amino acid and for their stability, while the size of the colonies growing on sulphur-rich and sulphur-deficient media were compared. Colonies growing better on the sulphur-rich medium were preliminarily isolated for further examination for their methionine content. Colonies isolated were maintained on yeast extract agar slants until fully examined.

1.3.2. Gamma irradiation. Suspensions of cell concentrations of 10^2, 10^3, 10^4, 10^5, 10^6 and 10^7 cm^{-3} were prepared as described in 1.3.1. Five cm^3 quantities from each dilution were transferred into presterilized glass ampoules in duplicate and were heat-sealed. The ampoules were irradiated in a ^{60}Co (89 kGy h^{-1}) instrument for 3.5, 7, 10.5, 14 and 17.5 min corresponding to radiation doses of 0.5, 1, 1.5, 2 and 2.5 kGy, respectively.

Ampoules of higher concentration were exposed to higher doses so that the survivors were countable when plated. Cells were then treated in the same manner as described in 1.3.1.

1.3.3. $NaNO_2$ treatment. This method was carried out as follows:

1. A Petri dish with pre-poured, solidified yeast extract medium was streaked with yeast to be treated and incubated at 30 °C for 24 h.

2. From the 24-h culture, a loopful was inoculated into a conical flask with synthetic broth of pH 4.5. The culture was shaken for 24 h at 30 °C.

3. The cells were centrifuged, then washed twice with 0.9% NaCl solution and centrifuged.

4. The cells were suspended in 0.9% solution of NaCl shaken for 2 h.

5. Amounts of 5 cm^3 were transferred in a series of 7 conical flasks. Five cm^3 of the $NaNO_2$ solution and 5 cm^3 of acetate buffer were added to each flask. Phosphate buffer was added to the first flask after 10 minutes, to the second after 20, 30, 60, 90 and 120 minutes, respectively.

The addition of phosphate buffer inhibits the action of $NaNO_2$. Therefore the actual $NaNO_2$ treatment time is that between the addition of $NaNO_2$ and the phosphate buffer. Then 10 cm^3 were centrifuged and cells were washed with a 0.9% NaCl solution.

6. Five cm^3 of the original suspension were diluted with distilled water to obtain the cell concentration of the treated samples and were also centrifuged.

7. Cells from each treatment and the control were suspended in 5 cm^3 synthetic broth and incubated at 30 °C for 72 h.

8. Viable cells were counted on plates of yeast extract medium and were further tested as in sulphur-rich and deficient media.

Only methionine-rich colonies were investigated, no investigation was carried out for auxotrophic mutants.

1.4. Protoplast fusion technique

Cells were propagated by the shaken culture method at 30 °C for about 10 h. Growth was stopped at the exponential growth phase which was necessary for producing high yield fusions. Cells were separated by centrifugation, the supernatant was discarded and precipitated cells were pretreated with a solution A (as in 1.2.9) in order to enhance the cell wall digestion. Cells were suspended in this solution for 14 min and were then centrifuged.

A fresh solution of 0.6 mol KCl and 1% snail enzyme was prepared. The solution was filtered using a filter paper of 0.22 nm porosity. The centrifuged cells were then mixed with 10 cm^3 of the snail enzyme solution.

The freeze-dried enzyme of *Helix pomatia* was used to digest the cell walls (FERENCZY et al., 1976). Holding the suspension at a constant temperature of 30 °C for 30–40 min caused 100% protoplast formation from the yeast cells. The formation of the protoplast and the digestion of the cell wall was followed up by microscopic examination.

Protoplasts were then separated from the enzyme solution by centrifugation and washed with 0.3 mol $CaCl_2$ solution. A dilution of a cell density of approximately 5×10^7 cm^{-3} was prepared, using the Bürker chamber from each parent strain.Then equal quantities of both dilutions were mixed together with 30% polyethylene glycol (PEG) Mw 4 000 and 0.6% $CaCl_2$ solution. The mixture was left to stand for 10 min. During this period cell aggregation was followed by microscopic examination. Dilutions of 10^2, 10^3 were prepared and 0.2 cm^3 of each was plated on 20 cm^3 pre-poured solidified OSY media in Petri dishes at 42–44 °C. Similarly, Petri dishes of OSY and OSM media were also inoculated, all incubated at 30 °C for 72 h, then examined and counted.

1.5. Determination of methionine content

1.5.1. Biological assay method. Thirty mg of dried yeast biomass was digested in sealed ampoules with 4 cm^3 of 6 *N* HCl, for 24 h at 105 °C. The acid was evaporated at 40 °C in an oven under vacuum for 24 h, the residue was then dissolved in distilled water and filtered. Methionine content was determined according to BARTON-WRIGHT (1972) by using *Leuconostoc mesenteroides* P50 auxotroph as a test organism.

1.5.2. Thin-layer chromatography. The acid hydrolysed yeast as in 1.4.1 was run on a thin-layer of silica-gel with a solvent of n-butanol–acetic acid–water (80 : 20 : 20). The chromatograms were finally checked by a video-densitometer (DÉVÉNYI, 1977).

1.6. Determination of total amino acid content

Yeast hydrolysed similarly as in 1.5.2 was used in an MIM (Hungary) Aminochrom type automatic amino acid analyzer.

1.7. Determination of the nucleic acid content

Twenty four mg of sand-ground dried yeast were heated for 20 min at 100 °C with 40 cm^3 of perchloric acid, the optical density of the centrifuged supernatant was then measured at 270 nm using Specord type photometer.

1.8. Statistical evaluation

All data presented are the mean of three replicates. The significance of differences was calculated by Student's method from which the significance was determined from the appropriate tables.

One asterisk (*) indicates a significant difference at a probability level of $P \geq 95\%$ while two asterisks (**) indicate that the difference was highly significant at a probability level of $P \geq 99\%$.

2. Results

2.1. Treatment with UV light

Candida guilliermondii 812 was treated in 32 cases with UV light. From the 2 080 colonies tested, 14 auxotrophic mutants were isolated. From these only one *Arg*$^-$ and one *Lys*$^-$ mutant were successfully maintained, the rest back-mutated after 1–3 weeks. From the total number of colonies tested on sulphur-deficient media, 25 colonies showed bigger sizes on the sulphur-rich

Table 1

Methionine, protein and nucleic acid content of 4 UV treated Candida guilliermondii 812 *strains compared to the parent strain*

UV treatment (S)	Methionine (% dry mass)	Total protein (% dry mass)	Nucleic acid (% dry mass)
	$\overline{X}_1$	$\overline{X}_2$	$\overline{X}_3$
Parent (70)	0.70±0.07	59.30±3.0	10.8±1.0
120	0.83±0.08*	41.20±2.1*	6.2±0.6*
180	0.80±0.08	52.00±2.5	5.6±0.6*
240	0.85±0.09*	43.00±2.1*	6.3±0.6*
300	0.88±0.09*	39.00±2.0*	7.3±0.7

* Significantly different from the value obtained in the parent at $P \geq 95\%$
$\overline{X}_1$, $\overline{X}_2$ and $\overline{X}_3$ are the means of 5 parallels with ± standard deviations

media. These were checked for their methionine content and only four of them were found to have a methionine content higher than that of the parent strain (Table 1).

Kluyveromyces fragilis 732 and 1068 and *Kluyveromyces marxianus* 857 were also treated with UV light. Individuals were selected according to their methionine content. *Kluyveromyces fragilis* 732 and *Kluyveromyces marxianus* 857 treated for 120 s showed the highest sulphur requirements. The slight increase in methionine content of *Kluyveromyces marxianus* 857 was impaired by a 30% increase in its nucleic acid content. While one colony from *Kluyveromyces fragilis* 1068 showed 80% increase in its methionine content compared to the parent strains, the nucleic acid content was not changed. Although *Kluyveromyces fragilis* 732 showed high frequency of colonies growing better on sulphur-rich media, no increase in their methionine content was noticed. Three auxotrophic mutants were also obtained, two from *Kluyveromyces fragilis* 732 and one from the 1 068, but all had reverted when examined after one week.

2.2. Gamma irradiation

Candida guilliermondii 812 and *S. lactis* 571 and 290 were treated with gamma radiation. From 1210 colonies of *Candida guilliermondii* and from 270 colonies of *Saccharomyces lactis* examined, neither methionine-rich nor auxotrophic mutants were obtained.

2.3. $NaNO_2$ treatment

Using this method neither auxotrophic nor methionine-rich mutants could be obtained out of 1400 colonies tested.

2.4. Protoplast fusion results

2.4.1. Protoplast fusion between auxotrophic markers of *Saccharomyces lactis* 571 and 290.

Selected colonies showed two fusion hybrids with a methionine content 70–75% higher than that of the parent strains, but their protein content dropped by 24–30%. Biotin content was nearly doubled in one of the fusions (Table 2).

2.4.2. Protoplast fusion of *Kluyveromyces lactis* auxotrophic markers.

Three mutants were used: 1223 His$^-$, 1224 His$^-$ and 1226 Met$^-$.

The fusion of 1223 + 1226 resulted in the selection of four colonies numbered 4, 11, 12 and 15 which showed a methionine content higher than that of the parents (Table 2). No significant change was noticed in the nucleic acid content.

The other fusion of 1226 + 1224 produced only one colony which was slightly higher in methionine content than that of the parents.

The protein content of all selected fusion hybrids was higher than that of the parents but they kept a nucleic acid content close to that of their parents (Table 2).

Table 2

Comparison of the main components of the parent strains with selected fusion hybrids

Yeast strain		Methionine (% dry mass)	Protein (% dry mass)	Nucleic acid (% dry mass)	Biotin (% dry mass)
Saccharomyces lactis NCYC 571		0.25	58.6	7.3	0.06
Saccharomyces lactis NIACY 290		0.29	57.0	7.3	0.07
Fusion hybrids:					
290 + 571 (1)		0.44*	43.3**	8.8	0.13
290 + 571 (2)		0.49*	40.1**	6.2	0.08
Kluyveromyces lactis	1223 His$^-$	0.52	41.0**	6.2	—
	1224 His$^-$	0.55	45.1**	5.2	—
	1226 Met$^-$	0.60	48.0*	7.1	—
Fusion hybrids					
1226 + 1223 (4)		0.61*	49.6*	6.5	—
(11)		0.95**	53.8	8.8	—
(12)		0.97**	54.7	6.7	—
(15)		0.67*	55.6	6.1	—
1226 + 1224 (2)		0.61	53.2	6.2	—

* Significantly different from the value of the parent strains at $P \geq 95\%$
** Highly significantly different from the value of the parent strains at $P \geq 99\%$

Table 3

Amino acid composition of 4 fusion hybrids compared to that of the parent strain

Amino acid	Parent strain	Fusion hybrids in percentage of dry mass[a]			
	1226	4	11	12	15
Lysine	3.36	3.61	4.47	4.67	3.9
Histidine	0.35	0.98	1.07	1.33	1.1
Arginine	2.37	2.16	2.39	2.66	2.21
Asparagine	5.2	3.87	3.7	4.65	4.59
Threonine	2.47	1.95	1.85	2.31	2.36
Serine	1.8	1.72	2.01	1.83	2.09
Glutamine	6.9	5.35	5.37	6.49	6.25
Proline	1.81	1.21	1.85	1.47	1.55
Glycine	2.38	1.85	1.53	1.92	1.97
Alanine	2.74	2.04	2.07	2.19	2.14
Valine	3.2	2.40	2.21	2.8	2.68
Methionine	0.6	0.61	0.95**	0.97**	0.67*
Isoleucine	3.31	1.91	1.61	2.27	2.22
Leucine	5.45	2.76	1.65	3.09	3.26
Tyrosine	2.8	0.99	0.93	0.97	1.18
Phenylalanine	1.86	1.7	1.63	2.01	1.96

[a] The data are the mean of three parallels
* Significantly different from the value of the parent strain at $P \geq 95\%$
** Highly significantly different from the value of the parent strain at $P \geq 99\%$

The total amino acid determination of the four fusions (Table 3) showed that the lysine content increased in three of them. Histidine increased very significantly in all fusions with a noticeable decrease in leucine and isoleucine.

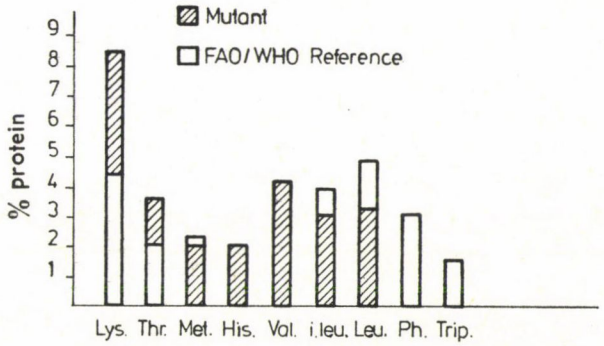

Fig. 1. Comparison of the amino acid content of fuson hybrid (11) with the FAO/WHO reference volume

In the case of the hybrid No. "11", the value of leucine and isoleucine dropped below the level recommended by FAO/WHO, but still the methionine content was well above the FAO/WHO reference value (Fig. 1).

3. Conclusions

In comparing the efficiency of the different methods and techniques in producing yeast with higher methionine and protein contents, the results are as follows:

3.1. UV treatment

Out of 2080 strains tested, two mutants with auxotrophic marker were successfully produced and were stable. The frequency, therefore, was about 0.1%. The stability of the auxotrophs was 14%. The highest methionine content was 25% higher than that of the parent strain.

3.2. Gamma irradiation

From 1480 colonies tested, the frequency of auxotrophic or methionine-rich mutants production was zero.

3.3. $NaNO_2$ treatment

Fourteen hundred colonies were examined without any success.

3.4. Protoplast fusion

Of the *Saccharomyces lactis* hybrids, only two were selected from 18 colonies tested with higher methionine content but they were lower in their protein content, while *Kluyveromyces lactis* showed higher frequency by giving out 4 mutants of 24 tested with protein and methionine contents higher than that of the parents.

The above results show that the UV treatment was the most efficient method compared with gamma radiation and $NaNO_2$ treatment. In addition to that the method was not as laborious as the gamma radiation and $NaNO_2$ treatments. $NaNO_2$ treatment was found to be very laborious and time consuming, this increased the probabilities of contamination which hindered the possibility of examining all the treated cells. This could be the reason for failing of this method to produce any mutants.

Gamma irradition did not prove to be more efficient than the $NaNO_2$ treatment as contamination was also frequently experienced, although a reasonable number of colonies were tested. Not only the different methods varied in their efficiency in this regard, but the different strains used varied also in their response to the same treatment. *Candida guilliermondii* 812 gave the highest number of auxotrophic mutants, some of which proved to be stable. While *Kluyveromyces fragilis* 1068 gave one mutant of highest methionine content compared to the original parent strain.

The results did not enable us to establish at which dose level the UV was more effective, and for this reason different levels of treatment were used throughout the experiment.

Compared with the UV treatment protoplast fusion proved to be of higher efficiency in producing mutants of higher methionine and protein contents.

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THE AVERAGE INTAKE OF Cu, Mn AND Zn MICRONUTRIENTS OF THE POPULATION OF HUNGARY IN 1978

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The aim of this study was to estimate the average daily dietary intake of the Hungarian population of Mn, Cu and Zn micronutrients. Practically all important components of the diet of the population have been analyzed partly in an earlier work of the author, partly in this work. Statistical data of the Central Statistical Office of Hungary about the annual consumption of various food components were utilized in the estimation of the micronutrient intake. The separated data of the Statistical Office for physical workers and for the intellectuals enabled to estimate the intake of the two classes separately, however the differences in the micronutrient intake were modest. The daily intake is about 11 mg Zn per day per person and this amount seems to be sufficient as the assumed requirement from Zn is between 10–15 mg per day per person. The situation is less favourable in the case of Cu, the intake of which amounts to 1.1 mg per day per person which seems to be marginal according to international recommendations. The intake of Mn amounts to less than 3 mg per day per person which seems to be again just on the margin of the recommended requirement.

The decreased intake of Cu and Mn is attributed to the decreased consumption of cereals and particularly the increased degree of refinement of cereal products as refined cereal products (white flour) contain much less micronutrients than whole grain wheat bread or brown sorts of bread.

It is well known that some micronutrients are essential for health, development and fertility of all living beings, inclusively man. Man is supplied with micronutrients in food and a very small part of them in water. The dietary habits changed gradually in consequence of industrial development and this change accelerated very significantly in the course of this century. There are very great differences between the dietary habits of developed nations and the nations in the lower brackets of the average national income. The significant development of the diet of the affluent nations has brought about unquestionably important benefits, e.g. supply of calories, animal proteins, vitamins, sugar, oil, fats, etc. Serious suspicions arise however that with this beneficial development some disadvantages might be associated.

Since the beginning of this century the change may be characterized by the fact that the consumption of cereals, complex carbohydrates decreased very substantially and the consumption of sugar, animal proteins and animal fats increased. Generally the consumption of cereal products supplies about 61% of the calories in the diet of the developing nations, however only about 31% in the diet of the affluent nations. (FOOD AND AGRICULTURAL ORGANIZATION OF THE UNITED NATIONS, 1977). The affluent nations obtain the

largest part of their calories in animal proteins, animal fats, oils, and sugar. The majority of our essential mineral requirements is supplied by cereal grains and their less refined products, e.g. whole grain bread, brown breads, etc. Not only the total consumption of cereals and cereal products decreased very substantially by affluency to less than half since the beginning of this century but within it the proportion of white refined cereal products (white bread, dry pastry, etc.) increased, at the expense of mineral elements.

Since it seemed that about 60–70% of Mn and about 30% of Cu content of the diet of affluent nations originated from cereals, the influence of milling and refining upon the micronutrient content of cereal products was investigated in a previous detailed study (SZALAY & MURÁNYI, 1982).

The Mn content of fine wheat flour with 0.55% ash content and about 72% extraction yield is only about 1/8 of that of the whole wheat grain and its Cu content amounts to about 1/3 of that of the grain.

The aim of this study is to determine the average daily intake of the Hungarian population from Mn, Cu and Zn micronutrients by utilizing the analytical determination of the content of various food sources carried out partly earlier (MURÁNYI, 1977) partly in this study and calculating the intake by utilizing the consumption data of households published by the Central Statistical Office (Hungary) (Household Statistics 1978, 1979).

Similar studies were carried out already in some countries. The book of UNDERWOOD (1971) is treating various data from the USA (TIPTON et al., 1966; SCHROEDER et al., 1966), Holland (BELZ, 1960) and the U.K. (HAMILTON et al., 1972/73). A comprehensive bibliographic survey is compiled from earlier – often very controversial – data in the book of SCHLETTWEIN-GSELL and MOMMSEN-STRAUB (1973).

The uptake of Mn, Cu and Zn in North Karelia (Finland) was already investigated earlier because there is the highest incidence of cardiovascular diseases in the world (SZALAY et al., 1982). Recently a study collecting all available data appeared on the intake of a large number of mineral elements in Finland (KOIVISTOINEN et al., 1980).

In order to find any causal relationship between any deficiency disease and the change of micronutrient intake, assumedly caused by affluency, it is necessary to estimate the intake of the average citizen and to estimate how it changed in the course of this century, further how it relates to the minimum requirement of the micronutrient in question as far as it is known at all.

1. Materials and methods

Samples of foods were collected from at least 6 different independent market sources. They were cleaned, pealed off if necessary, inedible parts were discarded as in households and then weighed first in the wet fresh state, then

after drying at 105 °C, finally they were ground and homogenized. 5 g samples were taken out from the much larger powdered sample, for ashing. Ashing was carried out at 550 °C and the ash was treated with cc HCl and evaporated. The residue was taken up and diluted to a standard volume with 0.1 *N* HCl. This solution was sprayed into an air-acetylene flame of a Beckman 485 Type atomic absorption spectrophotometer (AAS). The following wavelengths were used: 279.5 nm for Mn, 324.8 nm for Cu and 213.8 nm for Zn. The mean values of the six samples were utilized. A large number of analyses were carried out by MURÁNYI (1977) and her results are utilized. Results concerning cereals were taken from the previous publication of SZALAY and MURÁNYI (1982).

The accuracy of the analytical determinations is usually better than 5%, optimally about 1%. Data on the average consumption of the Hungarian population in the year 1978 were taken from the publication of the KÖZPONTI STATISZTIKAI HIVATAL (1979). This very detailed statistics is in principle suitable to determine the food consumption in kg per year per person for various food sources of the various occupational strata of society.

These statistical data were collected by interrogation of the population of about 241 settlements, each with more than 5000 inhabitants. Altogether 8500 households have been randomly selected. Part of the food was consumed outside of the households, in restaurants or working place canteens. This was taken into account in the statistics.

2. Results

Our results are demonstrated in Table 1 for consumption within the households of physical workers and in Table 2 for the strata of intellectuals. There are no significant differences between the results related to these two groups of population.

At the end of these Tables consumption outside the households (restaurants, working place canteens, etc.) are added.

In the first column the food sources are listed according to the data of the KÖZPONTI STATISZTIKAI HIVATAL (1979).

The determination of the average micronutrient intake meets with great difficulties and must be regarded as an approximative estimate, only. The cause of this does not lie with the analysis, because atomic absorption spectrometry is very useful, practical and reliable for the determination of these elements. The problem is inherent in the statistical data. The data werre collected by interrogation of a more or less well chosen sector of the population. In some countries and studies (e.g. USA), the total amount of food sold to the population is taken into consideration. However losses occur between the market and the consumer and thus the actual intake is less than the

Table 1

Mn, Cu, Zn micronutrient intake of the average Hungarian citizen in workers' households

Foodstuffs	Consumption (kg per year)	Consumption (g per day)	Mn	Cu	Zn	Mn	Cu	Zn
			concentration in ppm			intake in mg per day per person		
Pork	16.16	44.3	0.23	0.87	39.8	0.010	0.039	1.763
Beef and veal	1.56	4.27	0.40	0.55	59.5	0.002	0.002	0.254
Entrails	3.03	8.30	0.90	2.52	17.5	0.008	0.021	0.145
Mutton	0.80	2.19	0.80	0.80	12.0	0.002	0.002	0.026
Hen	15.34	42.0	0.23	0.27	12.1	0.010	0.011	0.508
Goose, duck	2.56	7.01	0.23	0.27	12.1	0.002	0.002	0.085
Meat products	17.12	46.9	0.23	0.87	39.8	0.011	0.041	1.867
Fish, tinned fish	1.47	4.03	0.57	0.90	6.97	0.002	0.004	0.028
Meats total	58.04	159				0.047	0.122	4.676
Eggs	219.8[a]	0.6[a]	14[b]	5.0[b]	582[b]	0.0084	0.003	0.349
Milk	82.39	226	0.03	0.04	3.30	0.0068	0.009	0.746
Cheese	1.36	3.73	0.22	0.32	36.6	0.0008	0.001	0.137
Butter	1.23	3.37	0.02	0.10	1.70	0.0001	0.0003	0.006
Fat	12.78	35.0	0	0.06	0.20	0	0.0021	0.007
Bacon	3.74	10.3	0	0.06	0.20	0	0.0006	0.002
Vegetable oils, margarine	3.43	9.40	0.01	0.03	0.73	0.0001	0.0003	0.007
Fats and oils total	21.18	58.0				0.0002	0.0033	0.022
Bread	85.53	229	5.15	0.62	6.48	1.18	0.142	1.484
Baker's ware	9.46	25.9	2.97	0.74	4.27	0.08	0.019	0.111
Bakery products total	92.99	255				1.26	0.161	1.595

Flour	23.89	65.5	5.45	2.37	6.94	0.357	0.155	0.455
Rice	3.90	10.7	10.5	1.40	23.0	0.112	0.015	0.246
Dried paste	2.20	6.03	1.52	1.76	8.60	0.009	0.011	0.052
Pastry	2.01	5.51	1.52	1.76	8.60	0.008	0.010	0.047
Total	32.0	87.7				0.486	0.191	0.800
Potatoes	45.80	125	0.88	0.72	2.73	0.111	0.091	0.344
Dry legumes, pulses	1.94	5.32	7.11	6.63	29.7	0.038	0.035	0.158
Nut, almonds, hazelnut, poppy	1.02	2.80	24.0	9.27	36.7	0.067	0.026	0.103
Sugar	19.05	52.2	0	0	0	0	0	0
Cabbage, savoy	7.50	20.6	1.84	0.34	2.76	0.038	0.007	0.057
Cabbage lettuce	2.11	5.78	2.05	0.58	4.02	0.012	0.003	0.023
Mushrooms	0.31	0.85	2.49	10.9	21.3	0.002	0.009	0.018
Cauliflower	1.07	2.93	1.12	0.38	5.03	0.003	0.001	0.015
Tomato	7.22	19.8	0.98	0.53	1.22	0.019	0.011	0.024
Carrot, turnip	8.25	22.6	2.97	1.42	3.25	0.067	0.032	0.074
Sorrel, spinach	0.57	1.56	7.79	0.87	9.12	0.012	0.001	0.014
Cucumber	6.22	17.0	0.67	0.43	1.44	0.011	0.007	0.025
Onion	6.71	18.4	1.28	0.63	4.58	0.024	0.012	0.084
French beans	2.07	5.67	2.08	0.65	2.58	0.012	0.004	0.015
Green peas	2.30	6.30	3.55	2.42	10.6	0.022	0.015	0.067
Green paprika	5.88	16.11	1.18	0.52	1.42	0.019	0.008	0.023
Others	3.89	10.7	1.89	0.98	3.87	0.020	0.011	0.041
Fresh vegetables total	54.10	148.0				0.261	0.121	0.481
Pickles	1.18	3.23	0.98	0.53	3.01	0.003	0.002	0.010
Sauerkraut	1.78	4.88	1.70	0.27	1.85	0.008	0.001	0.009

Table 1 (cont.)

Foodstuffs	Consumption (kg per year)	Consumption (g per day)	Mn	Cu	Zn	Mn	Cu	Zn
			concentration in ppm			intake in mg per day per person		
Preserved vegetables	1.81	4.96	2.27	1.57	7.00	0.011	0.008	0.035
Total	4.77	13.1				0.022	0.011	0.054
Apple	14.42	39.5	0.55	0.22	0.48	0.022	0.009	0.019
Peach	3.56	9.78	0.88	0.50	1.36	0.009	0.005	0.013
Apricot	1.44	3.95	0.82	0.62	1.42	0.003	0.003	0.006
Cherry	2.14	5.86	0.77	0.65	0.85	0.005	0.004	0.005
Water-melon	4.46	12.2	0.20	0.20	0.50	0.002	0.002	0.006
Cantaloup	0.44	1.21	0.30	0.50	1.30	0.0004	0.0006	0.002
Strawberry, raspberry, red currant	1.78	4.88	3.06	0.75	2.31	0.015	0.0037	0.011
Pear	2.77	7.59	0.48	0.68	1.18	0.004	0.0052	0.009
Sour cherry	1.82	4.99	1.13	0.82	1.10	0.006	0.0041	0.006
Plum	4.42	12.11	1.00	0.82	1.17	0.012	0.010	0.014
Grape	3.47	9.51	0.23	1.23	0.80	0.008	0.012	0.008
Other home fruits	0.47	1.29	0.54	0.74	2.55	0.0007	0.001	0.003
Fresh fruits total	41.20	112.9				0.087	0.060	0.102
Preserved fruit	1.25	3.43	0.88	0.64	1.25	0.003	0.002	0.004
Fruit juice, fruit syrup	2.01	5.51	1.24	1.04	2.19	0.007	0.006	0.012
Total	3.26	8.93				0.010	0.008	0.016
Tropical fruits	5.72	15.7	0.53	0.57	0.67	0.008	0.009	0.011

[a] piece
[b] μg per piece

Supplement to *Table 1*

Mn, Cu, Zn micronutrient intake in workers' households and the consumption outside the household total

	Consumption		Mn	Cu	Zn
	kg per year	g per day	intake in mg per day per person		
Meat products	65.77	180.0	0.053	0.138	5.294
Eggs	253.23[a]	0.6[a]	0.0084	0.003	0.349
Milk	88.80	243	0.0073	0.010	0.802
Fats	23.44	64.2	0.0002	0.004	0.024
Bread, bakery products	96.40	264	1.31	0.167	1.65
Flour, rice, dried paste	36.91	101	0.560	0.220	0.92
Sugar	20.87	57.2	0	0	0
Potato	59.68	164	0.15	0.12	0.45
Dry legumes, pulses	2.44	6.69	0.048	0.044	0.20
Nut, almonds, hazelnut, poppy	1.08	2.96	0.071	0.028	0.11
Fresh vegetables	58.56	160	0.282	0.131	0.52
Preserved vegetables	8.91	27.4	0.041	0.021	0.101
Fresh home fruits	42.69	117	0.090	0.062	0.106
Preserved home fruits, fruit juice	4.38	12	0.013	0.011	0.022
Southern fruits	5.79	15.9	0.0081	0.0091	0.0111
Coffee	2.9	7.95	0.053	0.013	0.040
Tea (in brew)	0.079	0.22	0.043	0.002	0.004
Vine	33.8	92.6	0.065	0.030	0.067
Beer	86	235.6	0.035	0.033	0.033
Beverages total			0.196	0.078	0.144
Chocolate	2.4	6.58	0.138	0.068	0.199
Micronutrient intakes total (mg per day per person)			2.98	1.12	11.0

[a] piece

amount of food sold. It is typically known of Hungary that some part of the bread is discarded by pealing off the crust or because of drying out of the leftovers, etc. Some losses arise also in the discussed microelements when the boiling water is discarded after cooking. Food products grown in the home garden cannot be properly estimated in food statistics, etc. Further, food statistics do not take into account the micronutrients. In the USA all cereal products are summarized under "cereals" i.e. grain equivalents, however the various cereal products contain very different amounts of micronutrients.

The result of such a statistical estimation relates to the "average citizen" and any real person might deviate very significantly from the average consumption according to his individual dietary habits.

Table 2

Mn, Cu, Zn micronutrient intake of the average Hungarian citizen in the households of intellectuals

Foodstuffs	Consumption (kg per year)	Consumption (g per day)	Mn	Cu	Zn	Mn	Cu	Zn
			concentration in ppm			intake in mg per day per person		
Pork	15.84	43.4	0.23	0.87	39.8	0.0099	0.038	1.727
Beef and veal	1.93	5.29	0.40	0.55	59.5	0.0021	0.003	0.315
Entrails	3.17	8.69	0.90	2.52	17.5	0.0078	0.022	0.152
Mutton	0.53	1.45	0.80	0.80	12.0	0.0012	0.0012	0.017
Hen	11.38	31.2	0.23	0.27	12.1	0.0072	0.0084	0.378
Goose, duck	2.14	5.86	0.23	0.27	12.1	0.0014	0.0016	0.071
Meat products	15.86	43.5	0.23	0.87	39.8	0.010	0.037	1.731
Fish, tinned fish	1.71	4.69	0.57	0.90	6.97	0.0027	0.0042	0.033
Meats total	52.56	144				0.0423	0.115	4.424
Eggs	201.40[a]	0.6[a]	14[b]	5[b]	582[b]	0.0084	0.003	0.349
Milk	91.41	250	0.03	0.04	3.30	0.0075	0.010	0.825
Cheese	2.87	7.86	0.22	0.32	36.6	0.0017	0.0025	0.288
Butter	1.97	5.40	0.02	0.10	1.70	0.0001	0.0005	0.009
Fat	8.38	23.0	0	0.06	0.20	0	0.0014	0.005
Bacon	2.21	6.06	0	0.06	0.20	0	0.0004	0.001
Vegetable oils, margarine	4.38	12.0	0.01	0.03	0.73	0.0001	0.0004	0.009
Fats and oils total	16.94	46.4				0.0002	0.0027	0.024
Bread	59.37	163	5.15	0.62	6.48	0.840	0.101	1.056
Baker's ware	12.90	35.3	2.97	0.74	4.27	0.105	0.026	0.151
Bakery products total	72.27	198				0.945	0.127	1.207

Flour	16.02	43.9	5.45	2.37	6.94	0.239	0.104	0.305
Rice	2.92	8.00	10.5	1.40	23.0	0.084	0.011	0.184
Dried paste	1.84	5.04	1.52	1.76	8.60	0.008	0.009	0.043
Pastry	2.89	7.92	1.52	1.76	8.60	0.012	0.014	0.068
Total	23.67	64.9				0.343	0.138	0.600
Potato	36.77	101	0.88	0.72	2.73	0.089	0.073	0.276
Dry legumes, pulses	1.19	3.26	7.11	6.63	29.7	0.023	0.022	0.097
Nut, almonds, hazelnut, poppy	0.92	2.52	24.0	9.27	36.7	0.061	0.023	0.093
Sugar	17.68	48.4	0	0	0	0	0	0
Cabbage, savoy	6.47	17.7	1.84	0.34	2.76	0.033	0.006	0.049
Cabbage lettuce	1.62	4.44	2.05	0.58	4.02	0.009	0.003	0.018
Mushrooms	0.50	1.37	2.49	10.9	21.3	0.003	0.015	0.029
Cauliflower	1.42	3.89	1.12	0.38	5.03	0.004	0.002	0.020
Tomato	8.17	22.4	0.98	0.53	1.22	0.022	0.012	0.027
Carrot, turnip	7.03	19.3	2.97	1.42	3.25	0.057	0.027	0.063
Sorrel, spinach	0.82	2.25	7.79	0.87	9.12	0.018	0.002	0.021
Cucumber	5.48	15.0	0.67	0.43	1.44	0.010	0.007	0.022
Onion	5.22	14.3	1.28	0.63	4.58	0.018	0.009	0.066
French bean	1.89	5.18	2.08	0.65	2.58	0.011	0.003	0.013
Green peas	2.03	5.56	3.55	2.42	10.6	0.020	0.014	0.059
Green paprika	6.81	18.7	1.18	0.52	1.42	0.022	0.010	0.027
Others	4.74	13.0	1.89	0.98	3.87	0.025	0.013	0.050
Fresh vegetables total	52.20	143				0.252	0.123	0.464
Pickles	1.30	3.56	0.98	0.53	3.01	0.004	0.002	0.011
Sauerkraut	1.26	3.45	1.70	0.27	1.85	0.006	0.0009	0.006

Table 2 (cont.)

Foodstuffs	Consumption (kg per year)	Consumption (g per day)	Mn	Cu	Zn	Mn	Cu	Zn
			concentration in ppm			intake in mg per day per person		
Preserved vegetables	2.11	5.78	2.27	1.57	7.00	0.013	0.009	0.041
Total	4.67	12.8				0.023	0.0119	0.058
Apple	16.78	46.0	0.55	0.22	0.48	0.025	0.010	0.022
Peach	5.41	14.8	0.88	0.50	1.36	0.013	0.007	0.020
Apricot	1.54	4.22	0.82	0.62	1.42	0.004	0.003	0.006
Cherry	1.95	5.34	0.77	0.65	0.85	0.004	0.004	0.005
Water-melon	5.44	14.9	0.20	0.20	0.50	0.003	0.003	0.008
Cantaloup	0.53	1.45	0.30	0.50	1.30	0.0004	0.0007	0.002
Strawberry, raspberry, red current	2.37	6.49	3.06	0.75	2.31	0.020	0.005	0.015
Pear	3.48	9.53	0.48	0.68	1.18	0.005	0.007	0.011
Sour cherry	1.97	5.40	1.13	0.82	1.10	0.006	0.004	0.006
Plum	4.28	11.7	1.00	0.82	1.17	0.012	0.010	0.014
Grape	5.34	14.6	0.83	1.23	0.80	0.012	0.018	0.012
Other home fruits	0.56	1.53	0.54	0.74	2.55	0.0008	0.001	0.004
Fresh fruits total	49.65	136				0.1052	0.073	0.125
Preserved fruit	1.81	4.96	0.88	0.64	1.25	0.004	0.003	0.006
Fruit juice, fruit syrup	2.34	6.41	1.24	1.04	2.19	0.008	0.007	0.014
Total	4.15	11.4				0.012	0.010	0.020
Tropical fruits	8.31	22.8	0.53	0.57	0.67	0.012	0.013	0.015

[a] piece
[b] μg per piece

Supplement to *Table 2*

Mn, Cu, Zn micronutrient intake of the households of intellectuals and their consumption outside of their households, total

	Consumption		Mn	Cu	Zn
	kg per year	g per day	intake in mg per day per person		
Meat products	66.97	184	0.054	0.147	5.653
Eggs	228.82[a]	0.63[a]	0.0088	0.0032	0.349
Milk	101.35	278	0.0083	0.011	0.917
Fats	21.10	57.8	0.00025	0.0034	0.030
Bread, bakery products	78.77	216	1.0309	0.139	1.317
Flour, rice, dryed paste	32.58	89.3	0.472	0.190	0.826
Sugar	20.81	57.0	0	0	0
Potato	62.65	172	0.152	0.124	0.470
Dry legumes, pulses	2.11	5.78	0.041	0.039	0.172
Nut, almonds, hazelnut, poppy	1.02	2.80	0.068	0.026	0.103
Fresh vegetables	60.35	165	0.291	0.142	0.535
Preserved vegetables	11.85	32.5	0.058	0.030	0.147
Fresh home fruits	52.08	143	0.11	0.077	0.131
Preserved fruits, fruit juice	6.20	17.0	0.018	0.015	0.030
Southern fruits	8.43	23.1	0.012	0.013	0.015
Coffee	2.90	7.95	0.053	0.013	0.040
Tea (in brew)	0.079	0.22	0.043	0.002	0.004
Vine	33.8	92.6	0.065	0.030	0.067
Beer	86.02	235.6	0.035	0.033	0.033
Beverages total			0.196	0.078	0.144
Chocolate	2.4	6.58	0.138	0.068	0.199
Micronutrient intakes total (mg per day per person)			2.66	1.11	11.3

[a] piece

These deviations might influence very much the intake of micronutrients e.g. bread consumption has a very strong influence on the intake of Mn. Individual deviations in the consumption of certain food sources might amount from 50% to some hundred percent.

Data in Table 1 and Table 2 and the supplements are related to the original fresh (wet) substances as they are sold, because the consumption statistics are related to them in this way. The micronutrient concentrations in ppm are related to the original substances, too. Some food sources require more detailed explanation. Entrails comprise liver, lung, heart, tongue, kidney, spleen, brain. The micronutrient concentrations given in the Table are mean values. Meat products comprise sausages, various meat delicacies

made in Hungary. They are calculated here for pork. The bread overwhelmingly consumed in Hungary is baked from wheat flour type BL 80, which has an ash content of 0.8%. Micronutrient concentrations are related to this kind of bread. Flour is sold as fine white household flour being the type BL 55, having an ash content of 0.55%. (See in this respect SZALAY & MURÁNYI, 1982.) Legumes, pulses, comprise here dry beans, dry peas, lentiles. Micronutrient concentrations are given as mean values of these 3 products. Other vegetables comprise asparagus, celery, red beet, horse radish, radish, kohlrabi, green onions, garlic, green paprika, pumpkin, squash. Mixed pickles comprise cucumbers, onions. Conserved vegetables include green peas and carrots. "Other home fruits" comprise gooseberry, quince-apple. Under the item of preserved fruits we used the mean value of all fruits listed in Tables 1 and 2. Tropical fruits comprise oranges, lemons, and bananas and the micronutrient values used are the mean values belonging to them.

Consumption in households is only a part of the total consumption. Consumption in restaurants, working-place canteens must be added. According to the data of KÖZPONTI STATISZTIKAI HIVATAL (1979) we added these factors of the micronutrient intake from these sources and in addition those originating from beverages (International Data of Consumption by the Population, 1980) to the intake of Table 1 and Table 2. The following values are obtained: The average daily intake in Hungary per head of the investigated micronutrients is the following:

	Mn	Cu	Zn
workers' households + supplements	2.98 mg	1.12 mg	11.0 mg
intellectuals' households + supplements	2.66 mg	1.11 mg	11.3 mg

There are no significant differences in the intake of the investigated two social strata. LINDNER-SZOTYORI and GERGELY estimate (1980) the micronutrient supply of the population of Hungary even lower, probably because they have investigated the whole Hungarian society not only these two strata.

3. Conclusions

In assessing the possible errors of these data, errors caused by the statistics must be considered. Errors of analyses are in comparison negligible. First of all the statistical data relate to the acquired quantity of food in household use. Substantial losses occur between market and consumption. Losses are due to pealing off potatoes, vegetables, etc. and discarding unedible parts

or to decay during storage. Some leftovers on the plate are not consumed. As the largest single source of Mn is bread, pealing off the crust and discarding the dried leftovers reduce substantially the intake of micronutrients. In calculation of some summarized items in Table 1 and Table 2 mean values are used for the micronutrient content of several food components comprised. Moreover, the influence of these simplifications is low.

Considering the factors of losses, treated above, the micronutrient intake data must be reduced. Home garden production has the opposite effect. Green vegetables, legumes, etc. are still grown in small gardens and evade the market. Similarly fruit consumption coming from the home garden is adding to the values treated in the statistics.

Essential human requirements in micronutrients are not exactly known. At first there are ethical considerations which prohibit a total withdrawal from human beings for experimental purposes. Such experiments can be carried out and are known on animals producing partly acute, sometimes chronic symptoms. The problem of human requirements is treated in the concise bibliographic work of SCHLETTWEIN-GSELL and MOMMSEN-STRAUB (1973), and by UNDERWOOD (1971), and BOWEN (1966).

Recommendations of the American Medical Association and the NATIONAL RESEARCH COUNCIL (1980) are based on earlier publications of various authors. They are the followings: 2.5–5.0 mg of Mn, 2.0–5.0 mg of Cu, 15 mg of Zn per day per person.

It is not the aim of this paper to treat the possible health consequences of deficiency. Concerning man, such consequences are known mainly for Cu and Zn deficiency, diseases occurring by unilateral nurishment, under extreme starvation conditions. No definite symptoms or diseases are known for Mn in humans according to SCHROEDER et al. (1966).

It is however the aim of this paper to judge how far the Hungarian diet satisfies the requirements in this respect.

No significant changes occurred in the supply of Zn in the course of changing to affluent diet, because the Zn supply is well distributed among food sources of plant and animal origin and dairy products. The value of 11 mg per day per person seems to be sufficient as the suggested requirement from Zn is between 10–15 mg per day per person in the various literary sources.

The situation is less favourable in the case of Mn. The Hungarian diet probably approximates the diet of the developed nations and offers only the minimal supply of Mn of somewhat less than 3 mg per day per person.

Individual Mn requirements might differ from the average due to congenital capacity of uptake of the digestive tract. Storage and homeostatic control of the level in human blood might be individually different. Not enough is known about the pathway and homeostatic control mechanism of Mn in the human body. The following can be however definitely stated:

We obtain the largest part of Mn supply from cereal products, from unrefined cereals, whole grain bread, brown bread, etc. Legumes, pulses provide a significant contribution. With the development of the standard of living, less is consumed from these food sources by the affluent nations. The consumption of animal proteins, dairy products, fats, oils, sugar, etc. increases, supplying almost 70% of the calory requirements. However, these food components contain very little Mn or almost none at all. These have been the main changes in the course of the last century. It seems that the decrease in the consumption of cereals and particularly the fact that within the group of cereal products the proportion of refined flour products increased, may be responsible for a disadvantageous turn. Unfortunately no reliable statistical data exist in this respect from the beginning of this century and even the modern statistical data are not compiled in view of the micronutrient content, thus these calculations are very rough.

The Cu supply of the average population seems to be insufficient. Most of literary data suggest a minimal requirement of 2 mg per day for adults (National Research Council, 1980.).

About 1.00 ± 0.1 ppm is the normal level of Cu in the blood of man and of most mammalia. This amount is stabilized by a very efficient homeostatic mechanism, in which the liver is the pooling organ and the bile removes the surplus into the great intestine. The role it plays and the defects caused by Cu deficiency are well known from veterinary experience and animal experiments. Similar defects occur in human population too, but they are not always causally correlated to Cu deficiency yet. It is possible that the homeostatic mechanism of man is extremely efficient in the case of Cu deficiency and perhaps it is able to retain the last reserves in the body if the supply is deficient.

If we compare the average intake of the population with the assumed minimal requirement, we should not forget the following fact. Any property of a statistically sufficiently large population can be approximately represented by a Gaussian distribution curve. E.g. in the case of the micronutrient intake the maximum of the curve would fall upon the mean value of the intake of the whole population. If the standard deviation of the Gauss function would be known we could calculate how large part of the population is consuming less than the half, resp. more than the double of the mean value. Those consuming less than the mean value are exposed to the consequences of deficiency. So if the present affluent diet supplies only about the minimum requirement then a significant part of the population might have a deficient supply.

At the beginning of this century, the population of the presently affluent nations consumed much more cereals than today. The gradual but large change in the dietary habits left the Zn supply largely unchanged or even somewhat improved, however the Mn supply and Cu supply decreased sub-

stantially. It is not sufficiently proved yet by direct experiments whether this depletion of the diet in them is causally correlated with the statistically increasing number of some diseases in affluent societies.

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ON THE RAFFINOSE CONTENT OF BEET MOLASSES

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The raffinose content of molasses samples taken from the twelve Hungarian sugar factories at the beginning and at the end, resp., of the sugar campaign 1980–81 were determined enzymatically. Clarification with the Carrez solution I and II as well as prolonged incubation (2 h) with galactose dehydrogenase were the prerequisites to obtain results with an average variation coefficient of 5.1%. Raffinose content in the molasses samples taken at the beginning of the campaign ranged from 0.305% w/w to 0.777% w/w and from 0.59% to 1.46% as related to polarimetrically determined sugar content. Out of the twelve samples taken at the beginning of the campaign six had raffinose contents below 0.5% w/w. In the molasses samples taken at the end of the campaign raffinose content ranged from 0.79% w/w to 2.01% w/w and from 1.69% to 4.11% as related to polarimetrically determined sugar content. Out of the twelve samples taken at the end of the campaign, six had raffinose contents above 1.5% w/w, i.e. surpassed the highest value measured at the beginning of the campaign. In the great majority of the sugar factories, the differences in raffinose content of the samples taken at the end and at the beginning of the campaign were highly significant ($P \geq 99\%$ or 99.9%). The increase in raffinose content was observed by the end of the campaign in all the molasses samples except for the one that had shown the highest raffinose content at the beginning of the campaign.

The world-wide shortage of motor car fuels and the ever increasing prices of gasoline rose the interest in fermentation alcohol as a possible substitute agent. The economic feasibility of the utilization of ethanol as motor car fuel depends on its production costs. These again can be reduced, among others, by increasing the yield and final concentration of alcohol in the mash. In Hungary, beet molasses is the exclusive raw material of ethanol production by fermentation. Although much has been done in the past to increase both yield and concentration of fermentation ethanol in molasses mashes by selecting an appropriate yeast strain and optimizing the conditions of fermentation (Vámos-Vigyázó, 1960), further improvements could be achieved by converting the entire raffinose content of molasses to alcohol. Raffinose, a trisaccharide composed of the monosaccharides glucose, galactose and fructose, is a common constituent of the sugar beet which accumulates, during the sugar manufacturing process, in the molasses. The *Saccharomyces cerevisiae* yeast strains used for molasses fermentation split off the fructose moiety of raffinose by virtue of their invertase activity, but, lacking melibiase (α-galactosidase) activity, are unable to convert the rest of the molecule, i.e. melibiose (glucose + galactose)

to fermentable sugars. Thus, 2/3 of the raffinose content of molasses remain in the mash as residual sugar. In the early '70-ies Japanese authors opened up new ways of raffinose utilization, applying an external source of α-galactosidase, to split raffinose into sucrose and galactose (McGINNIS, 1973; SUZUKI et al., 1972, 1973; SZÉP & VÁMOS-VIGYÁZÓ, 1980). This process, originally designed for sugar mills in order to increase sugar yield and promote crystallization, could be applied to distilleries as well. In this context it is essential to know the exact raffinose content of molasses. This, too, has been made possible lately, by applying an enzymatic method of analysis (ANON, 1979). The present paper deals with the enzymatic determination of the raffinose content of molasses and gives a comparison of its concentration in the samples from different Hungarian sugar factories taken at the beginning and at the end of the sugar manufacturing campaign.

1. Materials and methods

1.1. The molasses

Molasses samples were taken from the 12 Hungarian sugar mills at the beginning and at the end of the 1980–81 sugar manufacturing campaign. Sampling for analysis was performed after homogenizing the molasses by indirect heating to 50–60 °C under stirring to dissolve sugar sedimented on standing.

1.2. Preparation of the molasses solution

Five g of molasses were dissolved in 50 cm^3 60 °C distilled water, using a magnetic stirrer. The solution was clarified with 1 cm^3 each of the solutions Carrez I (30% w/v $ZnSO_4 \cdot 7H_2O$ in distilled water) and Carrez II (15% w/v $K_4Fe(CN)_3 \cdot 3H_2O$ in distilled water), allowed to cool to r.t., made up to 100 cm^3 and passed through filter paper. Aliquots of the filtrate were used for enzymatic raffinose determination and for polarimetric sucrose determination, respectively.

1.3. Enzymatic determination of raffinose

The underlying principle of the method is as follows: raffinose is split by α-galactosidase at pH 4.6 to galactose and sucrose. Then, in a second reaction, galactose is oxidized by galactose dehydrogenase at pH 8.6, in the presence of the oxidized form of nicotine adenine dinucleotide (NAD), to galactonic acid. Simultaneously, NAD is reduced to NADH which latter compound has sharp absorption maxima at 340 nm and 365 nm. The amount

of the NADH formed can be determined spectrophotometrically at either wavelength and, being equivalent to the raffinose transformed in the reaction, serves as its measure (ANON, 1979).

The determination itself was carried out exactly as described in detail in the leaflet of Boehringer (ANON, 1979; POLACSEK-RÁCZ et al., 1982) with the exception that the second reaction takes 2 h with molasses (in contrast to the 30 min required with raffinose pentahydrate model solutions).

2. Results

The enzymatically determined raffinose and the polarimetrically determined sugar contents of the molasses sampled at the beginning of the sugar campaign are given in Table 1.

According to raffinose content, molasses can be subdivided into two groups: in one group raffinose content is below, in the other it is above 0.5%. It is interesting to note that the molasses of all the sugar factories located in the western part of the country, i.e. in Transdanubia, belong to the latter group (Ács, Ercsi, Kaposvár, Petőháza and Sárvár). The sixth sample belonging to this group (Szerencs) originates from a factory in the northeastern

Table 1

Polarimetrically determined sugar and enzymatically determined raffinose contents in beet molasses samples taken at the beginning of the sugar campaign 1980–81 in twelve Hungarian sugar factories

Factory		Sugar content (w/w in %)	Raffinose content		
			(w/w in %)		related to sugar content (%)
Name	No		$\bar{x}$	$\pm s$	
Ács	1	51.4	0.606	0.00846	1.18
Ercsi	2	49.0	0.561	0.00161	1.14
Hatvan	3	50.8	0.492	0.00625	0.97
Kaba	4	51.2	0.491	0.0358	0.96
Kaposvár	5	52.4	0.695	0.0460	1.33
Mezőhegyes	6	46.4	0.450	0.0140	0.97
Petőháza	7	53.2	0.728	0.0804	1.36
Sarkad	8	46.8	0.492	0.00720	1.05
Sárvár	9	52.6	0.623	0.0324	1.18
Selyp	10	51.2	0.477	0.0111	0.93
Szerencs	11	53.4	0.777	0.0605	1.46
Szolnok	12	51.8	0.305	0.0404	0.59

$\bar{x}$ = mean of 4 paralel determinations; s = standard deviation

part of Hungary. The ratio of the highest ($0.78 \pm 0.04\%$) and the lowest ($0.31 \pm 0.04\%$) raffinose content of the population is 2.5 and within one group 1.6 and 1.4, respectively, i.e. the scatter of the values within the groups is similar.

The difference between the mean values of the two groups was shown, by Student's t test to be significant at a probabilitiy level of 99.9% ($t = 9.35$, $DF = 46$). As the variance within the groups proved homogeneous by the Cochran-test (SVÁB, 1973), the raffinose contents of the molasses of each group were compared by analysis of variance, the results of which are given in Table 2.

Table 2

Multiple comparison, by analysis of variance, of the raffinose contents of the molasses samples taken at the beginning of the sugar manufacturing campaign 1980–81 in twelve Hungarian sugar factories

Group A

Number of factory	3	4	6	8	10
4	0.0001				
6	0.042*	0.041*			
8	0.000	0.001	0.042*		
10	0.015	0.014	0.027	0.015	
12	0.187***	0.186***	0.145***	0.187***	0.172***

SD = 0.0349*; 0.0478**; 0.0651***

Group B

Number of factory	1	2	5	7	9
2	0.045				
5	0.089*	0.134***			
7	0.122**	0.167***	0.033		
9	0.017	0.062	0.072*	0.105**	
11	0.171***	0.216***	0.082*	0.049	0.154***

SD = 0.071*; 0.097**; 0.132***

The figures in the body of the Table indicate the differences in mean raffinose content of the molasses from the factories indicated by the numeration in the corresponding horizontal lines and vertical columns, respectively. The numeration of the factories is identical with that in Table 1. Group A and Group B: molasses of raffinose contents below and above 0.5%, respectively.

SD = significant difference; *, **, *** = significant at the levels of probability of 95%, 99% and 99.9%, respectively

Table 3

Polarimetrically determined sugar and enzymatically determined raffinose contents in beet molasses samples taken at the end of the sugar campaign 1980–81 in twelve Hungarian sugar factories

Factory		Sugar content (w/w in %)	Raffinose content	
Name	No		(w/w in %)[a]	related to sugar content (%)
Ács	1	59.0	1.06	1.80
Ercsi	2	44.6	1.24	2.78
Hatvan	3	54.6	2.01	3.68
Kaba	4	57.0	1.75	3.07
Kaposvár	5	50.0	1.74	3.48
Mezőhegyes	6	48.9	2.01	4.11
Petőháza	7	52.2	2.01	3.85
Sarkad	8	44.6	0.79	1.76
Sárvár	9	52.9	1.36	2.57
Selyp	10	50.7	1.76	3.47
Szerencs	11	50.8	0.85	1.69
Szolnok	12	54.2	1.34	2.47

[a] No replicates of measurements were carried out

Out of the molasses of the first group, raffinose content was significantly lower in the Szolnok sample than in the other five samples, while in the Mezőhegyes sample it was significantly lower than in the molasses from Kaba, Hatvan and Sarkad. The differences between the rest of the samples are within the limits of error.

Out of the molasses of the second group, the raffinose contents of the sample pairs from Ács and Sárvár as well as of those from Szerencs and Petőháza did not differ significantly. The raffinose contents of the majority of the samples were, however, different at probability levels of at least 95%.

The increasing order of raffinose content as related to polarimetrically determined sugar content was much similar to that related to molasses weight and was highest in the Szerencs sample (1.46%).

The pattern was entirely different in the samples taken at the end of the sugar manufacturing campaign (Table 3).

In this series the lowest raffinose concentration was 0.79% (w/w). This value was similar to the highest value found at the beginning of the campaign. Three samples had raffinose contents of about 2%. Thus, according to expectations, the range of raffinose content in molasses was shifted towards higher values by the end of the campaign. The rate of increase was different in the samples of the different factories. As raffinose content was assayed in the

samples from the end of the campaign without replicates, the results of this series of measurement were compared, by analysis of variance, to the mean values obtained for the samples from the beginning of the campaign, disregarding standard deviations (SVÁB, 1973). The results of the analysis of variance are given in Table 4.

With the exception of two sample pairs (Sarkad and Szerencs) the increase in raffinose content during the campaign was significant at the levels of probability of 99.9% (for 9 sample pairs) and 99% (for one sample pair), respectively. The ratios of the values measured at the end and at the beginning of the campaign ranged from 1.1 (Szerencs, not significant) to 4.5 (Mezőhegyes). The increase was more than fourfold in another two molasses (Szolnok and Hatvan). The ratio of the highest and lowest values of raffinose concentration was 2.5 in the samples taken at the end of the campaign, thus the scatter of the values remained the same as at the beginning of the campaign. Raffinose content as related to polarimetrically determined sugar content was above 3% in six out of the twelve samples taken at the end of the campaign.

Table 4

Analysis of variance of the raffinose contents in beet molasses samples taken at the beginning and the end, respectively, of the sugar manufacturing campaign 1980–81 in twelve Hungarian sugar factories

Cause of variance	Square sum of differences	Degree of freedom	Square sum of mean differences	Calculated value of F	Table value of F
Factory	1.1041	11			
Sampling time	5.1712	1	5.1712	44.12***	19.65
Interrelation	1.1889	11	0.1172		
Total	7.5642	23			

Differences in the raffinose contents of the molasses samples taken at the beginning and at the end of the campaign from the twelve factories

	1	2	3	4	5	6
Difference	0.45**	0.67***	1.46***	1.24***	1.04***	1.56***
	7	8	9	10	11	12
Difference	1.27***	0.29	0.75***	1.28***	0.06	1.02***

SD = 0.31*; 0.43**; 0.62***; (SD = significant difference)
For the explanation of the asterisks see Table 2
The numbers of the factories are identical with those given in Tables 1 and 3

One molasses sample (Szolnok) from the first series of measurements was kept in the laboratory throughout the campaign and analyzed for a second time at the end. Both raffinose and polarimetrically determined sugar contents were very similar (0.388% and 52.2%, resp.) to the values measured at the beginning of the campaign. The slight increase in both parameters might be due to evaporation.

3. Conclusions

The enzymatic raffinose determination as applied in the present study proved reliable and highly reproducible: the mean coefficient of variation calculated from the results of the first series of measurements was 5.1%.

It is interesting that the time requirement of the reaction catalyzed by galactose dehydrogenase is fourfold with molasses as compared to raffinose model solutions. The protracted enzyme reaction might be due to a soluble inhibitor present in the molasses or to one of the components of the clarifying solutions. The Carrez solutions were applied instead of lead acetate generally used in analytical work in sugar manufacturing as the latter had been shown to cause uncontrollable losses in the raffinose content of molasses (HOLLAUS et al., 1977).

Raffinose content varied in the molasses of the twelve Hungarian sugar factories to a considerable extent. The raffinose content of molasses originates entirely from the sugar beets. The raffinose extracted together with sucrose passes unchanged through the technological operations and accumulates nearly entirely in the molasses. This study did not deal with the effect, on the raffinose content in molasses, of growing conditions and postharvest handling prior to processing. The Hungarian molasses contained, in nearly every case, considerably more raffinose at the end of the beet processing campaign than at its beginning. The samples from the factory that produced the molasses of the highest raffinose content at the beginning of the campaign were an exception to this rule. The higher raffinose contents observed in the samples taken at the end of the campaign are obviously related to the accumulation of raffinose in the beets during storage. This accumulation depends on storage conditions, in the first place on temperature (VUKOV, 1971) and, under certain circumstances, also on the initial raffinose content of the beets.

The molasses samples taken at the end of the campaign had an average raffinose content of roughly 1.5% as related to weight and 2.9% as related to polarimetrically determined sugar content, the average of which was 51.6%. The utilization of the unfermentable two thirds of the raffinose content would thus increase the amount of alcohol obtained from 1 000 kg of molasses by 6 dm^3 (as calculated on a 60% conversion basis), i.e. the yield by 1.9 rel. %. According to these figures the utilization of the raffinose content of Hungarian

molasses seems worth to be dealt with. The data furnished by the analyses performed so far are, however, insufficient to judge of the feasibility of such a process. The influence of year, harvest and storage time of the sugar beets on the raffinose content of molasses are primary data to be established prior to considering the expenses of the α-galatosidase source and of enzymatic melibiose conversion.

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BOOK REVIEW

Dietary Fibre

G. G. Birch and K. J. Parker
(Eds)

Applied Science Publishers Ltd, London, 1983; 304 pages

The book contains the papers presented to the 13th Annual International Symposium held at the University of Reading, National Collage of Food Technology (Weybridge, Surrey, England, 29—31 March, 1982), under the auspices of a joint industry-university organizing committee.

The sixteen papers are published by prominent English and American authors. The role of dietary fibre in the aetiology and management of clinical disorders, such as diabetes mellitus and intestinal diseases, is discussed. Furthermore, the use of controlled addition of fibre to foods and the analytical determination of fibre components are described. Through wide-range and authentic information the cited references contribute to the results of research.

The collected papers are as follows: Testing the dietary fibre hypothesis; The development of the fibre hypothesis; The role of dietary fibre in food product formulation; Modern dietary fibre product development and nutrient bioavailability; Elevation of "fibre" in bread by guar addition; Advances in chemical characterisation and analytical determination of dietary fibre components; The chemistry of plant cell walls; Physical properties of fibre towards bile acids; Water and minerals; In vitro models of guar gum action; Blood glucose control using guar gum and similar materials; Dietary fibre as a tool of the clinician; Gastroenterological functions of dietary fibre; Bacteria, dietary fibre and chronic intestinal disease; Germ-free animals in studies of the nutritional importance of the gut microflora; The influence of dietary fibre on xenobiotic metabolism by gut bacteria.

For food technologists the book gives a comprehensive and detailed review on dietary fibre but it is valuable for dietetitians as well as for practising physicians.

I. Varsányi

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EDITORIAL ANNOUNCEMENT

We are pleased to inform our readers and subscribers that several internationally known experts of our discipline from various countries have accepted the invitation to join the Editorial Board of ACTA ALIMENTARIA. The activity of this expanded body starts with Volume 13. Simultaneously distribution of the journal will be shared between Akadémiai Kiadó, Budapest, Hungary and D. Reidel Publishing Co., Dordrecht, The Netherlands.

We hope that both these changes will contribute to improving the service of our journal for the progress of food science and technology and the growing use of contemporary examination methods.

We continue to look forward to receiving the critical remarks and suggestions of our readers as well as to their contribution of papers on subjects of international interest within the scope of our journal.

November 1983

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UTILIZATION OF CORN CONTAINING TOXIN F-2 BY MICROBIOLOGICAL TREATMENT

J. SAWINSKY-ACSÁDI

(Received: 31 July 1981; revision received: 14 April 1982; accepted: 15 April 1982)

In order to be able to utilize corn containing toxin F-2 the optimal growth conditions, the toxin F-2 decomposing capacity of the yeast strain *Candida intermedia*, were investigated and the products of metabolism were identified.

Since corn starch is broken down by enzymatic hydrolysis mainly into maltose, in order to develop the correct and most suitable technology model experiments were carried out using maltose as carbon source. To determine the optimal conditions of cultivation the orthogonal Latin square design scheme was applied.

Factors varied at 5 different levels were: urea and superphosphate concentration, rate of aeration, pH and temperature. Mathematical processing of the results of experiments revealed the following parameters to be optimal: urea 6 g dm^{-3}; superphosphate 3 g dm^{-3}; pH = 4.5; temperature 32 °C; aeration rate 500 $dm^3 h^{-1}$.

Applying the parameters found optimal, the cultivation was carried out in a medium of 30 mg kg^{-1} toxin F-2 content in hydrolysed corn. Then toxin F-2 was extracted from the biomass and the culture medium. By the aid of *Candida intermedia* 78% of toxin was digested and a metabolite formed. Toxin F-2 and the metabolite were separated by thin-layer chromatography. Based on the retention quotient and taking into account the related literature the metabolite was identified as zearalenol.

The biomass obtained by using *Candida intermedia*, adapted by passing several times, did not contain either toxin F-2, or its metabolite and the product of decomposition accumulated in the culture medium. In the course of *Candida intermedia* cultivation in a tube fermentor 28 g yeast biomass was obtained in the hydrolysate of 50 g corn containing 30 mg kg^{-1} toxin F-2. The protein content of the biomass amounted to 40.8%, the Morup–Ølesen chemical index (PV) was 48.49. The zearalenol content of the culture medium was 0.046 mg per 500 dm^3.

In recent years a great effort was made all over the world to study toxin F-2 and its metabolites.

In Hungary the utilization of corn containing toxin F-2, produced by *Fusaria* under unfavourable climatic conditions, is a great problem.

With the expansion of livestock raising increases the demand for fodder of good quality and rich in protein. One of the ways to satisfy this demand may be the cultivation of fodder yeast on various products and wastes of agriculture.

We attempted to utilize corn contaminated by toxin F-2 by culturing *Candida intermedia*, selected in preliminary experiments as a toxin decomposing strain, on corn hydrolysate and obtaining therefrom a biomass of high protein and vitamin content suitable for use as feedstuff.

Thus, while breaking down toxin F-2 the production of a biomass rich in protein was essayed, which would contribute to the covering of the feed protein shortage and provide a fodder of an amino acid composition more favourable than the original raw material.

1. Materials and methods

1.1. Materials

1.1.1. Corn. Healthy corn was used for the model experiments and a corn of 30 mg kg^{-1} toxin F-2 content for fermentation.

1.1.2. Reagents. Superphosphate, dried malt of brewing quality.

All the other reagents were of analytical grade.

1.2. Microbiological methods

1.2.1. Maintenance of yeasts. The yeasts were maintained on solid malt medium.

1.2.2. Media

1.2.2.1. Medium applied in model experiments. — Medium used in *Candida intermedia* cultivation serving to determine the quality of the nitrogen source.

The shaken cultures contained 1.5% maltose. In addition to 2 g dm^{-3} superphosphate as much of the nitrogen source as corresponded to 1.4 g nitrogen per dm^3. The following compounds were used as nitrogen source: KNO_3, NH_4NO_3, NH_4Cl, $(NH_4)_2SO_4$, $(NH_4)_2HPO_4$ and urea.

The fermentation period was 48 h.

Medium used in cultivation of *Candida intermedia* on malt. A loop-ful of *Candida intermedia*, grown on solid malt medium was suspended in 50 cm^3 of a nutrient medium containing 1.5% maltose, 1.5 g dm^{-3} urea and 2 g dm^{-3} superphosphate. The culture was shaken for 24 h at 30 °C. The yeasts were then centrifuged and an amount corresponding to 1 g dry yeast was transferred to 500 cm^3 nutrient medium containing 4% malt. Urea served as nitrogen source and superphosphate as phosphorus source.

1.2.2.2. Medium to grow Candida intermedia on digested and hydrolyzed corn containing toxin F-2. — A loop-ful of *Candida intermedia*, grown on solid malt medium was transferred to 50 g synthetic medium containing 1.5% malt. This was used as inoculum after shaking for 24 h at 32 °C and centrifuging.

The medium used to propagate the yeast contained 100 g hydrolyzed corn meal, 6 g dm^{-3} of carbamid and 3 g dm^{-3} of superphosphate. The inoculum amounted to 4% of corn.

1.2.2.3. Digestion and hydrolysis of corn. — Three hundred g of corn meal were suspended in 450 cm^3 water, 4.5 cm^3 of conc. H_2SO_4 were added and this

mass was autoclaved at 0.2 MPa for 30 min. After cooling 10% malt, related to the original dry mixture, was added, the pH was adjusted to 5.5 and it was kept at 60 °C for 1 h. The toxin content of the contaminated corn did not change during digestion.

1.2.3. Various methods of propagation

1.2.3.1. Propagation under shaking. — To each of 35 Erlenmeyer flasks of 250 cm^3 volume 50 cm^3 nutrient medium was transferred. The pH of the medium was controlled every hour and when necessary, was adjusted to the level required. The yeast culture was shaken at 180 r.p.m., corresponding to 30 mMol $dm^{-3}\,h^{-1}$ oxygen dilution rate.

1.2.3.2. Propagation in tube fermentor. — A jacketed tube fermentor of 1 dm^3 capacity was used. Air distribution was provided for by a G-1 glass filter. Each fermentor was filled with 500 dm^3 nutrient medium and the temperature required was maintained by an ultrathermostat. pH was controlled every hour and adjusted as needed. The amount of air, as established by rothameter, was 500 $cm^3\,min^{-1}$, corresponding to 260 mMol $dm^{-3}\,h^{-1}$ oxygen dilution rate.

1.2.3.3. Propagation in a fermentor of Biofer type. — When a larger amount of yeast was intended to be propagated a Biofer type fermentor, manufactured in Hungary, was used.

One fermentor unit consists of 2 Johan flask glass fermentors, fitted with automated temperature control, a blender of stabilized revolution per minute under continuous control, a foam level sensor and automated foam inhibitor and an air pressure stabilizer.

The instrument unit serves to measure the parameters characterizing the state of culture and consists of an instrument each for the measurement of diluted oxygen concentration, of the redox potential, of pH, of the CO_2 concentration, in the gas leaving the fermentor and of the temperature of the culture and the recorders belonging to each instrument.

For yeast propagation 7000 cm^3 nutrient medium was used in the glass fermentor. The introduced air stream provided an oxygen dilution rate of 300 $dm^{-3}\,h^{-1}$.

1.3. Physico-chemical methods

1.3.1. Toxin F-2 extraction from the raw material and its quantitative determination by chemical methods.

1.3.1.1. Extraction of toxin F-2 and purification. — Ten g of finely milled corn were extracted in a Soxhlet apparatus for 7 h. The solvent was evaporated in a Rotadest evaporator and the toxin containing the residue was purified by alkali treatment (Mirocha et al., 1974).

1.3.1.2. Identification by thin-layer chromatography. — Macherey–Nagel Polygram plates (with 0.25 mm Kieselgel layer) of 20×20 cm size, were used.

They were activated at 110 °C for 1 h. Chloroform–ethanol (95 : 5) was used as a developing system. Quantitative determination was carried out by comparing the spot of the unknown with the spot of the known substance. For the separation of toxin F-2 and its metabolites the following solvents were applied:

— chloroform—ethanol (97 : 3);
— chloroform—methanol (9 : 1);
— benzene—methanol (9 : 1);
— benzene—methanol—acetic acid (18 : 1 : 1);
— benzene—acetic acid (9 : 1).

To identify toxin F-2 the following methods, known from the related literature were used:

– Toxin F-2 under ultraviolet light showed a greenish-blue fluorescence at 254 nm, while pale blue fluorescence at 366 nm (MIROCHA et al., 1974).

– Spraying the plate with a 2% solution of 4-methoxi-benzene-diazonium-fluoroborate gives a reddish-brown discoloration after about 5 min. Spraying this spot with 0.1 *N* alcoholic potassium hydroxide solution it turns violet (SARUDI, 1974).

1.3.1.3. UV spectrophotometry. — A Specord-type spectrophotometer was used. To take the calibration curve crystalline toxin F-2 was applied. Optical density (OD) was determined in a methanol solution versus methanol (SARUDI, 1976). Toxin F-2 was purified by alkaline treatment and subsequent thin-layer chromatography.

1.3.1.4. Gas chromatography. — A Varian 1800 type instrument with flame ionization detector was used. Parameters: nitrogen carrier gas: 20 cm^3 min^{-1}; length of column: 1 m; inner diameter: 2.3 mm. The column was packed with Gas-Chrom Q, mesh 100–200 wetted with 3% OV-17 liquid phase.

Temperatures were: injector 285 °C; thermostat 180–280 °C at a program of 4 °C per min; detector 285 °C. The toxins were determined as trimethylsylil (TMS) ether derivatives (MIROCHA et al., 1974).

1.3.1.5. Protein determination. — To determine the protein content an automated Kjel–Foss apparatus was used.

1.3.2. Quantitatve determination of the bio-mass obtained in the model experiments and in fermentations with toxin infected corn. The amount of yeast was established by determining the dry matter content of cells.

In the model experiments to determine the dry matter content of cells the turbidity at 450 nm was measured with an extinctionmeter and the value obtained was converted for 100 cm^3 nutrient medium on the basis of the calibration curve. The correlation between the extinction values and the dry matter content of yeast cells was established by means of determining the dry matter content on a membrane filter.

In fermentations on digested and hydrolyzed corn the biomass was measured by centrifuging and drying to constant weight at 70 °C.

1.3.3. Determination of the sugar content. The method of Somogyi was used to determine the sugar in the fermentation liquor.

1.3.4. Quantitative determination of the amino acids. The amino acid composition of the biomass was established from the acidic hydrolysate with a Labor MIM Aminochrom type apparatus.

2. Results

2.1. Determination of the optimal parameters of fermentation

It was established in preliminary experiments that the toxin F-2 resistance of various yeast and bacterium strains was different in solid toxin containing medium. The microorganisms which were not growth-inhibited when cultivated on toxin containing solid medium were found to be able to decompose toxin F-2 in a medium containing F-2 infected corn digested under aerobic conditions and sacharified (SAWINSKY, 1977).

Of the strains studied *Candida intermedia* was selected and conditions of growth and its capacity to decompose toxin F-2 were examined.

Since the starch content of corn is degraded mainly to maltose by enzymatic hydrolysis, therefore model experiments were carried out to establish the exact technology. In these experiments maltose was used as carbon source.

According to the relevant literature in fermenting carbohydrate assimilating yeasts various nitrogen sources may be used. Thus, the effect of the quality of nitrogen sources on the growth of *Candida intermedia* was studied in preliminary experiments.

The correlation between the growth of *Candida intermedia* and the nitrogen source applied is shown in Table 1.

In order to establish the optimum conditions of propagating *Candida intermedia* on maltose the orthogonal Latin square design scheme as developed by BIRJUKOV (1968) was applied.

The following parameters were studied: temperature (26, 28, 30, 32 and 34 °C); pH (3.0, 3.5, 4.0, 4.5 and 5.0); urea (3, 4, 5, 6, and 7 g dm^{-3}); superphosphate (1.5, 2.0, 2.5, 3.0 and 3.5 g dm^{-3}); air flow (200, 300, 400, 500 and 700 $dm^3\ h^{-1}$).

The scheme applied is summed up in Table 2.

The experiments were carried out according to the above scheme. The results were used to calculate the effect of the level of factors on the basis of the following equations:

$$b_{ik} = \frac{m}{N} \Sigma Y_{ik} - \overline{Y} \qquad (1)$$

where

b_{ik} = the effect of k level of i factor upon the results of fermentation (protein content of biomass)
m = the number of levels ($m = 5$)
N = number of variants ($N = 25$)
Y_{ik} = value $\overline{Y}$ belonging to k level of i factor

$$\overline{Y} = \frac{1}{N} \Sigma Y \tag{2}$$

where

Y = result of a fermentation carried out at a given variation of factors
$\overline{Y}$ = average of the results of $N = 25$ fermentations carried out according to the mathematical scheme.

Table 1

Effect of the quality of the nitrogen source on the growth of Candida intermedia *in shaken culture*
(Fermentation was carried out in a medium containing 1.5% maltose at 30 °C for 48 h)

Nitrogen source	$\bar{x}$	$s_{\bar{x}}$
Urea	6.20	0.265
$(NH_4)_2HPO_4$	5.72	0.283
$(NH_4)_2SO_4$	5.65	0.265
NH_4NO_3	5.30	0.245
NH_4Cl	5.00	0.245
KNO_3	4.10	0.265

n = 4
$\bar{x}$ = mean values of growth (g dm^{-3})
$s_{\bar{x}}$ = deviation of the means

Significance of growth on various nitrogen sources

	KNO_3	NH_4Cl	NH_4NO_3	$(NH_4)_2SO_4$	$(NH_4)_2HPO_4$
Urea	***	***	**	*	*
$(NH_4)_2HPO_4$	***	**	Ø	Ø	
$(NH_4)_2SO_4$	***	*	Ø		
NH_4NO_3	***	Ø			
NH_4Cl	**				

Ø = there is no significant difference
* = difference significant ($P \geq 95\%$)
** = difference highly significant ($P \geq 99\%$)
*** = difference very highly significant ($P \geq 99.9\%$)

Table 2

Experimental design by the orthogonal Latin square scheme (5 × 5)

No. of scheme	Temperature (°C)	pH	Urea (g dm^{-3})	Superphosphate (g dm^{-3})	Air rate (dm^3 h^{-1})	Result of fermentation			
						Growth of biomass (g dm^{-3})		Protein content (%)	
						$\bar{x}$	s^2	$\bar{x}$	s^2
1	26	3.0	3.0	1.5	200	1.86	0.18	29.47	0.17
2	26	3.5	5.0	3.0	700	11.20	0.37	39.55	0.23
3	26	4.0	7.0	2.0	500	11.69	0.40	40.64	0.15
4	26	4.5	4.0	3.5	400	8.23	0.44	41.70	0.28
5	26	5.5	6.0	2.5	300	13.00	0.23	41.32	0.05
6	28	3.5	4.0	2.0	300	8.54	0.30	34.02	0.04
7	28	4.0	6.0	3.5	200	13.81	0.32	41.08	0.25
8	28	4.5	3.0	2.5	700	7.75	0.37	40.15	0.15
9	28	5.5	5.0	1.5	500	9.08	0.30	39.17	0.04
10	28	3.0	7.0	3.0	400	8.93	0.38	39.19	0.12
11	30	4.0	5.0	2.5	400	13.71	0.19	42.42	0.19
12	30	4.5	7.0	1.5	300	11.76	0.34	41.00	0.27
13	30	5.5	4.0	3.0	200	9.15	0.27	40.23	0.04
14	30	3.0	6.0	2.0	700	7.40	0.35	38.18	0.07
15	30	3.5	3.0	3.5	500	8.41	0.45	38.62	0.04
16	32	4.5	6.0	3.0	500	19.60	0.21	49.20	0.19
17	32	5.5	3.0	2.0	400	7.60	0.33	38.71	0.09
18	32	3.0	5.0	3.5	300	7.47	0.32	38.59	0.40
19	32	3.5	7.0	2.5	200	13.55	0.44	40.36	0.09
20	32	4.0	4.0	1.5	700	7.70	0.39	38.52	0.14
21	34	5.5	7.0	3.5	700	18.33	0.23	46.30	0.19
22	34	3.0	4.0	2.5	500	5.45	0.27	37.59	0.37
23	34	3.5	6.0	1.5	400	11.47	0.26	38.27	0.01
24	34	4.0	3.0	3.0	300	8.45	0.36	38.52	0.13
25	34	4.5	5.0	2.0	200	9.69	0.18	40.24	0.14

	biomass	protein
	$\overline{Y}=10.23$, $\bar{s}^2=0.32$	$\overline{Y}=39.72$, $\bar{s}^2=0.16$
	Confidence interval $\varepsilon=0.17$	Confidence interval $\varepsilon = 0.12$

The results of fermentation are the mean values of three parallel experiments

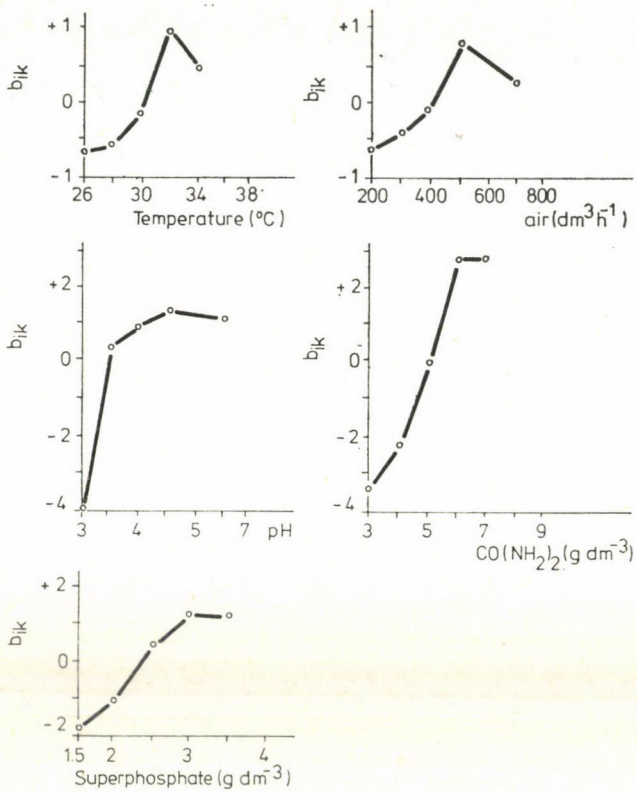

Fig. 1. Effect of the level of factors related to the growth of biomass (b_{ik}) as a function of the parameters of fermentation. $n = 3$; $\bar{s}^2 = 0.32$; $\varepsilon = 0.17$

The confidence interval of every factor was calculated by the following equation:

$$\varepsilon = t \sqrt{\frac{m \cdot \bar{s}^2}{N \cdot n}} \tag{3}$$

where

t = Student's value at $P = 95\%$ confidence level,

$\bar{s}^2$ = empirical variance.

The effect can be positive or negative depending on the level of factor improving or impairing yield (e.g. the growth of the biomass) in comparison to the mean value ($\overline{Y}$).

Values b_{ik} pertinent to the growth and protein content of the biomass were plotted against the levels of the factors studied. Figure 1 shows the effect of factor level on the growth of biomass.

The effect of factor level was calculated from the protein content of the biomass, then b_{ik} was plotted as a function of the factor levels studied.

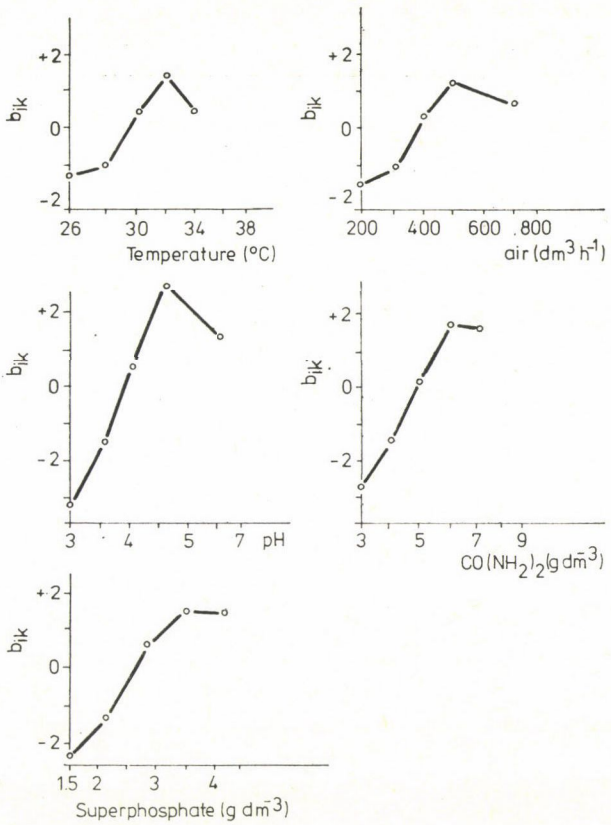

Fig. 2. Effect of the level of factors related to the protein content of the biomass (b_{ik}) as a function of the parameters of fermentation. $n = 3$; $\bar{s}^2 = 0.32$; $\varepsilon = 0.17$

Thus the optimum level of each factor was established and is shown in Fig. 2.

2.2. Analysis of the metabolite of toxin F-2

Candida intermedia was cultured on a medium found suitable in the model experiments, containing 30 mg kg^{-1} digested, hydrolyzed corn, in tube fermentor. It was established that toxin F-2 is decomposed while a metabolite is formed. In order to identify the metabolite the toxin and its metabolite were extracted from the yeast biomass, as well as from the medium. The extracts were exposed to alkaline purification then fractionated on silicagel plates. The following retention quotients were obtained in the solvents as given in Table 3.

Table 3

Retention quotients obtained in the solvents

Solvents	Zearalenone R_f	Metabolite R_f
Chloroform–ethanol (97 : 3)	0.65	0.28
Chloroform–methanol (9 : 1)	0.84	0.60
Benzene–methanol (9 : 1)	0.56	0.26
Benzene–methanol–acetic acid (18 : 1 : 1)	0.63	0.38
Benzene–acetic acid (9 : 1)	0.30	0.15

The metabolite gives the same colour reaction as toxin F-2 with 4-bromo--benzenediazonium-fluoroborate, 4-nitro-benzenediazonium-fluoroborate and 4-methoxibenzene-fluoroborate.

The thin-layer-chromatographic separation of toxin F-2 and its metabolite is illustrated in Fig. 3. On the basis of the retention quotient the compound was identified as zearalenone because the R_f values as obtained in the above solvent systems corresponded to those given by JACKSON and co-workers (1974), HOLDER and co-workers (1977), HAGLER and co-workers (1979), respectively.

Seventyeight percent of toxin F-2 was decomposed by *Candida intermedia*

Candida intermedia, adapted by sixfold passing, decomposed toxin F-2 and the metabolite accumulated in the ferment liquor. Our findings were supported by the investigations carried out at Minnesota State University, Department of Plant Pathology. On the basis of the data of these investigations, in the biomass obtained by fermentation in the Central Food Research Institute (Hungary) neither toxin F-2 nor its metabolite could be found while the medium contained 0.1 mg kg^{-1} zearalenol. Taking into account that 30 mg kg^{-1} toxin F-2 were contained initially in the medium and at the end of fer-

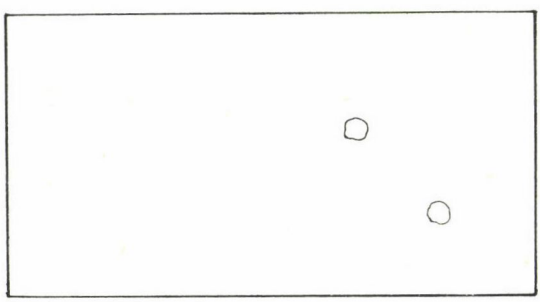

Fig. 3. Separation by thin-layer chromatography of toxin F-2 and its metabolite

Table 4

Balance of materials in Candida intermedia *cultivation on a medium containing 30 mg kg^{-1} toxin F-2 in corn digested and hydrolyzed*
(Fermentation was carried out in a tube fermentor with parameters as established in model experiments. Fermentation period: 16 h)

Amount of corn used: 50 g
Toxin F-2 in the corn: 1.5 mg

	$\bar{x}$	s_x
Starch content of corn (%)	62.1	0.72
Mass of dried biomass (g)	28.0	0.75
Protein in the biomass (%) related to dry matter	40.8	0.06
Toxin in the biomass		
F-2 (mg)	Ø	
Zearalenol (mg)	Ø	
Toxin in the medium		
F-2 (mg)	Ø	
Zearalenol (mg)	0.046	0.61

$\bar{x}$ = mean values of 4 parallel experiments
$s_{\bar{x}}$ = deviation of the means
Ø = not detectable by gas chromatography

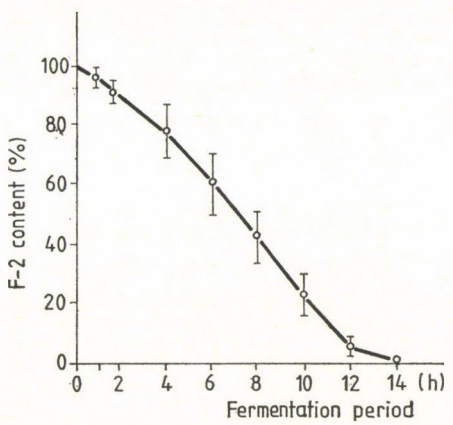

Fig. 4. Fermentation in a Biofer fermentor on digested corn contaminated by toxin F-2. Toxin F-2 content of the biomass related to the initial value

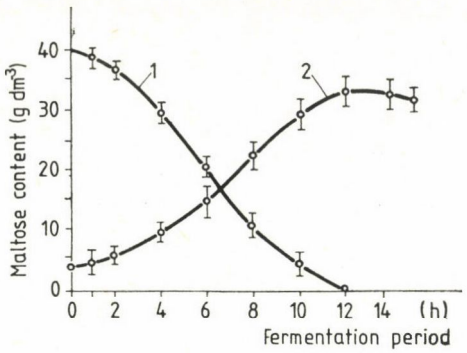

Fig. 5. Fermentation in Biofer fermentor on digested corn contaminated by toxin F-2. 1: Change of maltose content in the medium; 2: Growth curve of *Candida intermedia*

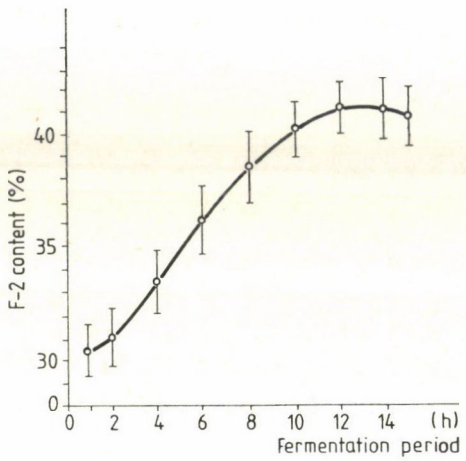

Fig. 6. Fermentation in Biofer fermentor on digested corn contaminated by toxin F-2

mentation 0.1 mg kg^{-1} zearalenol was found in it, it may be assumed that the strain used metabolized in the course of growth the toxin. However, this has yet to be proven.

The balance of materials is given in Table 4.

In order to produce a larger quantity of biomass and to be able to study the parameters of fermentation *Candida intermedia* was propagated in a Biofer type fermentor, with yeast passed six times.

The results of these experiments are illustrated in Figs. 4, 5 and 6.

Since adapted yeast was used in the experiments the lag period was very short. In the logarithmic phase the biomass increased rapidly and the

Table 5

Percentual composition of amino acids in the total amino acid of Candida intermedia *grown on a medium containing toxin F-2 contaminated corn digested and hydrolyzed*[a]

Essential amino acids	%	Non-essential amino acids	%
Lysine	6.1	Alanine	7.4
Leucine	9.7	Arginine	3.4
i-leucine	4.8	Asparagine	8.2
Threonine	5.6	Glycine	3.8
Triptophane	1.1	Glutamic acid	18.1
Valine	5.7	Histidine	3.1
Cysteine	0.1	Proline	8.0
Methionine	2.2	Serine	3.6
Phenylalanine	5.4		
Tyrosine	3.7		
Total:	44.4		55.6

[a] The Morup-Ølesen index (PV) = 48.49

sugar consumption was high. The protein content in the biomass increased intensively.

The protein content of the biomass was found to be 40.8%. Its amino acid composition and Morup-Ølesen chemical index (PV) are given in Table 5.

3. Conclusions

On testing the effect of various sources of nitrogen on the growth and protein content of *Candida intermedia* a significant difference was found between the results. Potassium nitrate proved to be significantly worse nitrogen source than urea, $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$, NH_4NO_3, NH_4Cl. The difference by t test between NH_4Cl and NH_4NO_3 was not significant, neither was it significant between NH_4NO_3 and $(NH_4)SO_4$, or NH_4NO_3 and $(NH_4)_2HPO_4$ (Table 1). Urea was found the significantly best nitrogen source. Thus urea was used in the experiments as nitrogen source.

The optimum conditions of the growth of *Candida intermedia* on maltose were established by the orthogonal Latin square design scheme of experiments. Under the given experimental conditions the following parameters were found optimal: pH 4.5, temperature of fermentation 32 °C, an aeration rate of

500 $dm^3\ h^{-1}$ in the tube fermentor, concentration of urea 6 g dm^{-3}, of superphosphate 3 g dm^{-3}.

By using the combination of these optimal parameters the optimal fermentation process was developed. Since in our experiment No. 16 the factors used in the scheme were the optimum values obtained by calculation, there was no need to carry out new experimental series.

The results show that the optimum parameters of growth are not inconsistent with the optimum parameters for the protein content of the biomass.

The optimum parameters of fermentation, as established in model experiments were applied in the experiments with toxin F-2 contaminated corn. Using *Candida intermedia*, adapted by passing several times, the biomass obtained did not contain either toxin F-2 or its metabolite. The zearalenol remained in the medium.

The attempts to establish whether alfa or beta diastereomer is the zearalenol obtained were so far not successful. To establish this seems important, because the literature shows the oestrogen activity of alfa-zearalenol three times as high as that of beta-zearalenol. The activity of the latter seems identical with that of zearalenone.

But even if it is presumed that the biologically more active form is produced during fermentation, the initial activity is reduced to one tenth of its original value and even this is not in the main product, the biomass, but in the ferment liquor, which may be considered as waste.

Thus, our results prove that corn contaminated with toxin F-2 is suitable for the production, by way of yeast fermentation, of feed protein.

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ASSAY INTO THE HEAT TREATMENT, STORAGE STABILITY AND MICROBIOLOGICAL CONDITION OF READY-TO-SERVE, PLASTIC-PACKED FOODS

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The properties of ready-to-serve foods and vegetable purees packaged on trays of polypropylene combined with polyvinylidene chloride were studied during storage. For the sake of comparison samples of the same food products were filled at the same time in glass jars and cans, too.

The trays and the polyester/aluminium/polypropylene laminate used to cover the trays tolerated well the heat and pressure load under sterilization. Data of measurement indirectly proved the rapid penetration of heat into the food on the plastic trays: under simultaneous heat treatment in the glass jars of 1 kg net weight $F_0 = 6.1$, while in the trays of 3 kg net weight $F_0 = 19.4$ heat load was given the pea puree.

Microbiological tests have shown the mesophilic aerobic total cell count to be below $10^2\ g^{-1}$ in everyone of the products. After pasteurization or sterilization none of the following microorganisms could be found in the products: coliforms, *Escherichia coli* I, sulfite reducing clostridia, staphylo- and enterococci, *Bacillus cereus*.

It was found that products packaged on trays of combined foil were significantly better after 2—3 months storage than those packaged on polypropylene trays ($P \geq 99\%$).

The shelf life of pea puree on the coated trays was 9 months. Ready-to--serve meals containing meat on single layer trays had a shelf life of 1–2 months at room temperature. By using coated foil their shelf life could be extended for another 1–2 months. The cause of a shelf life lower than expected was that the polyvinylidene chloride coating deteriorated rapidly under heat treatment and interaction with the food components and thus could not exert protective effect. Since the increase in shelf life by using coated trays does not compensate for the three-fold price of these trays in contrast to single layer trays, their use does not seem to be indicated. This investigation led to the conclusion that neither from the aspect of price, nor from that of keeping quality of foods compete polypropylene trays with the traditional packaging materials.

Ready-to-serve meals are considered generally to be suitable for consumption directly upon heating with or without the addition of water. From the point of quality and nutritive value quick-frozen foods are considered to approximate freshly prepared food. However, the costs and energy requirements of quick-freezing and refrigerated distribution imposes a burden even on the countries of highly developed industry. The quality of canned goods is lower than that of quick-frozen ones and the price of tin plate is continually rising causing problems mainly in the countries which have to rely on imports.

The development of plastic packaging for ready-to-serve meals started about the fifties. The two main trends of development were aimed at flexible

foils and semirigid trays. Depending on the purpose of utilization both systems can be made suitable for wrapping of pasteurized meals kept in cold storage or sterilized goods stable at room temperature, too.

In Hungary the use of polyethylene and polypropylene for wrapping heat treated food items was suggested already in 1962 by TELEGDY-KOVÁTS and SZILAS-KELEMEN in their book. Attention was called also to the low oxygen and flavour permeability of polyvinylidene chloride copolymer.

First industrial scale attempt to use a film of polyamide base for wrapping of meals with a keeping quality of 2–3 weeks in refrigerated storage was applied in the Nacka process. These products were used as institutional meals in hospitals and mines in Sweden (DELPHIN, 1968). According to LEEMAN (1975) the main advantage of using plastic pouches and trays lays in the short time of heat treatment required because of the thin layer of the goods packaged in them. He suggests as technical solution the application of preformed pouches or trays or the use of manufacturing lines where the filling and closing equipment is preceded by a machine which manufactures the pouches or trays on the spot from reels of plastic foil. He maintains that these wrapping materials can ensure a keeping quality of 1 or 2 months by increasing the thickness of the foil, by combining various kinds of foil or primarily by sandwiching an aluminium foil between two plastic films.

A packaging material made of 3 laminated layers, outside polyester, in the middle aluminium and on the inside polypropylene and called "retort pouch" was developed and successfully used first in the United States. Due to the excellent sealing capacity of the aluminium foil to light, gas and vapour this "retort pouch" gives a wrapping in which the sterilized meal can be kept without spoilage at room temperature for 1–2 years. Further advantages of plastic packaging, as pointed out by MERMELSTEIN (1976) are: because of the rapid heat penetration the food does not get overcooked when sterilized as in the traditional cans and jars, it does not need more than 5 minutes to warm the food prior to consumption and can be simply cut open with a knife or scissors. Thus it is useful in supplying people working in distant places, tourists, soldiers and single householders. In Japan already in 1974 500 million portions were marketed in this kind of pouch. In the United States first only the astronauts and soldiers were provided with food packaged in such pouches and only after permission has been granted by FDA was tried to sell it to the general public. In 1980 packaging 2–3 kg of food was started for institutional feeding purposes. This was aimed at replacing cans of 3 kg net weight. The, cost of a container for 1 gallon food using plastic pouches amounts to 0.27 $ while the same, using tin cans, amounts to 0.55 $ (BADENHOP & MILLEVILLE, 1980).

Further time and work saving can be achieved by using trays, because the food can be consumed directly from the tray which is then thrown away.

Thus the labour of washing up is saved and this is important for canteens suffering from labour shortage.

At the time of starting our work the building of a polypropylene plant in Hungary was in progress, therefore we wanted to find out how far would it be possible to replace the tin plate hitherto imported by this new plastic material made at home. A further aim was to compare the shelf life of foods wrapped in polypropylene or in polypropylene coated with polyvinylidene chloride. Data in the literature (MERZ, 1976) suggest that a polyvinylidene chloride coating reduces the permeability to air and thus permits of longer shelf life.

1. Materials and methods

1.1. Plastic wrappings

At the time of the experiments the Hungarian polypropylene factory was not working yet, therefore the material had to be imported.

1.1.1. Polypropylene trays. These were manufactured by Adolf Illig Maschinenbau GmbH. Co., (Heilbronn, FRG) from the polypropylene called "Carlona P" of the firm Shell.

The size of the trays was $208 \times 158 \times 30$ mm and they could hold 0.9–1.0 kg food. From here on these will be called PP trays.

1.1.2. M-pack trays. The trays were made of a 3-ply laminate by 4P Nicolaus Kempten (Göttingen, FRG). The outside and the inside layer was polypropylene homopolymer, while the sandwich layer was vinylidene chloride—vinyl chloride copolymer. Two different size trays were used. The 1 kg trays were of $208 \times 145 \times 50$ mm and the 2.5 kg trays were of $305 \times 238 \times 45$ mm. These trays will be called M-pack or PP-PVDC trays.

1.1.3. Covering foil. The trays were covered with Extruminium ST PETKP 12/12/75 PP laminated foil. This was also the product of the firm 4P Nicolaus Kempten. The outside layer was of a 12 μm polyester film, the middle layer was a 12 μm aluminium foil while the inside layer in touch with food a polypropylene foil of 75 μm thickness.

1.2. Traditional containers

The control samples were filled in jars of 850 cm^3 nominal capacity and in tin cans of cca 1 kg net weight.

1.3. Test samples

The ready-to-serve meals were prepared in the Debreceni Konzervgyár (Debrecen Canning Factory) in accordance with valid industrial material norms and standards. One dish, "Beef-steak in lecsó" (pieces of paprika pericarp

in tomato puree) was made—in a different way to the other dishes—according to the formula and in equipment as used in the catering trade.

1.3.1. Main dishes

1.3.1.1. Sauerkraut with pork meat. — This is a Hungarian speciality prepared from minced pork mixed with rice, sauerkraut spiced with ground paprika, onion, garlic and sour cream. When ready, 100 portions of the dish were filled in PP- and M-pack trays, resp., and 20 portions in jars as control samples. The half of the packages was sterilized, the other half pasteurized. The jars were sterilized.

1.3.1.2. Peas with pork chop. — The dish when cooked was treated in the same way as the above. Only the control samples were filled in cans instead of jars.

1.3.1.3. Beef-steak in lecsó. — When ready the dish was filled in 25 PP trays and 25 M-pack trays and all were pasteurized.

1.3.2. Pulped vegetables

1.3.2.1. Pea puree. — In addition to pulped peas it contained fat, salt, sugar, ascorbic acid and antioxidant. The product was filled in 50 M-pack trays of 2.5 kg capacity. Half of them was pasteurized, while the other half sterilized. 10 glass jars were also filled with the pea puree for control.

1.3.2.2. Carrot puree. — Additives were not added to the pulped vegetable. It was packaged and heat treated in the same way as the pea puree.

1.4. Experimental equipment and methods

1.4.1. Closing machine. The covering foil was heat sealed to the rim of the tray on a HSP 20—2 type machine borrowed from the firm Illig. The temperature of the sealing bar was 210–220 °C.

1.4.2. Heat treatment. Heat treatment was carried out in one of the 1.4 m^3 retorts of the factory. For pasteurizing the packs were kept in the closed retort for 40 min at 100 °C, for sterilization they were kept at 121 °C till the desired F_0 value was reached. In order to be able to control heat treatment the core temperature (this is the temperature measured at the slowest heating point i.e. at the geometric centre of the food) of the samples was measured every 2 minutes by means of an Ellab type electric thermometer connected to a copper-constantan thermocouple, and the F_0 value was calculated in the course of process. Since the retort was only for short periods at our disposal we were obliged to heat-treat the samples whether on trays of plastic or in traditional containers at the same time in the same retort. On this occasions the treatment was controlled so that even in the container of slowest heat penetration at least $F_0 = 3$, but possibly $F_0 = 5$–6 heat treatment shall be given. Thus, in packs where heat penetration was rapid a heat load much higher than required was given.

1.4.3. Storage. Pasteurized goods were stored at 3 ± 1 °C in Labor MIM (Hungary) 1292 (TX-1) type heating-cooling thermostats.

1.4.4. Microbiological methods. The total mesophilic aerobic cell and spore counts were determined in nutrient broth (OXOID/CM 1), the vegetative cells and spores of facultative anaerobic microbes on Holmann's medium by the most probable numbers (MPN) method. In accordance with the aim of our work we tried to detect microorganisms important from the aspects of food hygiene. To detect coliforms McConkey's lactose medium, and to establish the presence of sulfite reducing clostridia Takács–Narayan's semiliquid sulfite-agar medium was used. *Staphylococcus aureus* was detected on the selective medium of Baird–Parker according to the related HUNGARIAN STANDARD (1977). The media were prepared by dissolving the appropriate OXOID or HUMAN powdered nutrient.

The phase tests in the course of preparation of the ready-to-serve dishes were carried out by the microbiologists in Debrecen Canning Factory with the single difference, that instead of the nutrient broth they used TGE broth (Tripton-glucose-yeast extract).

Since in the preliminary experiments spoilage was not observed, microbiological tests were carried out at the beginning of storage and at the end of the guaranteed shelf life.

1 4.5. Sensory evaluation. The colour (appearance), aroma, taste, consistency of the product and the purity of the raw materials and the preparation of the dish were evaluated by sensory scoring on a 100 point scale generally used in Hungary. The sum of points scored for each property must not exceed 100. In HUNGARIAN STANDARD (1971) of the title "Preserved foods" it is specified that a product has to achieve a total score of at least 75 and a taste score of at least 26 points (of max. 40 points) to be marketable.

At the beginning of the storage period the pasteurized products were evaluated every two weeks, and the sterilized products every three weeks. Later the evaluations, particularly of the foods kept at lower temperature were less frequent. The panel consisted of 7–10 members.

The average scores were calculated and the rank sums, then the significance of differences between rank sums was calculated according to KRAMER (1960). Simultaneously with scoring triangular tests (BENGTSSON, 1953) were also carried out. These were very useful in deciding whether a difference in scores should be considered a change of quality or not. Namely, it is much simpler to come to a decision in a triangular test than to score 5 properties in a complicated scoring system.

1.4.6. Determination of the shelf life. The shelf life was determined graphically subsequent to regression analysis. The total score (sum of scores given individual sensory properties) was plotted against storage time. The intersection of the shelf life curve thus obtained and of the horizontal line drawn

from point 75 of the score axis (Y) was projected on the time axis (X). Since the deviation between individual scores and the average score exceeded 10% on some occasions we wished to determine the standard deviation of shelf life. Therefore, for the products where a linear correlation existed between the total score and the storage time, the regression line was constructed. The intersection of this and the horizontal line were projected on the time axis and this was considered the mean shelf life. From the standard deviation of the regression line the minimal and maximal shelf life was constructed, too.

1.4.7. Methods of chemical analysis. The two trays compared in these experiments differed mainly in their permeability to oxygen. Therefore it was found important to determine some chemical characteristics, which are suitable to follow up the oxidative changes in foods.

1.4.7.1. Determination of vitamin C. — The total vitamin C content (ascorbic acid + dehydroascorbic acid) was determined according to PETRÓ-TURZA (1977). The principle of the method is to oxidize with bromine the ascorbic acid in the sample to dehydroascorbic acid and this is then transformed with 2,4-dinitro-phenylhydrazine into osazon. In this second step the original dehydroascorbic acid content is turned into osazon, too. The disturbing osazons are separated by thin-layer chromatography on silicagel layer using benzene–aceton–pyridine (80 : 12 : 8) solvent. The dehydroascorbic acid osazon is quantitated by visually comparing the spots with those of parallel run DAS osazon of known concentration.

1.4.7.2. Determination of carotene. — The principle of the method used (KRAMER-FALUS, 1977) is that the sample to be examined is saponified with alcoholic potassium hydroxide then the carotenoids extracted by shaking several times with petroleum ether. The united extracts are then filled to mark in a volumetric flask and an aliquot, after desiccation with anhydrous Na_2SO_4 is applied to a Brockmann II aluminium oxide column.

The beta-carotene is then eluted from the aluminium oxide column with petroleum ether and thereby separated from the accompanying carotenoids. The absorption of the eluate at 430 nm was established on a GILFORD 2400 spectrophotometer. The beta-carotene content of the sample was calculated from the absorbance by equation $E^{1\%}_{1\,cm} = 198$, taking into account the dilutions.

1.4.7.3. Determination of thiobarbituric acid (TBA) number. — To characterize the autoxidation processes occurring in the fat content of the meat in the dishes the TBA numbers were determined. This determination is based on the colour reaction between malondialdehyde, formed in the course of lipid oxidation and thiobarbituric acid. The determination was carried out according to the distillation method of TARLAGDIS and co-workers (1960). The malondialdehyde content was established by means of the calibration curve plotted from the dilution series of the aqueous solution of 1,1,3,3-

-tetraethoxi propan (malondialdehyde tetraethyl acetal) (final concentration: 10^{-8} *M*). The result was expressed as the malondialdehyde in 1000 g sample.

2. Results

2.1. Results of heat treatments

An important aim of our work was to establish how a heat load can be ensured under plant conditions which enables the consumption and storage of plastic-packed foods free from hazard to health and from microbiological spoilage at the price of relatively small reduction in their hedonic and nutritive value.

Data of heat treatment are summarized in Table 1.

Table 1
Heat treatment of the products

Product	Formula of sterilization[b]	Sterilization equivalent (F_o)
Sauerkraut with pork		
PP tray	$\frac{24–72–32}{121}$	—
M-pack tray (1 kg capacity)	$\frac{24–72–32}{121}$	3.45
Glass jar	$\frac{30–80–70}{121}$	—
Snap beans with pork		
PP tray	$\frac{26–60–30}{121}$	24.52
M-pack tray (1 kg capacity)	$\frac{26–60–30}{121}$	4.39
Can	$\frac{20–105–H}{121}$	7.45
Carrot puree		
M-pack tray (2.5 kg capacity)	$\frac{22–70–H}{121}$	3.10[a]
Glass jar	$\frac{22–70–H}{121}$	7.31
Pea puree		
M-pack tray (2.5 kg capacity)	$\frac{22–70–H}{121}$	19.41
Glass jar	$\frac{22–70–H}{121}$	6.05

PP: polypropylene
M-pack: polypropylene-polyvinylidene-polypropylene foil
[a]: The thermocouple went wrong
[b] in the numerator: minutes necessary to heat the retort — minutes at maximum temperature — (H: minutes necessary to cool the retort, time was not established)
in the denominator: maximum temperature of the retort during processing

The Table, containing the formulae of sterilization and time equivalents at 121 °C (=F_0 values), shows that of the samples treated in the same retort at the same time the heat treatment was adjusted to those of slower heat penetration. Thus, when the snap bean dish on M-pack tray of slower heat penetration obtained the $F_0 = 4.4$ heat load, the same dish on polypropylene tray of quicker heat penetration was given a heat load of $F_0 = 24.5$, substantially higher than necessary. The same product packed in cans was sterilized separately. It was not difficult to set the sterilization equivalent $F_0 = 7.5$ near the value as given in the literature (WIRTH et al., 1975) Figure 1 shows unambiguously the advantage of the shallow plastic trays over the traditional cylindrical glass jars in the case of pea puree from the aspect of heat treatment.

Figure 1/a depicts the temperature of the retort and the core temperature in pea puree filled in glass jars and in 2.5 kg M-pack trays, respectively, as a function of sterilization time. Figure 1/b illustrates the lethal rate as calculated

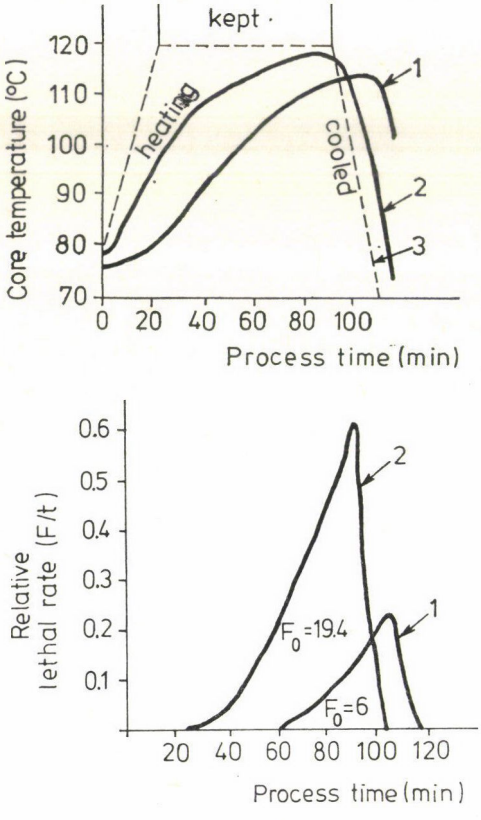

Fig. 1. Sterilization of "Pea puree" filled in glass jars and M-pack trays (polypropylene-polyvinylidenechloride-polypropylene). 1a. Core temperature curves; 1b. Sterilization curves. Curves: 1 = 5/4 glass jar; 2 = 2.5 kg plastic tray; 3 = autoclave

from the core temperature as a function of heat treatment time (when z = 10 °C). As it can be seen the temperature at the geometric center of the puree in jars follows the temperature of the retort slower than the puree on plastic trays and at a certain point of time is lower by about 10–15 °C than the core temperature of the latter. The temperature at the core of the jar reaches its maximum 12 minutes later than in the core of the plastic tray. It follows therefrom that while the pea puree in the jar obtained the heat load of $F_0 = 6.1$ equivalent, the pea puree on the trays suffered a heat treatment of $F_0 = 19.4$ equivalent.

2.2. Results of the microbiological tests

The microbiological pattern of the two ready-to-serve meals and two kinds of vegetable puree wrapped and heat treated in various ways are summed up in Tables 2 to 5.

In Table 2 data belonging to "Sauerkraut with pork" dish are given. The samples were tested directly upon cooking, after heat treatment and after 6 and 9 months storage. As shown by the data, the mesophilic aerobic total cell count of the cooked dishes was reduced by at least 1 order of magnitude by both pasteurization and sterilization. The last microbiological examination after storage of the sterilized foods was carried out at another institute. This may account for the slight increase in cell count.

The microbiology of the "Snap beans and pork" dish is summarized in Table 3. With this product a reduction in cell count due to heat treatment could not be observed. However, at the end of storage the aerobic mesophilic total cell count of every variant was lower than the initial one.

Table 4 shows the mesophilic aerobic total cell count of "Pea puree" which, in consequence of the high heat load ($F_0 = 19.4$), was reduced by 2 orders of magnitude. The effect of pasteurization was, of course, lower at both the initial and final point of time. Upon cooking the presence of coliforms was detected in "Pea puree" at a $10^1\ g^{-1}$ level, but they were killed by subsequent thermal processing.

"Carrot puree" was packaged also on M-pack trays of 2.5 kg capacity. Sterilization caused a drastic reduction of cell count, the product could be considered almost sterile (Table 5).

Summing up the results as shown in the four Tables, the viable cell count in both kinds of plastic wrappings did not exceed significantly the $10^2\ g^{-1}$ level either after heat treatment or after a storage period of at least 6 months. It is very important from the aspect of hygiene that microorganisms belonging in the group of staphylococci or enterococci, *Escherichia coli* I, coliform and sulfite-reducing clostridia could not be detected in any of the products after pasteurization or sterilization.

Table 2

Cell count in ready-to-serve "Sauerkraut with pork" at various stages of processing
Sterilized samples were stored at 22 °C; pasteurized samples at 3 °C

Wrapping	Stage of processing	Microbial count (g^{-1})							
		Mesophilic aerobic		Mesophilic anaerobic		Coli-forms	Sulfite-reducing clostridia	Staphylo-cocci	Entero-cocci
		total	spore	total	spore				
		count		count					
	Upon cooking	$4.3 \cdot 10^2$	$2.3 \cdot 10^1$	$5.5 \cdot 10^2$	10^1	1.3	neg	neg	neg
PP tray (1 kg capacity)	Upon sterilization	$2.3 \cdot 10^1$	2.3	<1	<1	neg	neg	neg	neg
	On the 169th day of storage (*b*)	$7.9 \cdot 10^1$	nt	neg	nt	nt	nt	nt	nt
PP-PVDC tray (1 kg capacity)	Upon sterilization	$1.25 \cdot 10^1$	2.3	<1	<1	neg	neg	neg	neg
	On the 169th day of storage (*b*)	$1.4 \cdot 10^2$	nt	$8 \cdot 10^1$	nt	nt	nt	nt	nt
PP tray (1 kg capacity)	Upon pasteurization	$2.3 \cdot 10^1$	2.3	<1	<1	neg	neg	neg	neg
	On the 259th day of storage (*a*)	$<1.8 \cdot 10^1$	nt	$<1.8 \cdot 10^1$	nt	neg	neg	neg	neg
PP-PVDC tray (1 kg capacity)	Upon pasteurization	$4.3 \cdot 10^1$	2.3	6.1	2.3	neg	neg	neg	neg
	On the 259th day of storage (*a*)	$<1.8 \cdot 10^1$	nt	$1.8 \cdot 10^1$	nt	neg	neg	neg	neg

Notes: PP: polypropylene; PP-PVDC: Polyvinylidene chloride coated with polypropylene; (*a*): determined by the author; (*b*): determined in the Institute for Canning and Paprika Industries; all the other determinations were carried out in the Canning Factory, Debrecen; neg: negative; nt: not tested

Table 3

Cell count in ready-to-serve "Snap beans and pork" at various stages of processing packaged in plastic trays or in traditional cans
Srerilized samples stored at 22 °C; pasteurized samples at 3 °C

Wrapping	Stage of processing	Microbial count, (g^{-1})							
		Mesophilic aerobic		Mesophilic anaerobic		Coli-form	Sulfite--reducing clostridia	Staphylo-cocci	Entero-cocci
		total	spore	total	spore				
		count		count					
	Upon cooking	$1.1 \cdot 10^2$	$2.3 \cdot 10^1$	10^2	10^1	neg	neg	neg	neg
PP tray (1 kg capacity)	Upon sterilization	$1.2 \cdot 10^2$	2.3	$3.3 \cdot 10^2$	2.3	neg	neg	neg	neg
	On the 168th day of storage (*b*)	$1.2 \cdot 10^1$	nt	1a	nt	nt	nt	nt	nt
PP-PVDC tray (1 kg capacity)	Upon sterilization	$2.3 \cdot 10^2$	2.3	10^2	<1	neg	neg	neg	neg
	On the 29th day of storage (*b*)	$2.9 \cdot 10^2$	nt	nt	nt	nt	nt	nt	nt
	On the 168th day of storage (*b*)	$1.2 \cdot 10^1$	nt	$2.3 \cdot 10^1$	nt	nt	nt	nt	nt
Cans	Upon sterilization	$2.3 \cdot 10^2$	2.3	10^2	<1	neg	neg	neg	neg
PP tray (1 kg capacity)	Upon pasteurization	$1.3 \cdot 10^1$	2.3	6	<1	neg	neg	neg	neg
	On the 259th day of storage (*a*)	$<1.8 \cdot 10^1$	nt	$1.8 \cdot 10^1$	nt	neg	neg	neg	neg
PP-PVDC tray (1 kg capacity)	Upon pasteurization	$1.2 \cdot 10^2$	$1.5 \cdot 10^1$	$5.5 \cdot 10^1$	5.5	neg	neg	neg	neg
	On the 259th day of storage (*a*)	$1.8 \cdot 10^1$	nt	neg	nt	neg	neg	neg	nt

For Notes see Table 2

Table 4

Microbial count in "Pea puree" at various stages of processing in plastic and traditional wrapping
Sterilized samples were stored at 22 °C; pasteurized samples at 3 °C

Wrapping	Stage of processing	Microbial count, (g^{-1})							
		Mesophilic aerobic		Mesophilic anaerobic		Coli-form	Sulfite-reducing clostridia	Staphylo-cocci	Escherichia coli I
		total	spore	total	spore				
		count		count					
	Upon cooking	$2.3 \cdot 10^2$	4.3	10^2	<1	$1.4 \cdot 10^1$	neg	neg	neg
PP-PVDC tray (2.5 kg capacity)	Upon sterilization	2.3	neg	nt	neg	neg	neg	neg	neg
	On the 167th day of storage (*b*)	<1	nt	4.7	nt	nt	nt	nt	nt
Glass jar	Upon sterilization	$2.3 \cdot 10^1$	2.3	nt	<1	neg	neg	neg	neg
PP-PVDC tray (2.5 kg capacity)	Upon pasteurization	$1.3 \cdot 10^1$	2.3	nt	<1	neg	neg	neg	neg
	On the 253rd day of storage (*a*)	$1.2 \cdot 10^2$	nt	$1.8 \cdot 10^1$	nt	neg	neg	neg	nt

For Notes see Table 2

Table 5

Microbial count in "Carrot puree" packaged in plastic and traditional wrapping
Sterilized samples were stored at 22 °C, pasteurized samples at 3 °C

Wrapping	Stage of processing	Microbial count (g^{-1})							
		Mesophilic aerobic		Mesophilic anaerobic		Coli-form	Sulfite-reducing clostridia	Staphylo-cocci	Escherichia coli I
		total	spore	total	spore				
		count		count					
	Upon cooking	$2.3 \cdot 10^2$	2.3	nt	<1	neg	0.4	neg	neg
PP-PVDC tray (2.5 kg capacity)	Upon sterilization	2.3	<2.3	nt	<1	neg	neg	neg	neg
	On the 167th day of storage (*b*)	<1	nt	2.3	nt	nt	nt	nt	nt
Glass jar	Upon sterilization	2.3	neg	nt	neg	neg	neg	neg	neg
PP-PVDC tray (2.5 kg capacity)	Upon pasteurization	$1.3 \cdot 10^1$	$1.2 \cdot 10^1$	nt	<10	neg	neg	neg	neg
	On the 253th day of storage (*a*)	$1.8 \cdot 10^1$	nt	$1.8 \cdot 10^1$	nt	nt	neg	neg	nt

For Notes see Table 2

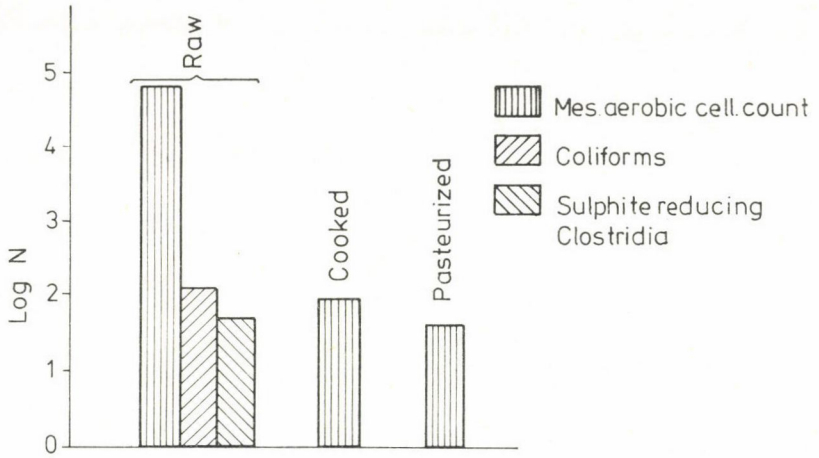

Fig. 2. Microbiological conditions of "Beef-steak in lecsó" packaged on polypropylene trays during preparation

The third ready-to-serve dish "Beef-steak in lecsó" was exposed to phase tests. The microbiological pattern of the product is illustrated in Fig. 2.

As it can be seen, the mesophilic aerobic total cell count of the raw materials was nearly 10^5 g^{-1}. Hundred and ten coliforms per g were found on the paprika slices and 46 sulfite reducing clostridia per g on the raw meat. Both kinds of microorganism were destroyed already by cooking and the mesophilic aerobic viable cell count was reduced below 10^2 g^{-1}. After this the cell reducing effect of pasteurization was minimal. The pasteurized samples contained directly after pasteurization a viable cell count of $4.2 \cdot 10^1$ g^{-1}.

In the refrigerator, at a temperature of 2–3 °C, on the 112th and the 211th day of storage a mesophilic aerobic vegetative cell count of 12 per gram was found. The results of microbiological tests in polypropylene trays and in trays of the combined foil coincided.

2.3. Results of chemical tests

2.3.1. Vitamin C analysis. Of all the products only the vitamin C content of the "Beef-steak in lecsó" was significant at the beginning of storage ($\geq$50 mg%). The pasteurized "Sauerkraut with pork" and the "Pea puree" contained about 3 mg% vitamin C while in the sterilized products only traces could be detected. Therefore the effect of packaging on the vitamin C content was studied only in "Beef-steak in lecsó" (Fig. 3).

The column diagram shows that at the time of the first determination the total ascorbic acid content of the product on M-pack trays was 56.5 mg%,

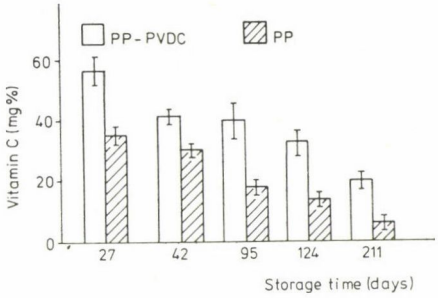

Fig. 3. Vitamin C content of "Beef-steak in lecsó" packaged on polypropylene trays (PP) and on polyvinylidene chloride combined with polypropylene M-pack trays (PP–PVDC), during storage at 3 °C

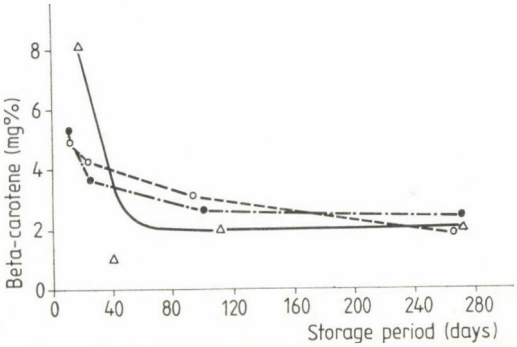

Fig. 4. Beta-carotene content of "Carrot puree" packaged on 2.5 M-pack trays (PP–PVDC), in glass jars and sterilized and on M-pack trays pasteurized, during storage at 3 °C. ○——○ PP–PVDC sterilized. ●— · —● 5/4 glass jar sterilized. △——△ PP–PVDC pasteurized

while on the PP trays it was 35.1 mg%. The determinations carried out later have also shown the vitamin C content of the product on the combined foil trays to be higher. At the last test, the samples on M-pack trays had a vitamin C content of 20 mg%, and on PP trays 6 mg%.

2.3.2. Quantity of beta-carotene in carrot puree. Figure 4 shows the beta-carotene content of "Carrot puree" on 2.5 kg M-pack trays after pasteurization and sterilization and that filled in glass jars and sterilized, during the storage period.

As it can be seen, the initial beta-carotene content of pasteurized "Carrot puree" was 8 mg%, while that of the sterilized samples 5 mg%. The beta-carotene content of the carrot puree after sterilization was the same whether on plastic tray or in glass jar and between the 12th and 103rd day of storage was reduced to the half of the original amount. The behaviour of beta-carotene after pasteurization differed from that observed after sterilization, the initial

amount was reduced to one fourth during the same period. After a storage period of 95–110 days the carotene content of the products, independently from wrapping, heat treatment and storage temperature was about 2–3 mg%. Storage was continued till the 266th day. During this period the carotene content of the sterilized carrot puree diminished on the plastic trays from 3 mg% to 1.8 mg%, while that of the other two samples did not change.

2.3.3. Results of TBA number determinations. It was not possible to follow up the change in the TBA number during the whole of the storage period, however, the tests carried out in the 5th or 6th month permit of having an insight in the character of change.

The malonaldehyde content of "Snap beans with pork" on PP and M-pack trays and in cans sterilized and stored at 22 ± 1 °C for 141 days is shown in Fig. 5.

As it can be seen, from the fat content of the meat on the polypropylene trays four times as much malonaldehyde was formed by oxidation than on the M-pack trays. The malonaldehyde content of the latter was about 20% higher than that of the product filled in cans and hermetically sealed.

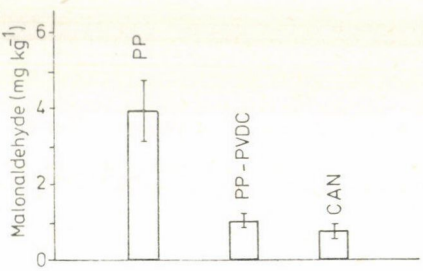

Fig. 5. Malonaldehyde content of "Snap beans with pork" packaged on polypropylene (PP) trays and on M-pack (PP–PVDC) trays and in cans and sterilized, after a storage period of 141 days at 22 °C

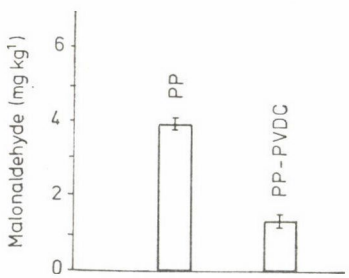

Fig. 6. Malonaldehyde content of the meat in the "Sauerkraut with pork" product packaged on polypropylene (PP) trays and M-pack (PP–PVDC) trays and pasteurized, after a storage period of 162 days at 3 °C

The malonaldehyde content of "Sauerkraut with pork" on PP and M-pack trays and stored at 3 °C for 162 days is shown in Fig. 6.

Here, too, the retarding effect of the combined tray on malonaldehyde formation is apparent.

2.4. Results of the sensory tests

2.4.1. Products sterilized and stored at 22 °C. In the sensory value of "Sauerkraut with pork" a definite change was observable in both plastic wrappings in the 6th week of storage. On this occasion the sample stored on PP tray scored 78.3, while the sample on the M-pack tray 85.3. On the 80th day of storage the total average score of the sample stored on PP tray was below the lowest acceptable level and according to ranking the total sensory value and taste value was significantly worse at the 99% probability level, than the sample on the M-pack tray (Fig. 7).

By triangular test 9 out of 10 panel members could distinguish between the products on the 2 kinds of tray ($P \geq 99\%$). All of them found the product

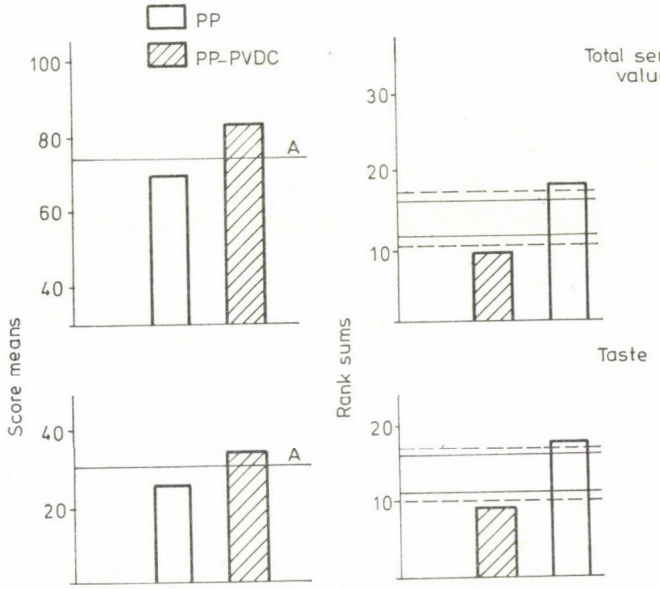

Fig. 7. Sensory evaluation by scoring and ranked on the basis of the scores of "Sauerkraut with pork" packaged on PP and PP–PVDC trays and sterilized, after 11 weeks of storage. (Storage temperature: 22 ± 1 °C; number of panelists: 9; A: minimum values of acceptability (HUNGARIAN STANDARD, 1971); PP: polypropylene tray; PP–PVDC: polypropylene combined with polyvinylidene chloride tray. Rank sums between the indicated limits do not differ significantly at 95 and 99% probability levels, resp. - - - Probability level: P = 99%. ——— Probability level: P = 95%

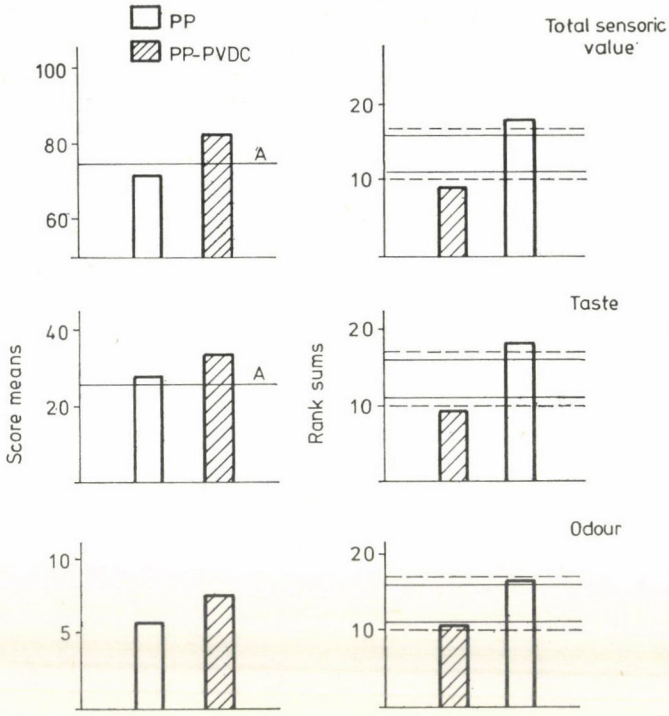

Fig. 8. Sensory evaluation by scoring and ranked on the basis of the scores "Snap beans with pork" packaged on PP and PP–PVDC trays, sterilized and stored for 91 days at 22 ± 1 °C. (Number of panelists: 9; PP: polypropylene tray; PP–PVDC: polypropylene combined with polyvinylidene tray; A: minimum of acceptability (HUNGARIAN STANDARD, 1971). Only the rank sums higher or lower than the limit values marked, differ significantly at the probability level of 95% or 99%. - - - - Probability level: P = 99%. ——— Probability level: P = 95%

on the M-pack tray better which means a very highly significant difference at the 99.9% probability level.

There was no difference observable in the"Snap beans with pork" samples on either kind of tray in the first 6 weeks of storage. Hereafter, the quality of the product on the PP tray started to deteriorate. In the sensory evaluation on the 91st day of storage this products scored 12 points less than the same product on M-pack tray. This difference was significant at the 99% probability level. The taste of the "Snap beans with pork" sample on PP tray was highly significantly lower ($P \geq 99\%$) and the odour significantly lower ($P \geq 95\%$) (Fig. 8).

The triangular test, carried out at the same time, supported the above finding: out of 9 panel members 7 made the correct difference and 6 found the M-pack better at a highly significant probability level ($P \geq 99\%$).

Since the quality of the "Snap beans with pork" sample on PP tray was below the required level it was not followed up further. However, the sample on M-pack tray was kept under control and in sensory evaluations on the 120th and 141st day it was compared to the canned sample. In the test on the 141st day of storage the M-pack with a total score of 77 was found acceptable, but in the triangular test out of 8 panel members 7 distinguished between the two kinds of packaging and 6 found the canned product better at the highly significant probability level of $P \geq 99\%$.

The initial high quality of the sterilized "Pea puree" is shown by the total score of 90–92 obtained 2 weeks after packaging. In later tests the score of the product on M-pack trays was always lower than in the glass jars. The difference in the score was only a few points. Panel members found metallic, salty and caramel off-flavour which was probably due to the fact that the product on the plastic tray was given a heat treatment about three times as high as needed. However, in spite of this, the "Pea puree" in M-pack scored 81 after 160 days storage.

Since "Carrot puree" was prepared without the addition of fat and/or seasonings and therefore had an undistinctive flavour, it was scored only on three occasions. The product on M-pack was found to be a few points better. The difference was not significant.

2.4.2. Pasteurized products, stored at 3 °C. In consequence of the lower heat treatment the pasteurized purees were of a lighter, fresher colour and taste. Because of the lower storage temperature their quality remained unchanged for a longer period than that of the sterilized ones.

Of the ready-to-serve dishes only "Beef-steak in lecsó" was of good quality after pasteurization. This exceptionally good quality was due to the fact that it was manufactured at the end of summer when fresh ingredients were available. In sensory tests held on the 11th and 20th day of storage the product on PP trays scored a few points higher than on the M-pack tray. However, on the 69th day the latter preceded by 7 points the former which scored still 82. In the last test on the 124th day of storage the product on PP trays, because of its darker colour and slight by-taste, scored only 76.

The quality of the other pasteurized ready-to-serve dishes was not satisfactory. In order to have homogeneous samples both the samples to be sterilized and those to be pasteurized were taken from the same batch, whereas in canning practice the samples for sterilization are cooked only to 50—70% doneness. The low heat treatment of pasteurization did not finish off cooking, thus the ingredients remained under-done and the flavouring substances did not penetrate the product. Particularly low was the quality of "Snap beans with pork", therefore, this product was not suitable for sensory evaluation.

The defects of preparation did not impair to the same extent "Sauerkraut with pork", therefore, the pasteurized product did not deviate so much

from the sterilized one, produced and tested on the same day. The difference between them amounted to a few tenth of points. In the 6th week of storage the M-pack sample scored 3 points more than the other plastic packed sample. In the 23rd week the difference was already 13 points and the PP-packed sample with its 72 points was not worth further testing. The M-pack sample scored 81.6 in the 37th week of storage.

2.5. Storage life of the products

The total score of "Snap beans with pork" on PP trays and sterilized is shown as a function of storage time in Fig. 9.

The mean and standard deviation of the storage life of this product, on the basis of the regression line, was found to be 80 $\pm$ 25 days at 22 $\pm$ 1 °C storage temperature. The linear correlation between the sensory value and the storage period was the closest ($r = 0.78$) with this product. The significance of this is also worth noting ($P \geq 99.9\%$). The relatively low standard deviation of shelf life shows the good agreement of the judgement of panel members. "Snap beans with pork" on M-pack trays maintained its quality for 90 days. The regression line could be fitted only to the scores of sensory tests carried out after 90 days, when the product started to deteriorate. On the basis of this straight line the shelf life of "Snap beans with pork" on M-pack trays

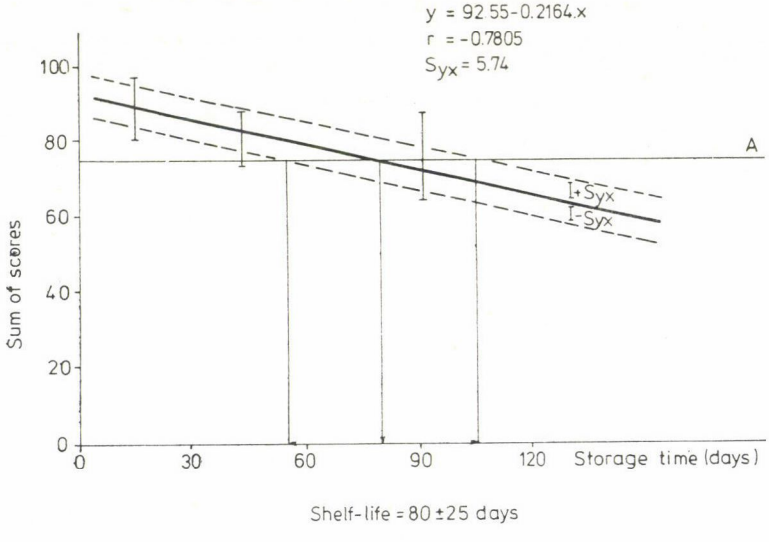

Fig. 9. Determination of the shelf life of "Snap beans with pork" packaged on PP trays, sterilized and stored for 80 days at 22 $\pm$ 1 °C, on the basis of the regression line describing the change in quality. Vertical bars represent the range of scores. A: minimum of acceptability (HUNGARIAN STANDARD, 1971). The correlation coefficient (r) was significant at a 99% probability level

is estimated to be 124 ± 60 days. Figure 10 shows the shelf life curve of sterilized "Pea puree" on 2.5 kg M-pack trays.

The shelf life of 274 ± 110 days proves this product to be substantially more stable than the above mentioned ready-to-serve dishes. The standard deviation of shelf life has increased in comparison to that of "Snap beans with pork", which is partly due to the slower rate of deterioration. The correlation coefficient of the change in time is only 0.47, but due to the 40 data used in its evaluation it is still highly significant ($P \geq 99\%$).

In the case of "Pea puree" the mild pasteurization temperature and the low storage temperature (3 °C) ensured a nearly doubled shelf life of 528 ± 184 days in comparison to that of the sterilized product (Fig. 11).

The parameters characterizing the correlation are the same for both products but because of the better initial quality and higher stability a longer axis section and a less steep slope belongs to the regression equation of the pasteurized "Pea puree".

In the case of the pasteurized and sterilized "Sauerkraut with pork" product with the sensory evaluations carried out at premeditated points of time the kinetics of change in quality could not be followed up as closely as with the other food products. The correlation coefficient of the sterilized "Sauerkraut with pork" on PP trays was $r = 0.35$ which was still significant at the probability level of 95%. For the same product on M-pack trays no

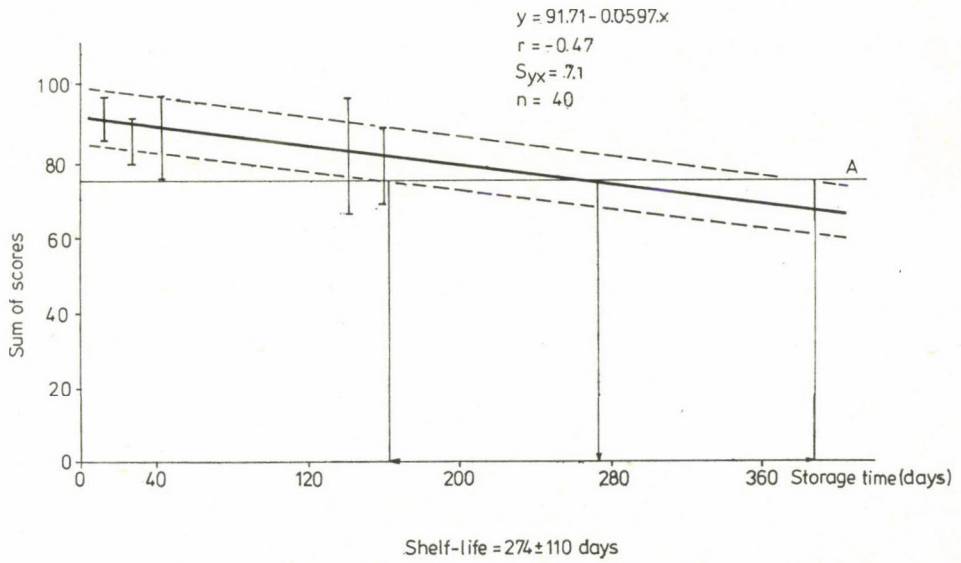

Fig. 10. Determination of shelf life of "pea puree" packaged on PP–PVDC trays, sterilized and stored at 22 ± 1 °C, on the basis of the regression line describing the change in quality. A: minimum of acceptability (HUNGARIAN STANDARD, 1971). Vertical bars represent the range of scores. The correlation coefficient (r) was significant at a 99% probability level

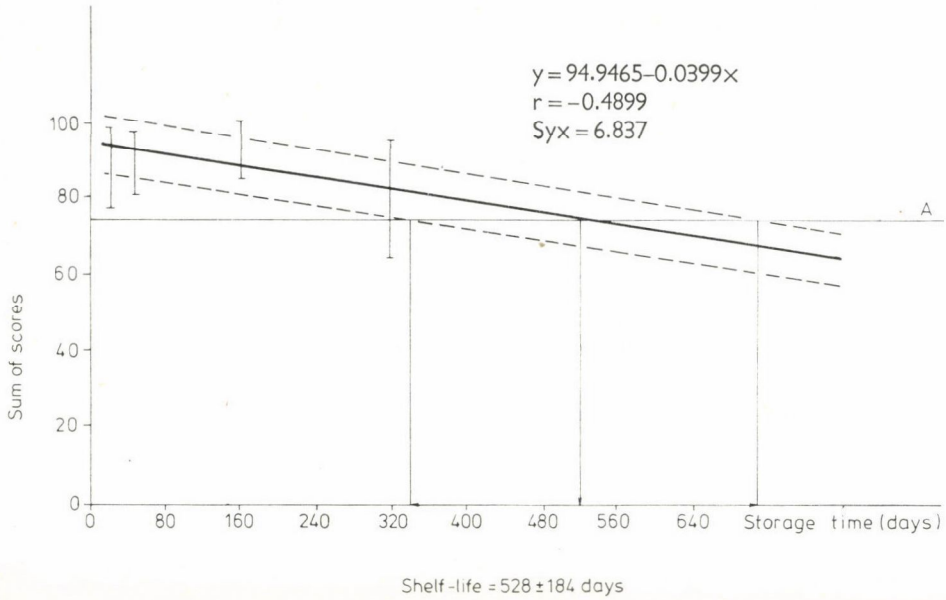

Fig. 11. Determination of the shelf life of "Pea puree" on 2.5 kg PP–PVDC trays, pasteurized, stored at 3 ± 1 °C, on the basis of the regression line describing the change in quality. A: minimum of acceptability (HUNGARIAN STANDARD, 1971). Vertical bars represent the range of scores. The correlation coefficient (r) was significant at a 99% probability level

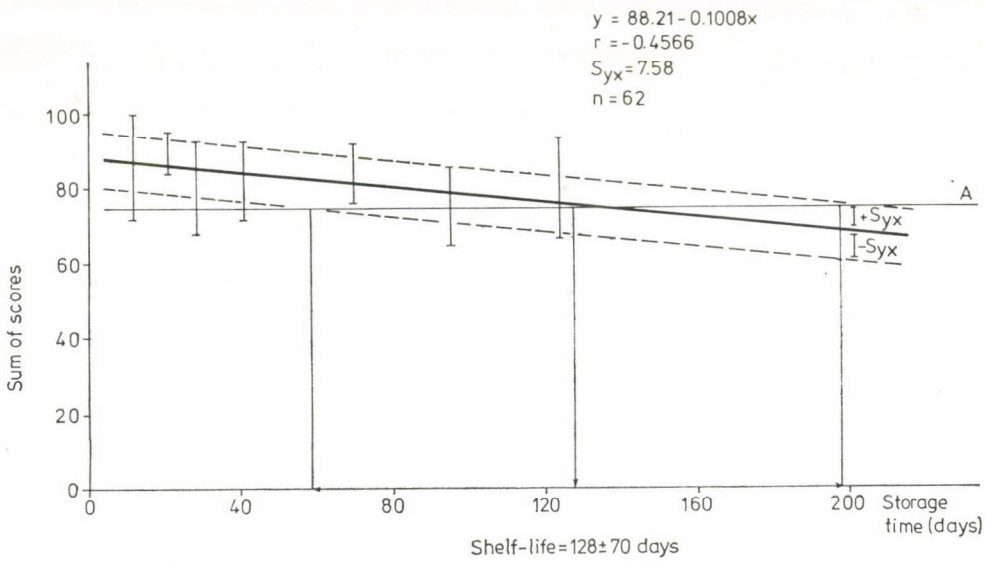

Fig. 12. Determination of the shelf life of "Beef-steak in lecsó", pasteurized and stored at 3 ± 1 °C, on the basis of the regression line describing the change in quality. A: minimum of acceptability (HUNGARIAN STANDARD, 1971). Vertical bars represent the range of scores. The correlation coefficient (r) was significant at a 99% probability level

correlation could be found, thus the shelf life was estimated on the basis of the curves connecting the average scores.

Thus, the shelf life of sterilized "Sauerkraut with pork" on PP trays was estimated to be 60 days, while that on M-pack trays 88 days. The pasteurized product on PP trays had a shelf life of 124 days and on M-pack trays it scored still 81.6 points on the 261st day of storage.

The pasteurized "Beef-steak in lecsó" was exposed 7 times to sensory evaluation during storage. Thus the equation of the regression line was calculated from 60 data (Fig. 12).

The Figure shows that even in the case of a product of good quality a large standard deviation of the average score may exist. Shelf life as seen in the figure is 128 ± 70 days. The applicability of the linear regression is proven by the fact that the slope of the line deviated from 0 at the probability of $P \geq 99.9\%$. The product on M-pack trays was still of excellent quality (total score 88) when that on the PP trays fell out. Because of the small amount of available product storage could not be continued and thus the shelf life could not be established. Considering the other products on M-pack trays the shelf life may be estimated as 2–3 times as long as that on PP trays.

3. Conclusions

It can be established that apart from some slight deformations both the single-layer and combined polypropylene trays, as well as the "Extruminium" wrapping foil as investigated in this study proved to be stable exposed to a heat treatment of 121 °C in an old type retort.

It was proven by measurements that the heat penetration in plastic trays is much quicker than either in traditional cans or glass jars. By applying plastic trays this was the first occasion of sterilizing 2.5 kg packs of vegetable puree without heat damage. The problem of sterilization of foods characterized by viscous heat conduction was considered solvable by LUND (1977) also by the correct selection of the geometry of the container.

The factory autoclaves were at our disposal for limited periods only, thus, the different types of packs could not be sterilized one by one. In consequence some of the types of packs obtained a heat treatment 2–3 times higher than needed and the adequate treatment time could not be established. SZCZEBLOWSKY & NEBESKY (1978) found that the required heating time for trays of the same size as used in these experiments was 93 min, while for 3 kg capacity traditional cans it was 215 min, which means a more than 50% saving of time.

On heat treating trays of 1 kg capacity it appeared that the food on M-pack trays higher by 20 mm, obtained a heat load one third of that obtained on

the PP trays (Table 1). This shows that the shape of the tray in itself does not garantee the rapid heat penetration. The key problem of developing the appropriate geometry is the correct proportion of the height of the tray to its length and width.

The results have shown the microbiological condition of the products manufactured, preserved and stored by us to meet the specifications of hygiene as laid down in the norms. It was proven by phase tests that heat sensitive microorganisms causing the spoilage of food were destroyed by pasteurization or sterilization and in the case of foods of low pH even by cooking. The cell count of survivors did not exceed the order of $10^2\,g^{-1}$ either in the case of sterilized goods under control for 6 months or of pasteurized goods under control for 9 months. This means also that the wrapping consisting of polypropylene base trays and Extruminium foil protected the food against reinfection. Bits of food on the edge of trays which might have served as gates of reinfection could easily be removed prior to manual sealing. When developing a technology of machine-sealing the elimination of contamination of the tray edges is of importance (TAENDLER, 1976).

Polypropylene trays combined with polyvinylidene chloride provided even at ambient temperature a better protection of the original colour, flavour and taste of the foods than those made of polypropylene only. This meant also an extended shelf life. The more than 8 month shelf life of the sterilized "Pea puree" at ambient temperature is certainly an advantage. However, the 1 to 2 months increase in the shelf life of ready-to-serve dishes is not in proportion with the price of the combined trays. According to the related literature the shelf life of ready-to-serve dishes is highly dependent on the oxygen permeability of the plastic foil. Some authors attach hopes to the use of polyvinylidene chloride because of its good gas tight property (EICHNER, 1978). The analyses of ascorbic acid and TBA number carried out in the present study permit the conclusion that oxidative changes are to some extent inhibited by the application of foil containing PVDC.

Simultaneously with our experiments the members of the Institute of the Plastics Industry carried out stress and permeability tests on the trays used in our experiments (CSER-SZÜCS & CZVIKOVSZKY, 1979). This tests have shown the gas permeabilty factor of the M-pack to be one fourth of that of the PP trays prior to contact with food. After heat treatment this increased twofold and increased further during storage. In about 5 months time the permeability of the two kinds of tray was practically the same. The scanning electronmicroscopic pictures have shown that the food components, presumably mainly the fats, have permeated the inner PP layer, reached the PVDC layer and loosened it. This loosening caused the gas tightness to be lost.

From the aspect of economy it is worth noting that some of the ready-to-serve dishes, when pasteurized and stored at low temperature maintained

their good quality for three months on the cheaper simple polypropylene trays, too.

*

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RESULTS OF EXPERIMENTS AIMED AT INCREASING THE FILTERABILITY OF MAIZE STARCH SYRUP

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Present scientific knowledge recommends that 40–60% of the human energy requirement should be covered by carbohydrate. One form of carbohydrate suitable for human consumption could be starch syrup manufacture by the acidic or enzymatic depolymerization of starch suspension. The paper deals with the problems involved in refining the hydrolysate obtained after acidic processing.

It was found that an easily filtered starch hydrolysate can be produced if the suspension is mechanically homogenized in the course of degradation and is heated simultaneously. Large-scale batch converters operate under quite different conditions, so even unchanged starch granules are to be found in the hydrolysate produced in them. Under such conditions the filterability can only be improved by previously removing the suspended matter. For this purpose the author developed a clarifying apparatus with three outlets, with which the filtering capacity of the starch syrup factory where the experiment was carried out was increased from 20 t to 30 t per day.

In accordance with present scientific knowledge it is recommended that 40—60% of the human energy requirement should be covered by carbohydrates. The majority of this should be starch, with at least a third, but at most 50% being made up of sugars (SÓLYOM, 1981). A certain amount of starch is, however, depolymerized and marketed as starch syrup. This is used in the manufacture of sugar, since the dextrin content of the syrup prevents saccharose from recrystallizing and moderates the sweet taste. It is also used widely in fruit preserves, medicinal and dietetic preparations, and in meat products (GRAEFE, 1965; SUZUKI, 1970; PALMER, 1973).

Starch solutions are easily retrograded, and this causes certain difficulties when processing and refining the hydrolysates prepared from the solutions, since this always involves filtration (BORUCH & PIERGALSKY, 1979).

As starch syrup production developed into a large-scale industry the original filter bags were replaced by filter presses. Handling the filter presses involves hard, physical work in a hot environment, as in the sugar industry. Considerable progress has been made in the sugar industry towards eliminating this (CZIRFUSZ, 1967; SZABÓ, 1967). The present paper deals with experiments aimed at modernizing the processing of starch syrup.

1. Materials and methods

1.1. Materials

Maize starch of industrial origin with a starch content of 99.5%; sulphuric acid and calcium carbonate of technical quality; industrial filter cloth, type 4733-I-72.

1.2. Methods

1.2.1. Determination of reducing sugar content. The total reducing sugar content of the hydrolysates was determined iodometrically according to Schoorl and Regenbogen and expressed as dextrose equivalent (DE).

1.2.2. Determination of suspended matter. The quantity of suspended components in the hydrolysates was determined using corrugated filter paper of type Macherey-Nagel 615/1/4 and a filtration system especially designed for the purpose. Exactly 100 cm^3 starch solution were poured onto the filter, and the amount of clear filtrate obtained was subtracted from this value. The difference between the two figures gave the suspended matter content as a wet volume percentage.

1.2.3. Calculation of permeability constant. Filtration is a complex flow process which can be described, under certain conditions, according to Darcy's law (SZABÓ, 1966; SZABÓ, 1968). The law states that the capacity of a filter depends on the following factors:

$$Q = \frac{k_d F \Delta P}{LM}$$

where

Q	=	volume rate	($m^3\ s^{-1}$)
k_d	=	permeability constant	(m^2)
F	=	surface of filter	(m^2)
ΔP	=	difference in pressure	(Pa)
L	=	thickness of filter layer	(m)
M	=	dynamic viscosity	(Pa s)

Since the factor k_d in the numerator is proportional to the capacity, i.e. to the rate of flow, it can be used as a numerical characteristic of the material being filtered

2. Experimental results

2.1. Preliminary laboratory tests

The oligosaccharide content of starch syrup increases the viscosity and thus in accordance with Darcy's law, reduces the capacity of the filter. In order to determine the correlation a series of experiments was conducted

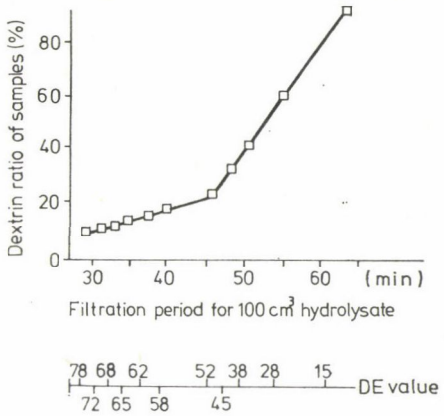

Fig. 1. Changes in the filtration time of laboratory hydrolysates as a function of the dextrin ratio of the samples. The dextrin ratio of the samples was determined according to GRAEFE's data (1965) in correlation with the reducing sugar content of the filtrate

using maize starch hydrolysates with the same initial concentration (32 g per 100 cm^3) but differing reducing sugar contents. The dextrin contents associated with various DE values were determined on the basis of data published by GRAEFE (1958) (Fig. 1).

On the horizontal axis the time required to filter 100 cm^3 hydrolysate is given in minutes. Below this, the DE values of the samples can be seen; these are, of course inversely proportional to the filtration time. The points plotted are the results of four parallel measurements with a maximum standard deviation of 1.2%.

The low standard deviation values are an indication of the efficiency of the laboratory filtration system designed for the purpose of reliable comparisons (Fig. 2).

The ground glass vessel (1) ensures not only the rapid exchange of the measuring cylinder in which the clear filtrate is collected, but also the vacuum connection. The rust-proof wire screen used to brace the filter cloth and the sealing rubber ring are placed on the lower, threaded funnel shaped section. After the upper, cylindrical section is screwed on, it is impossible for unfiltered starch syrup to enter the measuring cylinder. The filtration of the samples took place at a constant pressure difference of 0.9 bar.

An analysis of the data in Fig. 1 shows that there is a change in the filtration properties of starch syrup at a DE value of 52, i.e. at a dextrin ratio of 22%. (The 40 DE syrup manufactured industrially has a dextrin ratio of 32% and a filtration time of 50 min.) The permeability constant (k_d) for the sample with the longest filtration time and a DE value of 15, was 6.6 m^2. The equation for the steep regression line from 15–52 DE is $y = -162 + 4.1\,x$,

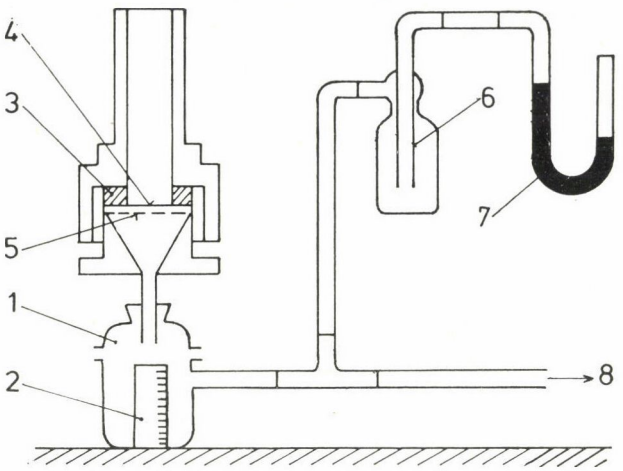

Fig. 2. Laboratory apparatus for the measurement of filtration rate. 1. Ground glass vessel, 2. collecting cylinder for filtrate, 3. rubber sealing ring, 4. filter cloth, 5. wire screen for bracing the filter cloth, 6. ground glass safety vessel, 7. U-tube manometer, 8. vacuum connection

while the equation for samples from 52–78 DE is $y = -13.2 + 0.76\,x$. The closeness of the correlations is indicated by the correlation coefficient, which is 0.98 in both cases.

2.2. Preliminary experiments with a layered bag filter

For the purpose of the experiment a bag filter model fitted in a pressure-resistant cylinder was prepared with the following technical characteristics:

— internal diameter of the pressure-resistant cylinder	400 mm
— internal height of the pressure-resistant cylinder	650 mm
— diameter of circular filter unit	320 mm
— useful filter surface	13.2 dm^2

The great advantage of this type of filter over the filter press is that the filters can be cleaned without taking the apparatus apart, so it is labour-saving. (Utilization is only practicable, however, where the residue forming a layer on the filter is not of value, or is not required for reprocessing.)

In order to approximate practical conditions, the model filtration unit was tested using industrial hydrolysate. The filter cloth was stretched over the wire screen and the actual precoat was formed in a stream of water using crude cellulose from a paper factory. Later, to avoid dilution, the 10–15 g cellulose pulp used for each experiment was mixed with the hydrolysate and measurements were not begun until the filtrate became clear. The precoat

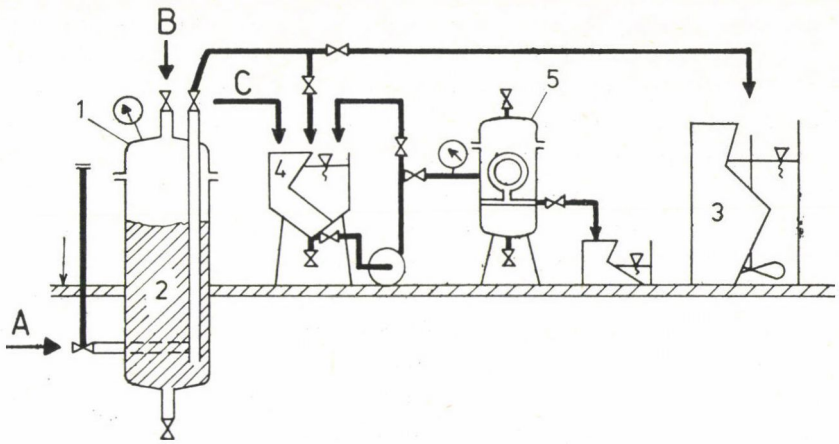

Fig. 3. Supply of materials to the layered filter, and its place in the experimental system. 1. batch reactor, 2. hydrolysate in the reactor, 3. contact tank for neutralization with active carbon, 4. tank for the preparation of experimental material, 5. bag filter; A. steam, B. starch suspension, C. filter aid

thus formed was 1.5–2.0 mm thick when moist and 0.6–0.8 mm thick when dry. (When calculating the permeability constant the moist layer thickness was taken into consideration.) The cellulose quantity mentioned here was sufficient for the formation of a coherent precoat. It was not considered practicable to add larger quantities of cellulose for fear of reducing the capacity. The position of the precoat and how it is supplied with material in the industrial system are shown in Fig. 3.

The batch reactor is marked (1) in the figure. When it is in operation, acidic water is first added to the pressure resistant cylinder, where it is heated to boiling by passing steam through it. The starch suspension is poured slowly into this boiling acidic solution. The rate is regulated manually. After adding the required quantity of starch the tap is closed and the reaction mixture is put under pressure using a reduced supply of steam. The course of hydrolysis is followed up using the iodine test, then, once the desired value has been reached, the hydrolysate is carried into the collecting tank (3) by the overpressure from the reactor. Samples were taken from the extraction tube in the position shown in the figure and collected in the tank (4). Here the H_2SO_4 solution was neutralized with $CaCO_3$ to pH 6.5. Before filtration the crude starch syrup was homogenized by recirculating with the aid of the feeder pump. After this the cylinder of the precoat filter was filled and then deaerated. Filtration was carried out at a constant pressure difference of 1 bar. Of the various experiments carried out, the course of one experimental cycle is illustrated in Fig. 4.

It can be seen that the amount of filtrate obtained in the last four hours of filtration was only one quarter of the capacity measured in the first five hours. Due to the conditions under which the measurements were taken, no difference could be found between the data measured during operation. Thus, the reproducibility of the results can be considered identical to the unreliability ($\pm 6\%$) of the determination.

In seeking the reasons for the poor performance the functioning of the batch reactor was also subjected to examination. It was found that flakes and lumps of starch not degraded were present in the neutralized hydrolysate, due to the fact that with batch feeding the degradation of starch is not uniform. It may happen that the starch suspension added at the beginning of the cycle is already hydrolysed, while the last addition is not gelatinized yet. This means that a hydrolysate prepared from an average sample may contain unchanged granules of starch as well as dextrin, maltose and glucose molecules. Microscopic examinations demonstrated not only the presence of starch granules, but also the fact that the $CaSO_4$ formed during neutralization takes the form of crystal needles under the given reaction conditions, rather than the holohedral crystal pattern found in nature. These loosely-packed needles break up on the blades of the pump and, instead of forming a useful layer, they impair permeability. This is the only possible explanation for the extremely low (0.39) permeability constant (k_d) calculated on the basis of data of the model filter.

It was also established that some of the substances extraneous to the syrup rose to the surface, while others settled at the bottom. In about 10 min the middle layer became clear.

The results outlined above led to the conclusion that the technical parameters of filtration in starch syrup manufacture can only be improved if the supernatant particles are previously removed.

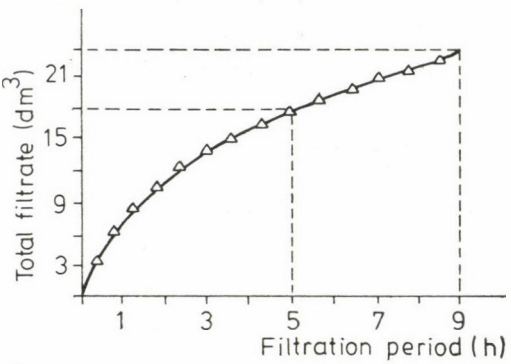

Fig. 4. Illustration of the increase of the filtrate in a 9-hour experiment using 10 g cellulose. Constant pressure difference was 1 bar

2.3. Large-scale experiments on a clarifying system with three outlets

To prove the correctness of the above assumption a large-scale clarifying system with three outlets was designed, in which the sediment, the supernatant impurities and the liquid phase could be separately drawn off (Fig. 5).

As can be seen, the path of flow was designed in such a way that it promoted the separation of the sediment and the supernatant impurities. Matter which rose to the surface was removed via channel and sliding bolt (2), after being previously drawn to the side. The sediment was removed by means of sliding bolts (3). Input and the removal of the middle phase were carried out continuously through openings (1) and (4) respectively. The main operational characteristics were as follows:

— useful volume	10 (m^3)
— flow rate	$2.8 \cdot 10^{-3}$ ($m\ s^{-1}$)
— maximum volume rate	10 ($m^3\ h^{-1}$)
— density of hydrolysate	1140 ($kg\ m^{-3}$)
— dynamic viscosity	$2 \cdot 10^{-3}$ (Pa s)

In designing the apparatus particular attention was paid to ensuring that the Reynolds number should not exceed 116 even at maximum volume rate. This eliminated (with a safety margin of approx. 20-fold) the likelihood of turbulence, which would have obstructed the separation of the suspended matter.

The operation of the apparatus was checked by taking hourly samples. Samples taken from the upper, middle and lower layers were filtered without any filter aid, as in the laboratory experiments described in section 2.1. In this manner, 40% (v/v) suspended matter was found in the upper layer, 3% (v/v) in the middle layer and 10% (v/v) in the lower layer. The data are the mean values of samples taken over five days; the greatest standard deviation was $\pm 3.15\%$.

As a result of removing the suspended matter from the lower and upper layers, the filtering capacity of the factory unit where the experiment took place rose from 20 t to 30 t a day. The same ratio is reflected by the permeability constants, which were as follows:

— filtration without the separating system	$k_d = 2.8$ (m^2)
— after the introduction of the separating system	$k_d = 4.3$ (m^2)

These latter figures confirm the statement that permeability constants determined on the basis of Darcy's law can be used for the quantitative comparison of filtration characteristics.

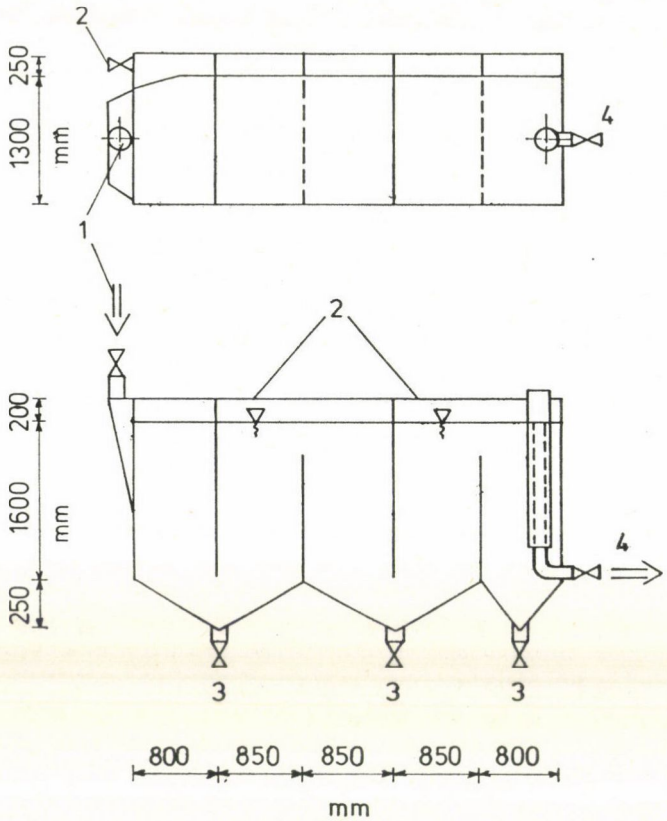

Fig. 5. Outline and connections of a clarifying system with three outlets. 1. inlet of neutralized, diluted, syrup, 2. mechanical removal of supernatant impurities, 3. periodic draining off of sediment mixed with $CaSO_4$ crystals, 4. middle layer drawn off for filtration

The supernatant impurities and sediment separated can be utilized in two ways, depending on whether the hydrolysate is introduced into the separating system in the acidic or neutral state. In the acidic state the carbohydrate-rich sediment can be recirculated into the converter, thus reducing the amount of waste. In this case the apparatus must be made of acid-resistant material. If the hydrolysate is previously neutralized the sediment cannot be recirculated into the converter due to pollution with $CaSO_4$, but the upper phase, which makes up the greater proportion, can be used as fodder without any further treatment. The apparatus used in the experiments was made of 10 mm thick aluminium plates of 99.9% purity.

The position occupied by the separation system in the syrup manufacturing line is shown in Fig. 6.

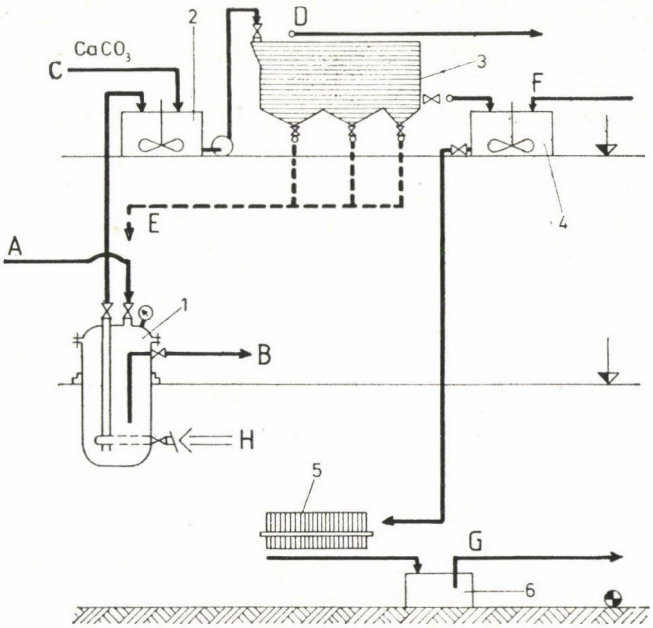

Fig. 6. Process for clarifying neutralized, diluted syrup. 1. batch reactor, 2. neutralization of syrup containing sulphuric acid, 3. clarifying system, 4. storage tank for collecting and mixing middle layer, 5. filter presses, 6. tank for storing diluted syrup after filtration; A. feeding, B. sampling, C. diluted syrup, D. for fodder purposes, E. periodic drainage, F. fuller's earth + active carbon, G. to the evaporator, H. steam

3. Conclusions

As a result of the experiments the conclusion was reached that, unlike the sugar industry, which uses crude juices resembling molecular solutions and containing a minimum amount of suspended matter, the starch syrup industry will not be able to use bag filters for the time being, despite the fact that these are more up-to-date than filter presses. This is primarily due to the deficient degradation of the starch suspension. This is also indicated by changes in the k_d factors of the Darcy equation for the materials tested. In summary, these changes are as follows:

- in the laboratory, for a hydrolysate prepared with perfect mechanical mixing and a DE value of 15 $k_d = 6.60$
- for an industrial hydrolysate filtered through a model bag filter (40 DE) $k_d = 0.39$
- for an industrial hydrolysate filtered through a filter press (40 DE) $k_d = 2.80$
- for an industrial hydrolysate filtered after treatment in a clarifying system with three outlets $k_d = 4.30$

A comparison of the first two figures shows that a homogeneous hydrolysate can be prepared by means of adequat mechanical mixing and by heating the whole suspension at the same time. Under factory conditions, however, the hydrolysate produced differs considerably from this ideal. In the course of the experiments the precoat which had to be added to the layered filter further reduced the capacity. Thus, when using a batch reactor the filtration characteristics can only be improved by previously removing the majority of the suspended matter.

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ACCUMULATION AND DISTRIBUTION OF ELEMENTS IN PLANTS (PAPRIKA)

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Various samples of Hungarian paprika plant (leaf, stem, root, fruit with 20% seeds, fruit without seeds) and corresponding soils were analysed for their Br, As, K, Mn, Cr content by Neutron Activation Analysis and for Hg by Flameless Atomic Absorption Spectrometry.

The concentration values found for Br ranged between 0.67–19.05 ppm (fruit), 4.79–11.97 ppm (leaf), 4.67–9.50 ppm (stem), 5.65–15.67 ppm (root), 4.04–7.48 ppm (soil, earth), for As 0.03–1.48 ppm (fruit), 0.01–1.28 ppm (leaf), 0.45–1.49 ppm (stem), 2.27–6.15 ppm (root), 6.80–7.48 ppm (soil, earth), for K 17 430.30–23 365.28 ppm (fruit), 17 058.46–31 989.30 ppm (leaf), 14 258.32–37 683.77 ppm (stem), 10 518–17 651.39 ppm (root), 8 922.04–10 195.78 ppm (soil, earth), for Mn 9.07–15.34 ppm (fruit), 28.07–59.76 ppm (leaf), 8.95–19.09 ppm (stem), 28.07–64.43 ppm (root), 156.65–238.94 ppm (soil, earth), for Zn 14.932–28.229 ppm (fruit), 13.543–20.149 ppm (leaf), 12.995–17.772 ppm (stem), 25.705–74.144 ppm (root), 34.700–45.494 ppm (soil, earth), for Cr 0.443–4.868 ppm (fruit), 0.772–3.539 ppm (leaf), 0.648–2.969 ppm (stem), 4.373–10.710 ppm (root), 18.010–25.528 ppm (soil, earth), for Hg 0.014–0.025 ppm (fruit), 0.008–0.017 ppm (leaf), 0.006–0.017 ppm (stem), 0.017–0.024 ppm (root), 0.067–0.080 ppm (soil, earth).

The values are reported in ppm (dry weight). Statistical analysis showed:

– no significant differences in the content of trace elements (Br, As, Zn, Hg, Mn) in leaves, stems, roots,

– the content of K in stems and leaves in generally significantly decreased after three months,

– the content of Cr in roots and stems significantly increased after three months,

– in the majority of the cases, increasing in the seed content of the samples tended to reduce their K, Mn, Br content except Zn, As, Cr, Hg.

A number of years ago when the concept of trace elements was being developed, the field of mineral studies was a popular one for research scientists, but since that time, research effort has dwindled in relative terms. Recently however, there has been a revival of interest in mineral research with the discovery that the elements selenium, chromium, nickel, tin and vanadium long considered only to have toxic properties are now required at least by man and some animals (PETERSON, 1972). These researches together with those on the so-called pollution elements, lead, mercury, arsenic and cadmium have provided a new impetus for mineral research. Although considerable advances have been made in the fields of minerals in man and animals, fewer studies have been initiated into the accumulation of these elements in plants and their possible roles.

The elements cobalt and zinc have long been known to be essential for the growth of plants and animals and provided us with some well-known examples of mineral accumulation. *Thlapsi calaminare* (syn. *T. alpestre*) perhaps the best known example, is an accumulator of zinc. *Becium homblei* (formerly *Ocinum homblei*) is a well-established copper accumulator occurring in Zambia and Rhodesia. Indicator plants for zinc are also known from the *Caryophyllaceae* but others are found from the genera *Compositae, Cruciferae, Gramineae, Plumbaginaceae, Rutaceae* and *Violaceae* (BOWEN, 1966; CANNO, 1960). Cobalt rich soils in Katanga have given rise to *Crotalaria cobalticola*, a plant which probably accumulates the greatest amount of cobalt known to date.

In this article we wish to consider the accumulation of several elements (Br, As, K, Mn, Zn, Cr, Hg) and their distribution within the paprika plant as well as in the soil.

1. Materials and methods

1.1. Samples

The dried and ground samples of paprika plant (leaf, stem, root, fruit with 20% seeds, fruit without seeds from various times) and corresponding soils were supplied by the Paprika Research Station, Kalocsa, Hungary (Table 1).

1.2. Neutron activation analysis

Samples weighing from 0.2 to 0.4 g were sealed in quartz ampoules and irradiated for 26 hours at a thermal neutron flux of $7 \cdot 10^{13}$n $\cdot$ cm^{-2} s^{-1}, in Core of ASTRA-Reaktor. After a waiting period of 4 weeks, the samples were counted (long half-life elements ^{51}Cr, ^{65}Zn). For short half-life elements: – samples weighing from 0.08 to 0.15 g were sealed in PVC ampoules and irradiated for 30 minutes at a thermal flux of $7 \cdot 10^{13}$n cm^{-2} s^{-1}.

After waiting 1 hour the samples were counted (^{82}Br, ^{76}As, ^{42}K, ^{56}Mn). Samples were measured with a Canberra 4000-channel analyser connected to a 80 cm^3 Ge(Li) detector (resolution 1.7 keV at 1332 keV).

1.3. Atomic absorption spectroscopy

Perkin-Elmer AAS Model 450 connected with Mercury Analysis System was used for determination of Hg.

1.3.1. Combustion. The samples were burned in an apparatus with small surface made of quartz in a stream of pure oxygen. Ignition was achieved by means of IR radiators.

Ignition and flow of oxygen were turned on at the same time by pressing a button. After combustion both IR radiators were moved downwards. Now

Table 1
Paprika cultivars studied in this report

Variety	No.	Samples and date of sampling
	1	soil 15-10-1979
	2	leaf 20-07-1979
	3	leaf 15-10-1979
	4	stem 20-07-1979
KD-601	5	stem 15-10-1979
	6	root 20-07-1979
	7	root 15-10-1979
	8	fruit with 20% seeds 15-10-1979
	9	fruit without seeds 15-10-1979
	10	soil 15-10-1979
	11	leaf 20-07-1979
	12	leaf 15-10-1979
	13	stem 20-07-1979
K-V-1	14	stem 15-10-1979
	15	root 20-07-1979
	16	root 15-10-1979
	17	fruit with 20% seeds 15-10-1979
	18	fruit without seeds 15-10-1979
	19	soil 15-10-1979
	20	leaf 20-07-1979
	21	leaf 15-10-1979
	22	stem 20-07-1979
K-504	23	stem 15-10-1979
	24	root 20-07-1979
	25	root 15-10-1979
	26	fruit with 20% seeds 15-10-1979
	27	fruit without seeds 15-10-1979
	28	soil 15-10-1979
	29	leaf 20-07-1979
	30	leaf 15-10-1979
	31	stem 20-07-1979
KM-622	32	stem 15-10-1979
	33	root 20-07-1979
	34	root 15-10-1979
	35	fruit with 20% seeds 15-10-1979
	36	fruit without seeds 15-10-1979

Table 2a

Average values in ppm ($\bar{x}$) and standard deviations ($\pm s$) of bromine, arsenic, potassium and manganese contents in powdered paprika samples

No. of sample	Br		As		K		Mn	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
1	4.89	0.47	11.42	1.28	9 763.58	1 091.41	158.39	10.15
2	11.97	1.01	0.01	0.01	26 454.49	505.18	59.26	6.65
3	7.49	1.74	1.19	0.01	25 827.76	1 594.67	57.45	0.30
4	8.76	0.74	0.86	0.16	22 512.49	87.60	18.03	0.25
5	7.45	1.62	0.80	0.18	14 530.20	609.31	10.79	1.30
6	15.67	0.70	5.87	0.60	14 848.47	628.62	59.69	0.76
7	8.58	0.53	6.15	0.77	15 245.14	791.44	62.38	2.33
8	0.67	0.37	1.17	0.37	18 713.23	1 438.58	11.13	1.18
9	1.07	0.10	0.70	0.08	23 120.69	657.57	12.99	0.71
10	7.48	1.59	6.80	0.99	10 012.59	164.97	164.22	5.41
11	4.83	1.14	0.91	0.07	31 676.02	2 270.44	56.76	0.57
12	4.79	0.42	1.28	0.11	19 046.84	276.04	31.73	3.51
13	4.67	0.78	0.58	0.13	37 683.77	3 708.41	17.44	0.65
14	5.65	0.61	1.49	0.71	20 026.90	1 583.82	8.95	0.91
15	11.30	3.01	2.46	0.22	13 287.42	720.73	59.65	0.64
16	6.41	0.82	2.51	0.02	14 613.34	545.09	64.43	3.52
17	12.13	0.32	1.48	0.08	19 479.33	433.64	10.04	0.62
18	18.68	0.74	0.62	0.00	21 614.17	149.87	15.34	1.14

they were heating the acid (20% $HClO_3$, 7% $HClO_4$ Merck Art. 10,741) in the test tube. During this "reflux heating" the inlet of oxygen was closed. After 20 minutes all residues from combustion were dissolved in the acid (MORSCHES & TÖLG, 1955).

1.3.2. A 100 cm^3 sample was treated with nitric and sulfuric acids in the presence of potassium permanganate to oxidize all of the mercury present to the mercuric (Hg^{2+}) form. The excess permanganate is reduced with hydroxylamine hydrochloride and then the mercury is reduced to metallic mercury with stannous chloride. An aerator is placed in the sample solution. A circulating pump moves the air (trapped in the system) through the solution, thus evaporating the mercury and carrying the vapor through the absorption cell. Mercury vapor in atomic form absorbs the 253.7 nm radiation emitted from the light source (hence the therm, Flameless Atomic Absorption).

The change in energy is then detected and read out in the usual way on the atomic absorption spectrophotometer (HATCH & OTT, 1968).

Table 2b

Average values in ppm ($\bar{x}$) and standard deviations ($\pm s$) of bromine, arsenic, potassium and manganese contents in powdered paprika samples

No. of sample	Br		As		K		Mn	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
19	6.82	0.67	7.45	0.77	10 195.78	336.94	156.65	6.49
20	5.05	0.62	0.92	0.10	30 778.88	2346.07	40.04	6.65
21	6.29	0.41	0.58	0.05	19 642.62	586.75	28.07	0.30
22	6.35	0.66	0.74	0.09	21 934.67	1953.47	12.60	2.31
23	7.01	0.35	1.40	0.71	14 396.41	1034.53	10.88	0.94
24	9.73	1.36	2.27	0.06	16 697.91	1294.48	43.00	2.31
25	7.64	0.99	2.62	0.17	10 942.55	85.33	53.35	1.27
26	12.78	0.51	1.48	0.08	17 430.30	1754.94	9.30	0.45
27	13.65	0.70	0.03	0.01	23 365.28	1871.37	14.53	0.24
28	4.04	0.65	6.89	1.05	8 922.04	322.82	238.94	4.02
29	9.28	1.12	0.03	0.01	31 989.30	640.47	46.29	1.05
30	6.08	0.13	0.05	0.05	17 058.46	911.76	32.03	3.84
31	6.66	0.80	0.45	0.07	26 113.40	2228.28	19.09	1.39
32	9.50	1.30	0.68	0.06	14 258.32	1634.54	11.13	0.63
33	10.30	1.51	2.78	0.53	17 651.39	530.39	28.07	2.44
34	5.65	0.92	3.28	0.54	10 518.02	1095.93	35.97	1.24
35	10.90	0.56	0.84	0.11	18 773.60	1729.17	10.03	1.22
36	19.05	0.78	0.28	0.05	20 731.25	1436.80	9.07	0.33

2. Results

The mean values and standard deviations of the trace or minor element content in different samples can be found in Tables 2a, 2b, 3 showing bromine, arsenic, potassium, manganese, zinc, chromium and mercury content of the paprika plant (leaf, stem, root, fruit with 20% seeds, fruit without seeds) and corresponding soils. Statistical analysis of significant differences between paprika plant particles (leaf, stem, root, fruit with 20% seeds, fruit without seeds) was calculated using the *t*-test (SACHS, 1972).

We also have found a different trace element content between paprika with 20% seeds and without seeds (KARIMIAN-TEHERANI et al., 1979; KARIMIAN-TEHERANI et al., 1975).

As can be seen in the majority of the cases the trace element content of the samples without seeds appeared to be significantly higher than that of the samples containing 20% seeds (except Zn, As, Cr, Hg).

Table 3

Average values in ppm ($\bar{x}$) and standard deviations ($\pm s$) of zinc, chromium and mercury contents in powdered paprika samples

No. of sample	Zn		Cr		Hg	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
1	43.399	4.149	25.528	1.204	0.071	0.004
2	13.543	1.691	1.006	0.128	0.017	0.001
3	16.313	2.471	1.290	0.124	0.011	0.001
4	17.752	1.676	2.002	0.051	0.016	0.001
5	15.672	0.397	1.163	0.142	0.010	0.001
6	65.048	3.311	8.628	0.757	0.019	0.001
7	49.043	4.798	5.679	0.812	0.017	0.001
8	23.156	0.346	3.980	0.113	0.021	0.002
9	17.174	0.078	0.520	0.091	0.014	0.001
10	34.700	3.531	22.221	2.194	0.072	0.005
11	20.149	0.486	1.058	0.134	0.017	0.001
12	15.472	0.698	1.117	0.288	0.011	0.001
13	15.383	0.887	0.648	0.141	0.016	0.003
14	12.995	0.061	2.349	0.400	0.014	0.001
15	74.144	4.748	6.872	0.201	0.023	0.001
16	38.228	1.922	10.710	0.281	0.019	0.002
17	21.139	0.359	2.855	0.049	0.023	—
18	18.231	1.189	0.443	0.069	0.016	0.001
19	44.607	2.251	18.010	3.433	0.067	0.001
20	15.464	0.960	3.539	1.053	0.017	0.002
21	15.182	1.505	2.317	1.295	0.012	0.001
22	17.362	0.620	0.737	0.120	0.017	0.001
23	13.745	0.289	1.490	0.310	0.014	0.001
24	48.990	2.594	4.722	0.285	0.024	0.001
25	37.891	5.469	5.512	0.208	0.020	0.002
26	20.585	0.037	2.550	0.375	0.025	0.001
27	14.932	0.370	0.450	0.071	0.017	0.001
28	45.493	1.191	24.869	0.821	0.080	0.001
29	19.645	5.588	1.170	0.522	0.012	0.001
30	21.883	7.373	0.772	0.184	0.008	0.001
31	17.084	0.357	1.033	0.121	0.008	0.001
32	15.566	0.113	2.969	0.404	0.006	0.001
33	69.967	4.267	4.373	0.641	0.018	0.001
34	25.705	2.442	6.212	0.317	0.017	0.001
35	28.229	0.712	4.868	0.638	0.020	0.001
36	17.995	0.681	0.846	0.070	0.016	0.001

Figure 1 shows accumulation and distribution of elements (ppm) in paprika plant and soil. The values found for bromine were 0.67–19.05 ppm (fruit), 4.79–11.97 ppm (leaf), 4.67–9.50 ppm (stem), 5.65–15.67 ppm (root), 4.04–7.48 ppm (soil), for arsenic 0.03–1.48 ppm (fruit), 0.01–1.28 ppm (leaf), 0.45–1.49 ppm

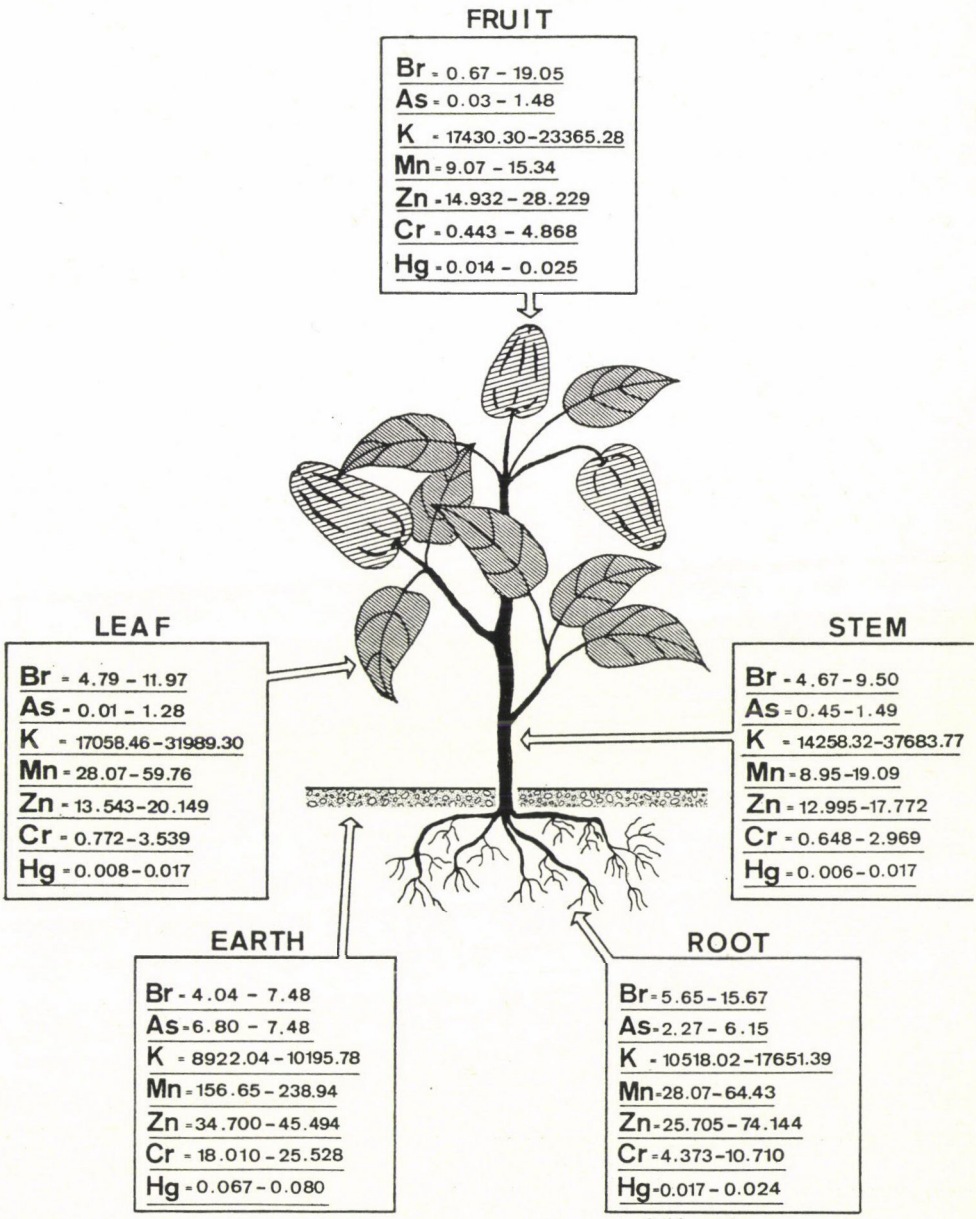

Fig. 1. Accumulation and distribution of elements (ppm) in plant (paprika) and soil

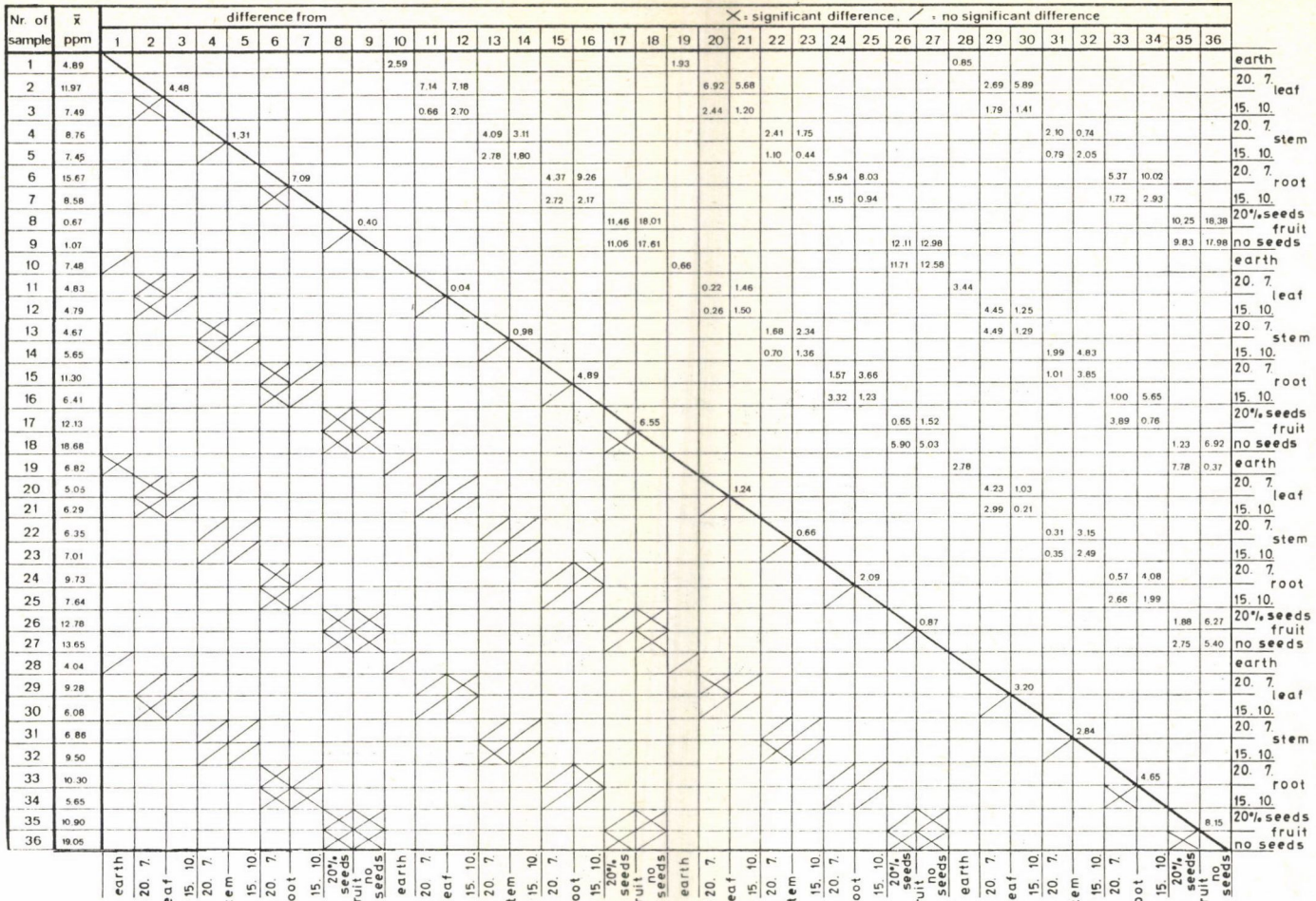

Fig. 2. Statistical analysis of significant differences for Br content
× significantly different; / non-significantly different

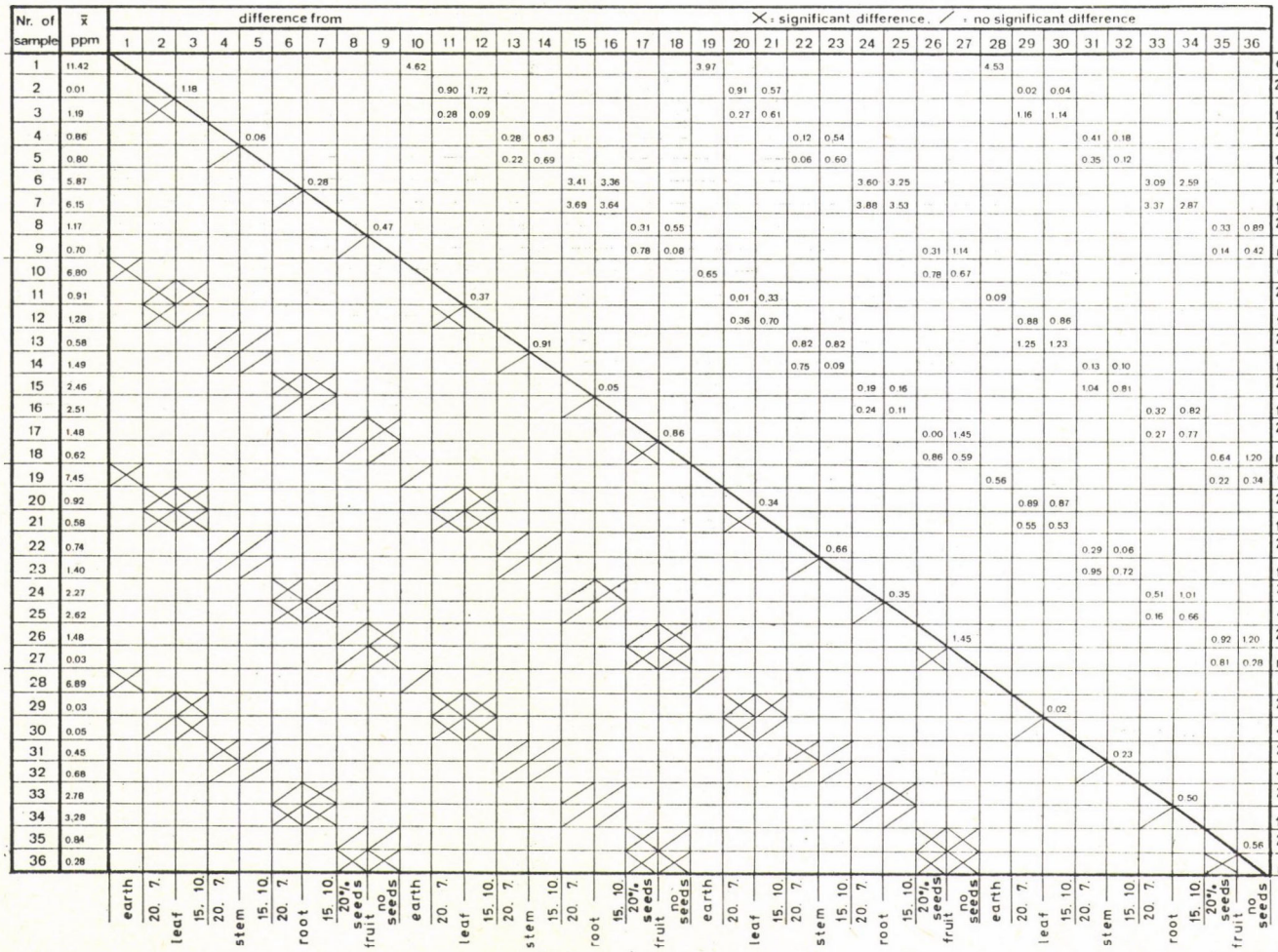

Fig. 3. Statistical analysis of significant differences for As content
× significantly different; / non-significantly different

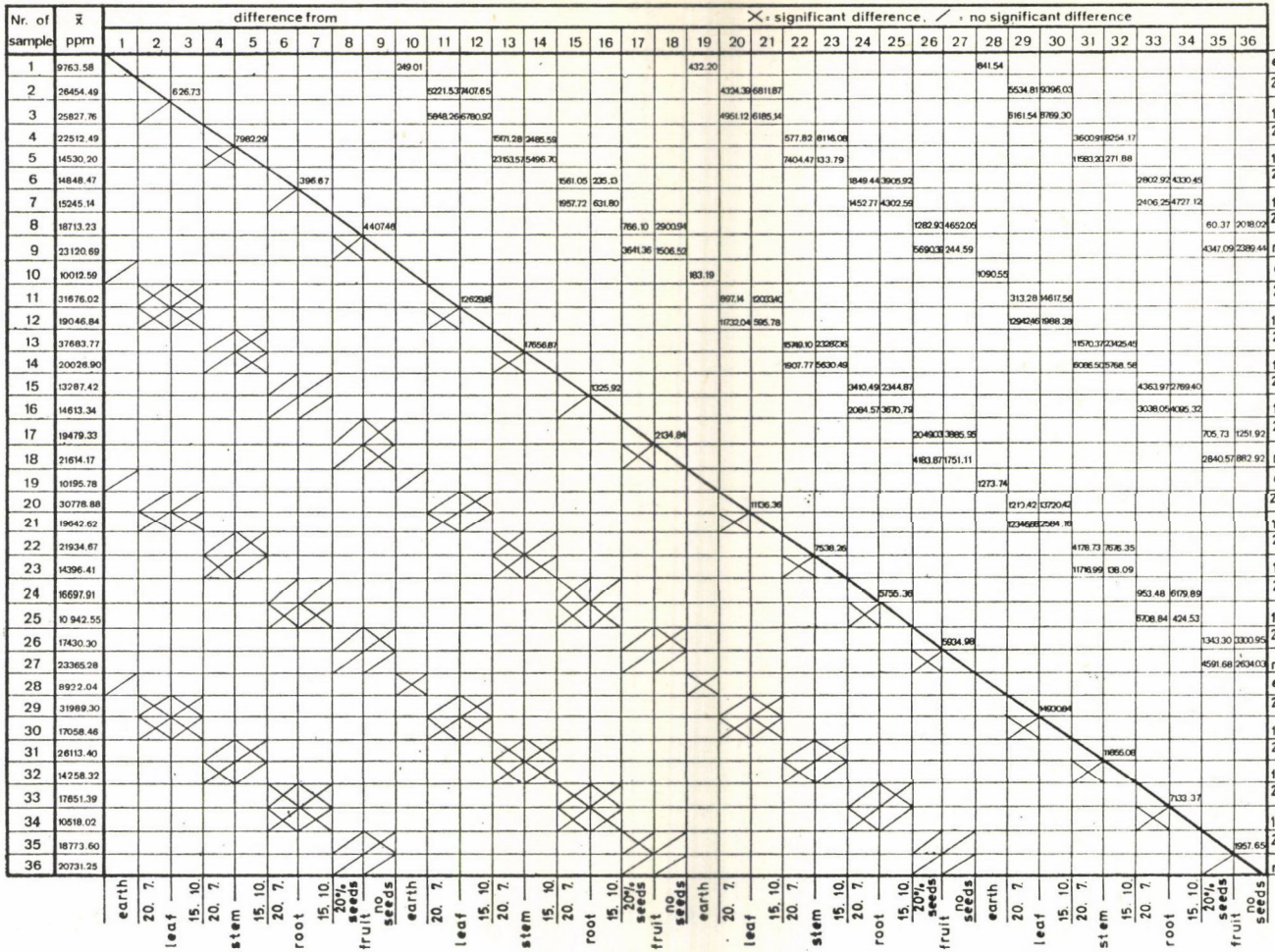

Fig. 4. Statistical analysis of significant differences for K content
× significantly different; / non-significantly different

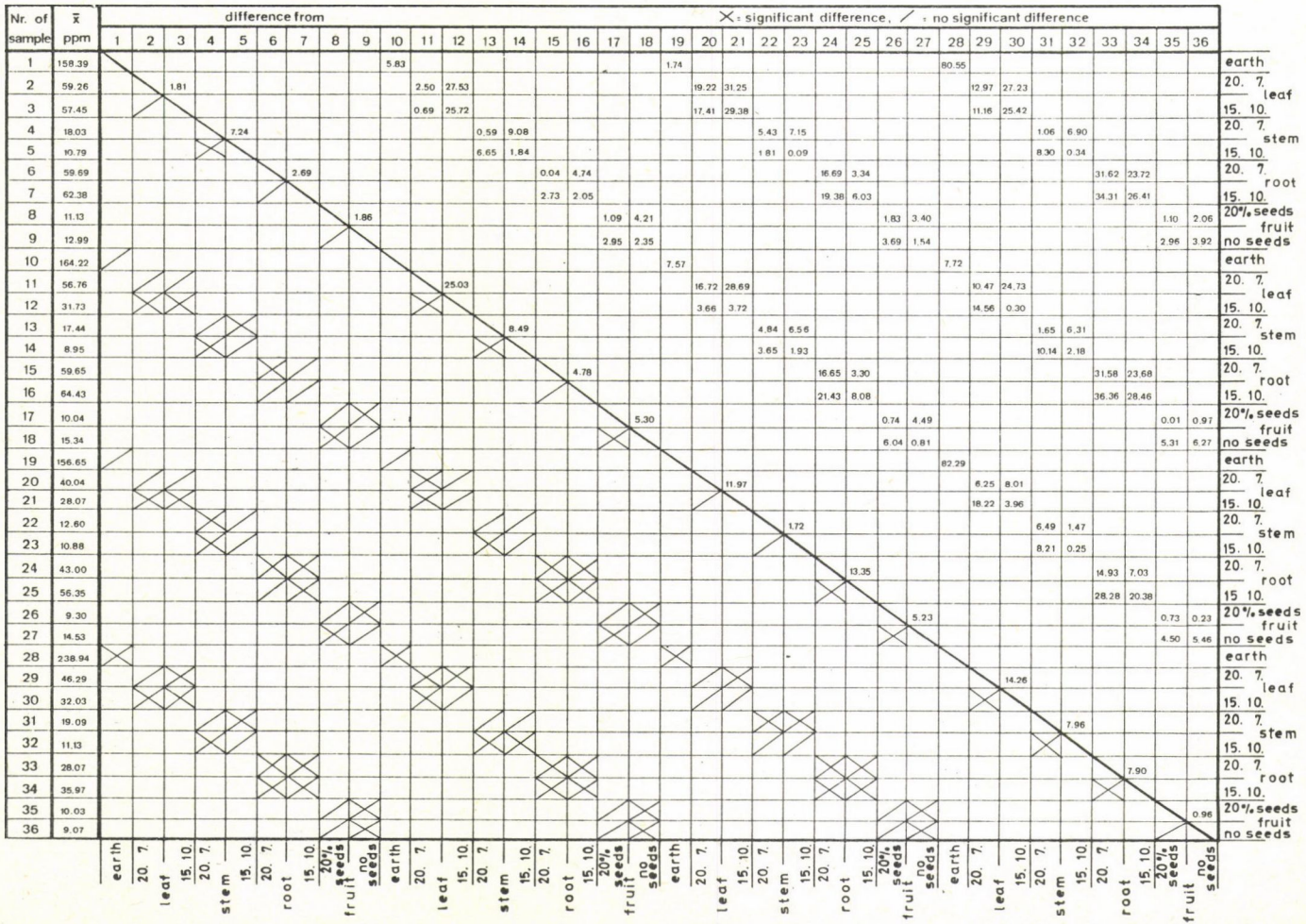

Fig. 5. Statistical analysis of significant differences for Mn content
× significantly different; / non-significantly different

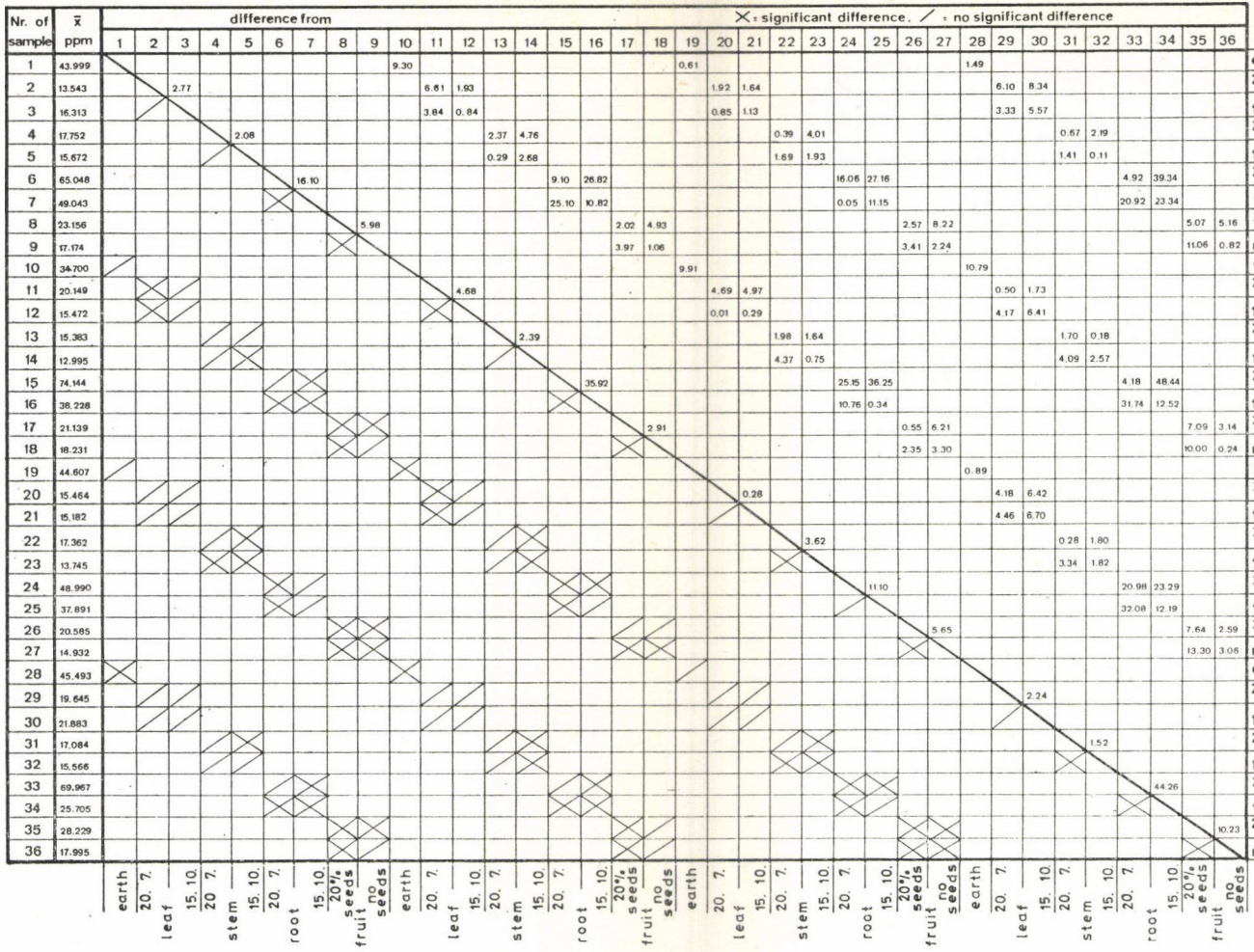

Fig. 6. Statistical analysis of significant differences for Zn content
× significantly different; / non-significantly different

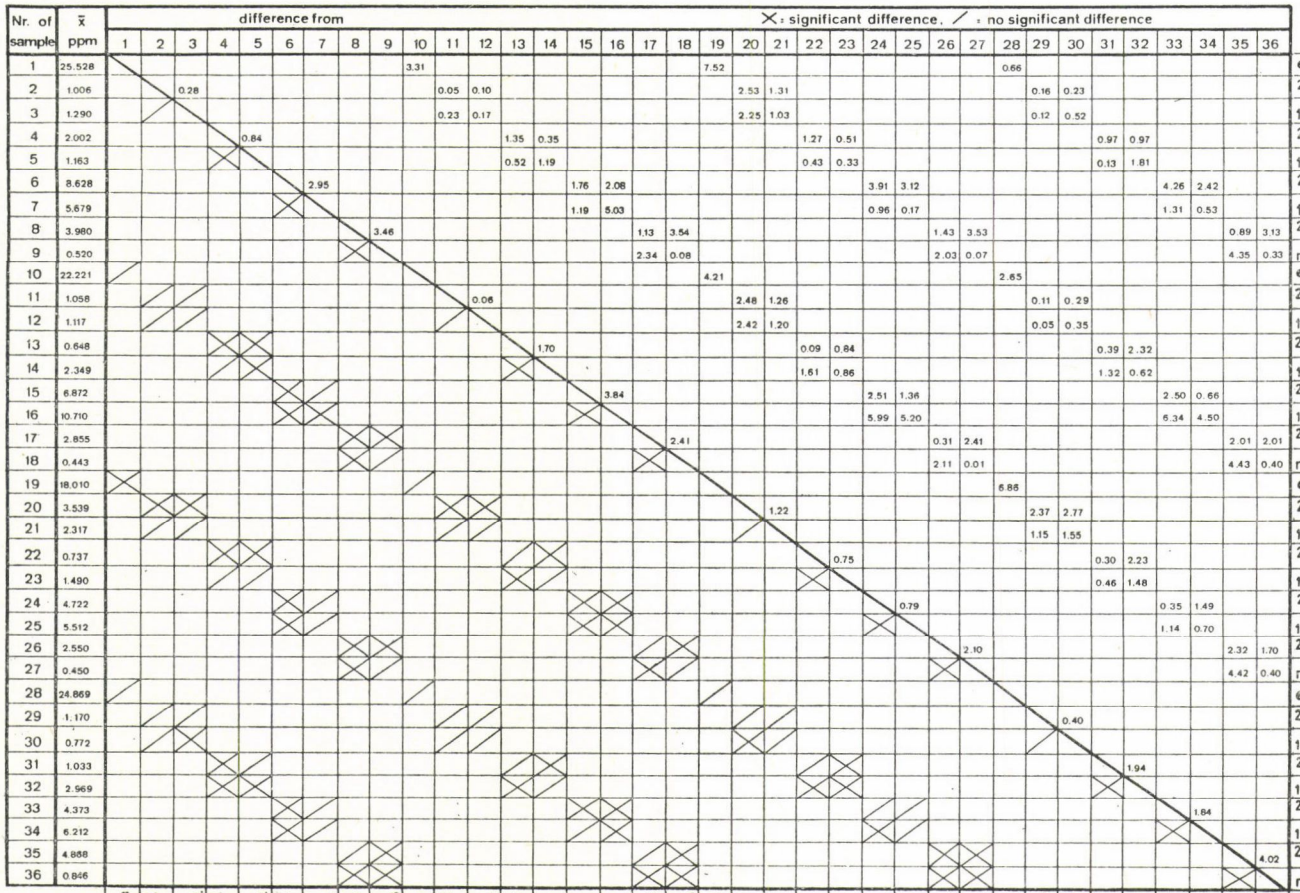

Fig. 7. Statistical analysis of significant differences for Cr content
× significantly different; / non-significantly different

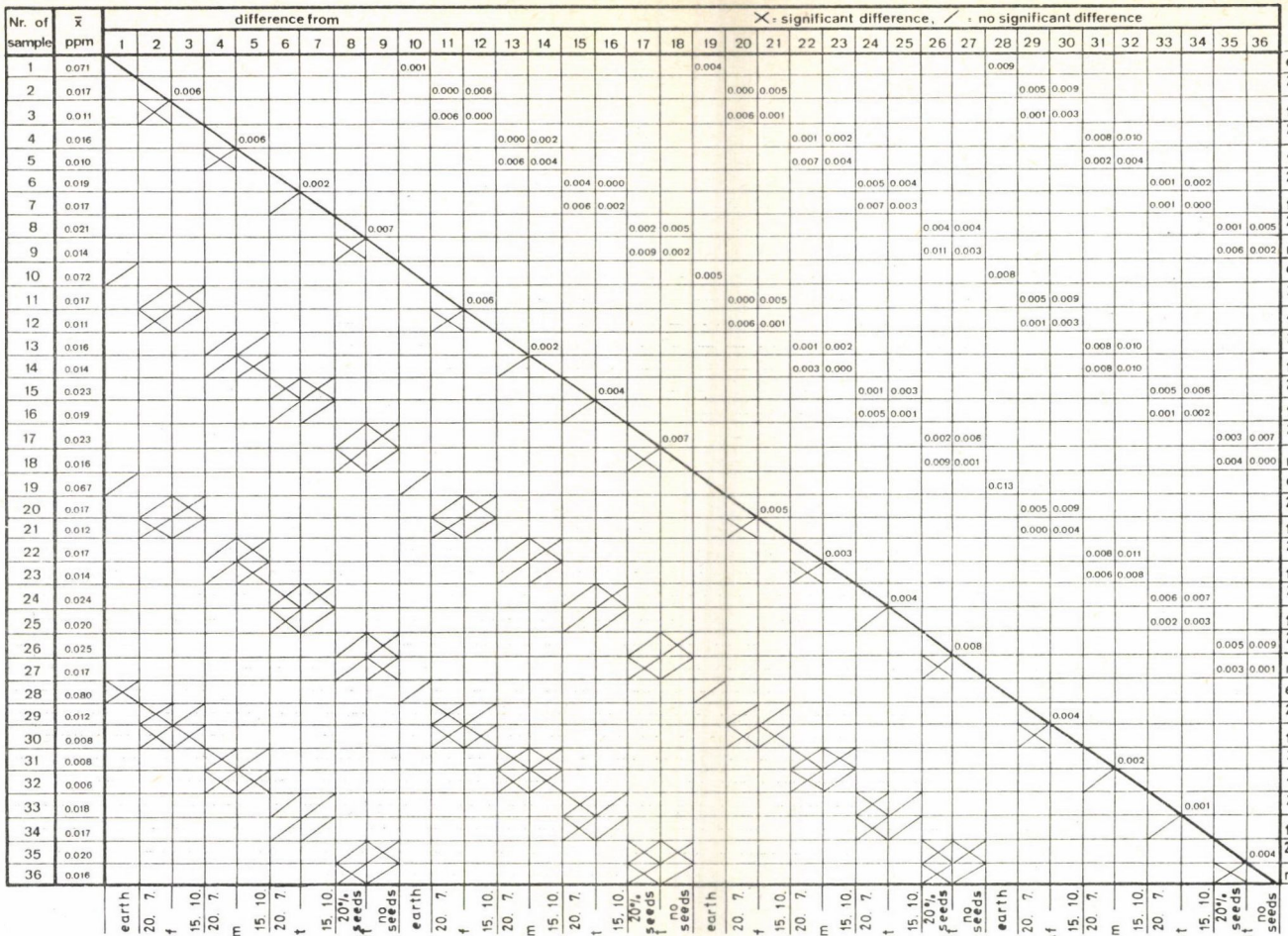

Fig. 8. Statistical analysis of significant differences for Hg content
× significantly different; / non-significantly different

Table 4

Content of 7 trace elements in the leaves of paprika plant (4 cultivars)

Trace element		Leaves				No. of cultivars in the "higher" or "lower" or "not differing" categories			Total
		KD 601	KV 1	K 504	KM 622	►	◄	◆	
Br		►	◆	◆	◆	1	0	3	4
As		◄	◄	►	◆	1	2	1	4
K		◆	►	►	►	3	0	1	4
Mn		◆	►	◆	►	2	0	2	4
Zn		◆	►	◆	◆	1	0	3	4
Cr		◆	◆	◆	◆	0	0	4	4
Hg		►	►	►	►	4	0	0	4
No. of trace elements in the higher or lower or not differing categories	►	2	4	3	3	12			
	◄	1	1	0	0		2		
	◆	4	2	4	4			14	
Total		7	7	7	7				28

► = indicates higher trace element content in samples of 20-7-1979
◄ = indicates higher trace element content in samples of 15-10-1979
◆ = indicates statistically non-significant difference

Table 5

Contents of 7 trace elements in the stems of paprika plant (4 cultivars)

Trace element		Stems				No. of cultivars in the "higher" or "lower" or "not differing" categories			Total
		KD 601	KV 1	K 504	KM 622	►	◄	◆	
Br		◆	◆	◆	◆	0	0	4	4
As		◆	◆	◆	◆	0	0	4	4
K		►	►	►	►	4	0	0	4
Mn		►	►	◄	►	3	0	1	4
Zn		◆	◆	►	►	2	0	2	4
Cr		►	◄	◄	◄	1	3	0	4
Hg		►	►	►	◆	2	0	2	4
No. of trace elements in the higher or lower or not differing categories	►	4	2	3	3	12			
	◄	0	1	1	1		3		
	◆	3	4	3	3			13	
Total		7	7	7	7				28

► = indicates higher trace element content in samples of 20-07-1979
◄ = indicates higher trace element content in samples of 15-10-1979
◆ = indicates statistically non-significant difference

Table 6

Contents of 7 trace elements in the roots of paprika plant (4 cultivars)

Trace element	Roots				No. of cultivars in the "higher" or "lover" or "not differing" categories			Total
	KD 601	KV 1	K 504	KM 622	▶	◀	◆	
Br	▶	◆	◆	▶	2	0	2	4
As	◆	◆	◆	◆	0	0	4	4
K	◆	◆	▶	▶	2	0	2	4
Mn	◆	◆	◀	◀	0	2	2	4
Zn	▶	▶	◆	▶	3	0	1	4
Cr	▶	◀	◀	◀	1	3	0	4
Hg	◆	◆	◆	◆	0	0	4	4
No. of trace elements in the higher or lower or not differing categories ▶	3	1	1	3	8			
◀	0	1	2	2		5		
◆	4	5	4	2			15	
Total	7	7	7	7				28

▶ = indicates higher trace element content in samples of 20-07-1979
◀ = indicates higher trace element content in samples of 15-10-1979
◆ = indicates statistically non-significant difference

Table 7

Contents of 7 trace elements in the fruits of paprika plant (4 cultivars)

Trace element	Fruits				No. of cultivars in the "higher" or "lower" or "not differing" categories			Total
	KD 601	KV 1	K 504	KM 622	▶	◀	◆	
Br	◆	◀	◆	◀	0	2	2	4
As	◆	▶	▶	▶	3	0	1	4
K	◀	◀	◀	◆	0	3	1	4
Mn	◆	◀	◀	◆	0	2	2	4
Zn	▶	▶	▶	▶	4	0	0	4
Cr	▶	▶	▶	▶	4	0	0	4
Hg	▶	▶	▶	▶	4	0	0	4
No. of trace elements in the higher or lower or not differing categories ▶	3	4	4	4	15			
◀	1	3	2	1		7		
◆	3	0	1	2			6	
Total	7	7	7	7				28

▶ = indicates higher trace element content in samples with 20% seed
◀ = indicates higher trace element content in samples without seeds
◆ = indicates statistically non-significant difference

(stem), 2.27–6.15 ppm (root), 6.80–7.48 ppm (soil), for potassium 17 430.30–23 365.28 ppm (fruit), 17 058.46–31 989.30 ppm (leaf), 14 258.32–37 683.77 ppm (stem), 10 518.02–17 651.39 ppm (root), 8 922.04–10 195.78 ppm (soil), for manganese 9.07–15.34 ppm (fruit), 28.07–59.76 ppm (leaf), 8.95–19.09 ppm (stem), 28.07–64.43 ppm (root), 156.65–238.94 ppm (soil), for zinc 14.932–28.229 ppm (fruit), 13.543–20.149 ppm (leaf), 12.995–17.772 ppm (stem), 25.705–74.144 ppm (root), 34.700–45.494 ppm (soil), for chromium 0.443–4.868 ppm (fruit), 0.772–3.539 ppm (leaf), 0.648–2.969 ppm (stem), 4.373–10.710 ppm (root), 18.010–25.528 ppm (soil), for mercury 0.014–0.025 ppm (fruit), 0.008–0.017 ppm (leaf), 0.006–0.017 ppm (stem), 0.017–0.024 ppm (root), 0.067–0.080 ppm (soil).

Data shown in this report are in good agreement with those presented in an earlier one (KARIMIAN-TEHERANI et al., 1981; KARIMIAN-TEHERANI et al., 1983) and for the values recently reported in literature. MORRISON (1967) found that Zn content in sugar beet leaves, the Br and Mn content is very greatly depending upon the species of plant investigated (BOWEN, 1967; UNDERWOOD, 1962). DAVIES (1980) has reviewed the distribution of trace elements in soil profiles. Mercury tends to be enriched in surface, organic rich horizons from 0.33 to 0.07 ppm in depth 0–600 mm. We also have found 0.06 to 0.104 ppm.

3. Conclusions

The accumulation and distribution of trace elements in soils and in various parts of paprika plants (leaf, stem, root, and fruit (ground paprika made with 20% seeds, without seeds, resp.) was studied in July and October.

It was found that the content of bromide, chromium, manganese, zinc and arsenic in the leaves did not change significantly within the period of the experiment, whereas potassium and mercury decreased significantly from July to October.

In the stems bromide, arsenic, zinc and mercury were not affected by the time of season, but potassium and manganese decreased from July to October and chromium increased significantly.

In the roots the content of bromide, arsenic, potassium, manganese and mercury did not change during the three months period, zinc decreased and chromium increased significantly.

It cannot be concluded definitely whether the enrichment of the elements occurs in the pod tissue of the fruit or in the seeds, although in the majority of the cases, the trace elements content of the samples without seeds appeared to be significantly higher (K, Mn, Br) than that of the samples containing 20% seeds (except Zn, As, Cr, Hg).

*

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DETERMINATION OF TRACE AND HEAVY METALS IN PAPRIKA AND SOIL BY NEUTRON ACTIVATION ANALYSIS AND ATOMIC ABSORPTION SPECTROSCOPY

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Various samples of Hungarian spice paprika plant (treated with Wuxal) and corresponding soils were analysed for their Fe, Cr, Zn, Co, Br, As, K, Mn and La (only in soil samples), Hg content. Statistical analysis showed significant differences in the trace element contents between most cultivars and soils. There is a higher amount of trace elements in samples treated with Wuxal than in controls with the exception of As where there is more in the controls.

Mostly the same situation is observed in samples of soil as in paprika. Exception is the non-significant difference in the case of Fe and Co.

Differences in mercury content between treated and untreated paprika samples were not significant.

Various developments in agriculture and technology can affect the trace element content of foods. The introduction of new varieties of plants, the use of agricultural chemicals, alterations in feeding practices used for farm animals and other new techniques designed to increase food production and yields may affect the composition of basic food items (RAO MATURU, 1980).

The trace element content of foods may vary significantly according to species and age. The variety of fruit will thus determine the trace element content of the fresh or canned fruit, or the juice. Similarly, the trace element content of bread, cereal and other baked foods depends on the variety of wheat, rye or oats used. GREAVES and ANDERSON (1936) reported that the copper content of sixteen varieties of wheat grown in the same soil under similar conditions ranged from 5.0–16.7 mg per kg.

Age also seems to affect the trace element content of foods. Younger animals have a higher copper concentration in organs and muscle meats than do older animals of the same species. LENSE and co-workers (1938) found egg yolks from 3 to 4-year-old hens to contain 3490 ppm less copper than those from 2-year-old hens. The same is also true for plants. Amounts of copper present are higher in the greener and younger portions of plants (AKOPDZHANIAN, 1937). The trace element content of the soil and the soil type may determine the amount which a plant absorbs beyond the level necessary for growth. The availability of the trace element in the soil may depend upon its oxidation state and the pH of the soil. This may affect the concentration of the trace

elements in fruits, vegetables and cereals as well as the amounts available to herbivorous animals in forage.

Food grown in the seleniferous areas of Venezuela may contain high amounts of selenium (JAFFE et al., 1972). On the other hand, foods from Sweden (LINDBERG, 1958) and Egypt (MAXIA et al., 1972) have been reported to have rather a low selenium content compared with the levels found in the USA (SCHROEDER et al., 1970; MORRIS & LAVENDER, 1970); also MILLER and MITCHELL (1931) found that the amounts of copper in spinach and lettuce depend on the quantity and availability of the trace elements in the soil.

Differences due to geographical location probably depend on contamination of water, air or soil with the trace element. Variations observed in the selenium content of foods grown in Sweden, Egypt and the USA are a good example of how geographical location significantly affects the trace element content of foods (ABDULIAH et al., 1972; HILTNER & WICHMAN, 1919). MADER and MADER (1937) reported that the use of certain fertilizers containing trace elements will increase the trace element content of the soil and this significantly affects the trace element content of foods.

As part of an attempt to increase the understanding of the distribution of elements both geographically and throughout the food chain (KARIMIAN-TEHERANI et al., 1975; KARIMIAN-TEHERANI et al., 1979) we measured some elements in Hungarian paprika and corresponding soils.

1. Materials and methods

1.1. Material

The different varieties of paprika plant and soil samples treated with Wuxal (fertilizer with trace elements) were supplied by the Paprika Research Station, Kalocsa, Hungary (Table 1).

1.2. Methods

1.2.1. Neutron activation analysis. The dried samples (ground paprika and soil) weighing from 0.2 to 0.4 g were sealed in quartz ampoules and were irradiated for 26 hours at a thermal neutron flux of $7 \cdot 10^{13}$n cm^{-2} s^{-1}, in Core of ASTRA-Reaktor. After a waiting period of 4 weeks, the samples were counted (long half-life elements ^{60}Co, ^{59}Fe, ^{51}Cr, ^{65}Zn). For short half-life element samples weighing from 0.08 to 0.15 g were sealed in PVC ampoules and irradiated for 30 minutes at a thermal flux of $7 \cdot 10^{13}$n cm^{-2} s^{-1}.

After waiting 1 hour the samples were counted (^{82}Br, ^{76}As, ^{42}K, ^{56}Mn, ^{140}La).

Samples were measured with a Canberra 4000-channel analyser connected to a 80 cm^3 Ge(Li) detector (resolution 1.7 keV at 1332 keV).

Table 1

Paprika cultivars and soil studied in this work

No.	Paprika samples			Soil samples
	variety	treatment	grinding	
1	K-D-522	Wuxal	with 20% seeds	K-M-622 Wuxal
2	K-M-622	Wuxal	without seeds	K-M-622 control
3	K-M-622	control	with 20% seeds	K-D-601 Wuxal
4	K-M-622	control	without seeds	K-D-601 control
5	K-D-601	Wuxal	with 20% seeds	K-504 Wuxal
6	K-D-601	Wuxal	without seeds	K-504 control
7	K-D-601	control	with 20% seeds	
8	K-D-601	control	without seeds	
9	K-504	Wuxal	with 20% seeds	
10	K-504	Wuxal	without seeds	
11	K-504	control	with 20% seeds	
12	K-504	control	without seeds	

1.2.2. Atomic absorption spectroscopy. Perkin–Elmer AAS Model 560 connected with Mercury Analysis System was used for determination of Hg.

1.2.2.1. Combustion. – The samples were burned in an apparatus with small surface made of quartz in a stream of pure oxygen. Ignition and flow of oxygen were started at the same time by pressing a button.

After combustion both IR radiators were turned downwards. Now they were heating the acid in the test tube. During this "reflux heating" the inlet of oxygen was closed. After 20 minutes all residue from combustion were dissolved in the acid (MORSCHES & TÖLG, 1966).

1.2.2.2. Procedure. – A 100 cm^3 sample is treated with nitric and sulfuric acids in the presence of potassium permanganate to oxidize all the mercury present to the mercuric (Hg^{2+}) form. The excess permanganate is reduced with hydrosylamine hydrochloride and then the mercury is reduced to metallic mercury with stannous chloride. An aerator is placed in the sample solution. The air (trapped in the system) is driven through the solution by a circulating pump, thus evaporating the mercury and carrying the vapor through the absorption cell. Mercury vapor in atomic form absorps the 253.7 nm radiation emitted from the light source (hence the term, Flameless Atomic Absorption).

The change in energy is then detected and read out in the usual way on the atomic absorption spectrophotometer (HATCH & OTT, 1968).

Table 2

Average value ($\bar{x}$) and standard deviations ($\pm s$) of the iron, chromium, zinc and cobalt contents in ppm related to the dry matter in powdered paprika samples

No. of sample	Fe		Cr		Zn		Co	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
1	113.07	2.69	1.77	0.62	29.64	1.94	5.47	0.85
2	130.82	2.96	0.53	0.27	18.35	0.73	8.50	0.66
3	109.22	3.88	1.92	0.33	21.59	1.97	4.89	0.79
4	120.15	3.99	0.25	0.11	16.24	1.30	4.84	0.95
5	129.31	7.89	3.90	0.30	22.48	1.73	7.55	0.84
6	109.72	7.67	0.53	0.20	18.18	1.26	4.65	0.72
7	115.00	3.00	2.64	0.47	19.50	0.59	4.38	0.82
8	99.62	0.88	0.52	0.17	17.05	0.97	3.28	0.36
9	109.35	5.63	2.88	0.29	27.89	0.96	3.97	0.24
10	122 91	3.26	0.35	0.27	15.80	1.26	6.15	0.28
11	99.72	1.62	0.93	0.06	22.89	2.54	3.90	0.38
12	111.95	2.37	0.48	0.21	15.30	2.04	3.78	0.57

number of parallels (n) = 8

Table 3

Average value ($\bar{x}$) and standard deviation ($\pm s$) of bromine ($n = 5$) arsenic ($n = 5$), potassium ($n = 6$) and manganese ($n = 6$) contents in ppm related to the dry matter in powdered paprika samples

No. of sample	Br		As		K		Mn	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
1	14.47	0.55	0.79	0.07	27 403.33	1 224.83	11.47	0.51
2	12.08	0.72	0.25	0.02	32 212.21	841.86	11.10	0.57
3	12.98	0.29	0.85	0.05	25 198.32	1 747.94	9.92	0.51
4	10.80	0.52	0.33	0.02	23 357.06	850.73	9.89	0.74
5	14.80	0.64	0.98	0.02	24 088.58	1 787.63	11.77	0.89
6	24.64	0.46	0.25	0.02	27 051.39	573.22	20.07	0.74
7	13.54	0.54	0.95	0.02	19 346	1 380.24	10.82	0.76
8	22.28	1.39	0.37	0.01	24 312.71	1 726.89	15.87	0.83
9	16.24	0.38	1.01	0.06	23 534.03	2 131.44	10.50	0.75
10	14.98	0.70	0.22	0.02	24 219.47	2 600.09	13.15	0.72
11	12.97	0.85	0.60	0.04	21 668.06	1 647.33	10.07	0.52
12	14.08	0.16	0.45	0.03	26 237.66	992.46	14.24	0.82

n = number of parallels

2. Results

The mean values and standard deviations of the trace or minor element content in different samples can be found in Tables 2, 3, 5 showing the iron, chromium, zinc, cobalt, bromine, arsenic, potassium, manganese and mercury content of the powdered paprika samples (treated with Wuxal and control).

Table 4 shows the average value and standard deviation (s) of bromine, arsenic, potassium, manganese, lanthanum, iron, chromium, zinc, mercury and cobalt content in different soil samples. The significance of differences between samples with and without seeds (Wuxal, control) was calculated using the *t* test (SACHS, 1972).

Data shown in this report are in good agreement with those presented in earlier papers (KARIMIAN-TEHERANI et al., 1975; KARIMIAN-TEHERANI et al., 1979).

As can be seen, the iron content is much higher than that of the other trace metals. It is not, however, inordinately high for plant samples. CHATEFIELD and ADAMS (1940), and KAPELLER (1971) for example, have measured the iron content of many vegetables and found it to vary from 6.6 to 76 ppm wet weight. MAYER and GORHAM (1951), and CANNON (1960) have also reported that normal iron content may be as high as 160 ppm.

Co may vary from 0.5 ppm in a non-accumulator to as high as 1.8% dry weight in an accumulator species such as *Crotolaria cobalticola* (PETERSON, 1972). Br and Mn also vary greatly depending upon the species of plant investigated (BOWEN, 1967; UNDERWOOD, 1962).

We have found a different trace element content in soil treated with Wuxal and control. The data varied for arsenic from 4.9 to 8.63 ppm, for potassium from 7 245.08 to 10 616.81 ppm, for manganese from 263 to 339.19 ppm, for lanthanum from 20.20 to 27.09 ppm, for iron from 15 805.67 to 16 381.55 ppm, for chromium from 25.46 to 29.19 ppm, for zinc from 38.66 to 55.87 ppm, for cobalt from 5.29 to 5.60 ppm.

Data in Table 4 show a good agreement with values recently given in literature (KARIMIAN-TEHERANI et al., 1981). DAVIES (1980) has reviewed the distribution of trace elements in soil profiles. Mercury tends to be enriched in the surface layer organic-rich horizons from 0.33 to 0.07 ppm in depth 0.600 mm. We also have found 0.06 to 0.104 ppm.

3. Conclusions

In the paprika samples and corresponding soil samples treated with Wuxal there is significant difference in the amounts of trace elements studied (Tables 6, 7, Figs. 1–8 for paprika samples; Table 8, Figs. 9–13 for soil samples).

Table 4

Average value ($\bar{x}$ ppm) and standard deviation ($\pm s$) of bromine ($n = 5$), arsenic ($n = 5$), potassium ($n = 4$), manganese ($n = 4$), lanthanum ($n = 5$), iron ($n = 4$), chromium ($n = 4$), zinc ($n = 4$), mercury ($n = 2$) and cobalt ($n = 4$) in powdered soil samples

Samples	Br		As		K		Mn		La	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	x	$\pm s$	$\bar{x}$	$\pm s$
K 504 Wuxal	9.48	0.39	4.9	—	9 844.5	1 683.79	339.19	26.59	27.09	0.62
K 504 control	6.35	0.42	5.32	0.88	7 245.08	1 301.62	263.00	10.03	20.20	0.85
KD 601 Wuxal	6.82	0.45	8.48	0.88	9 808.88	513.88	319.46	7.81	24.05	0.39
KD 601 control	4.45	0.46	8.64	1.05	7 586.75	1 493.85	278.84	7.74	20.43	1.03
KM 622 Wuxal	3.29	0.33	5.67	0.99	10 616.81	1 112.92	331.58	22.78	25.40	1.29
KM 622 control	1.16	0.16	7.12	0.50	7 445.39	1 584.72	284.90	12.66	24.41	0.57

Samples	Fe		Cr		Zn		Co		Hg	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
K 504 Wuxal	16 375.94	174.67	28.67	1.96	48.05	1.61	5.60	0.09	0.089	0.002
K 504 control	15 940.83	385.84	26.83	1.62	40.78	0.41	5.51	0.12	0.064	0.005
KD 601 Wuxal	16 332.53	427.76	26.22	1.55	55.87	3.89	5.29	0.36	0.091	0.004
KD 601 control	15 885.39	683.90	26.21	1.78	44.46	2.54	5.36	0.25	0.072	0.022
KM 622 Wuxal	15 805.67	691.82	25.46	1.53	46.03	1.93	5.32	0.35	0.104	0.002
KM 622 control	16 381.55	517.62	29.19	2.25	38.66	2.00	5.51	0.16	0.097	0.003

Table 5

Average value ($\bar{x}$) of two measurements and standard deviation ($\pm s$) of the mercury contents in ppm related to the dry matter in powdered paprika samples

No. of sample	Hg	
	$\bar{x}$	$\pm s$
1	0.019	<0.001
2	0.013	0.002
3	0.013	0.002
4	0.013	0.001
5	0.022	0.002
6	0.012	<0.001
7	0.013	0.005
8	0.013	<0.001
9	0.020	0.002
10	0.019	0.001
11	0.016	0.003
12	0.016	0.002

Table 6

Effect of treatment on the content of 8 trace elements in paprika (without seed, 3 cultivars)

Trace element	Varieties			No. of cultivars in the "higher" or "lower" or "not differing" categories			Total
	KM 622	KD 601	K 504	▶	◀	◆	
Fe	▶	▶	▶	3	0	0	3
Cr	▶	◆	◆	1	0	2	3
Zn	▶	▶	◆	2	0	1	3
Co	▶	▶	▶	3	0	0	3
Br	▶	▶	▶	3	0	0	3
As	◀	◀	◀	0	3	0	3
K	▶	▶	◆	2	0	1	3
Mn	▶	▶	◀	2	1	0	3
No. of trace elements in the "higher" ▶	7	6	3	16			
or "lower" or "not differing" ◀	1	1	2		4		
categories ◆	0	1	3			4	
Total	8	8	8				24

▶ = indicates higher trace element content in samples treated with Wuxal

◀ = indicates lower trace element content in samples treated with Wuxal

◆ = indicates statistically non-significant difference

Table 7

Effect of treatment on the content of 8 trace elements in paprika (with 20% seed, 3 cultivars)

Trace element	Varieties			No. of cultivars in the "higher" or "lower" or "not differing" categories			Total
	KM 622	KD 601	K 504	▸	◂	◆	
Fe	▸	▸	▸	3	0	0	3
Cr	◆	▸	▸	2	0	1	3
Zn	▸	▸	▸	3	0	0	3
Co	◆	▸	◆	1	0	2	3
Br	▸	▸	▸	3	0	0	3
As	◆	▸	▸	2	0	1	3
K	▸	▸	◆	2	0	1	3
Mn	▸	◆	◆	1	0	2	3
No. of trace elements in the "higher" ▸	5	7	5	17			
or "lower" or "not differing" ◂	0	0	0		0		
categories ◆	3	1	3			7	
Total	8	8	8				24

▸ = indicates higher trace element content in samples treated with Wuxal

◂ = indicates lower trace element content in samples treated with Wuxal

◆ = indicates statistically non-significant difference

The significance of differences is of various extent.

The enrichment of the trace elements occurs in the treated paprika without seeds as well as paprika with 20% seeds, although, in the majority of the cases, the trace element content in the samples treated with Wuxal appeared to be significantly higher than that in the control (except As). We have found a significant difference in soil samples treated with Wuxal as well as paprika samples (except Fe, Co).

Paprika soil samples were analysed for their mercury content. The significance of differences between paprika and corresponding soil samples, whether treated or untreated was found to be negligible.

The work was carried out within the frame of the Austrian–Hungarian scientific bilateral agreement.

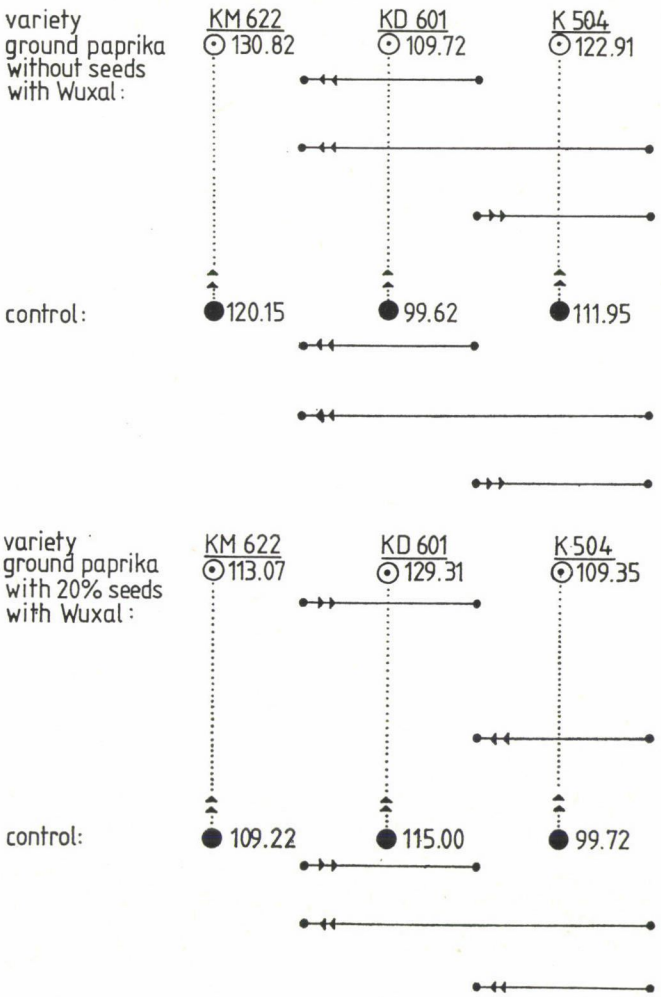

Fig. 1. Differences in Fe content of powdered paprika samples calculated by *t*-test
▲ ▲ = trace element content significantly higher in samples treated with Wuxal (t = 0.05)
◄ ◄ = trace element content significantly higher in this cultivar (t = 0.05)
► ► = trace element content significantly lower in this cultivar (t = 0.05)
◄ = trace element content higher in this cultivar (t = 0.05)

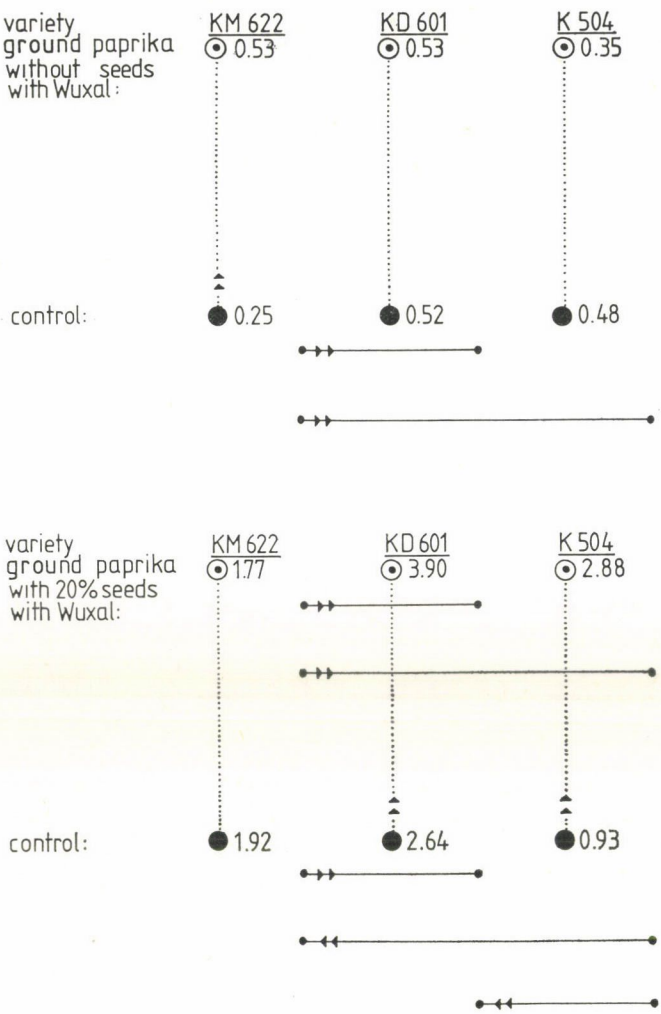

Fig. 2. Differences in Cr content of powdered paprika samples calculated by *t*-test

▲ = trace element content significantly higher in samples treated with Wuxal (t = 0.05)
◆ = difference non-significant
◄ = trace element content higher in this cultivar (t = 0.05)
▲ = trace element content higher in samples treated with Wuxal (t = 0.05)
▼ = trace element content lower in samples treated with Wuxal (t = 0.05)
►► = trace element content significantly lower in this cultivar (t = 0.05)
◄◄ = trace element content significantly higher in this cultivar (t = 0.05)

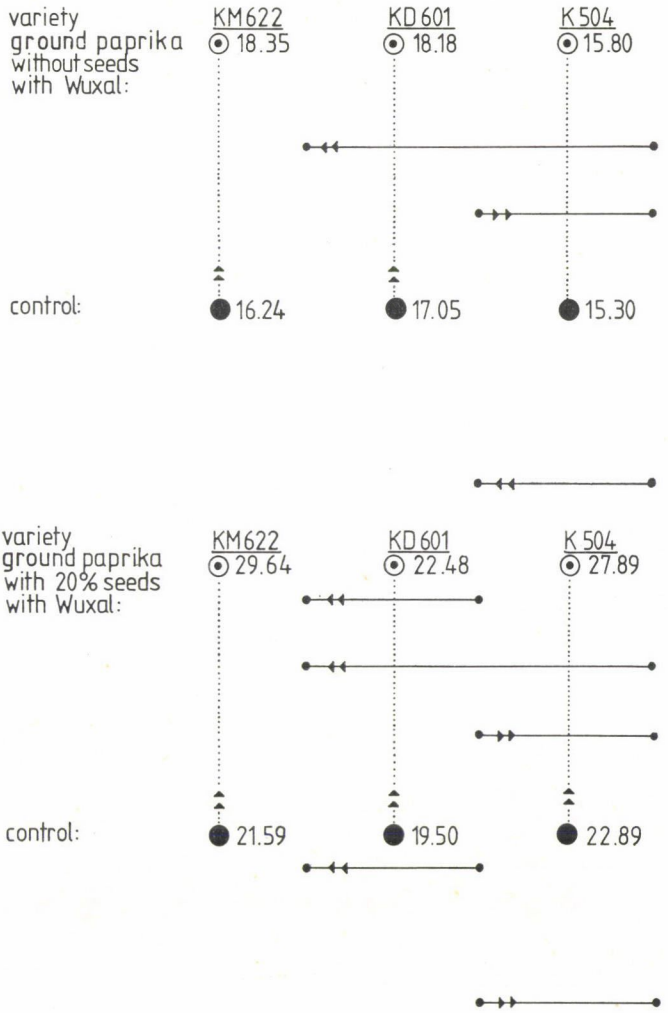

Fig. 3. Differences in Zn content of powdered paprika samples calculated by *t*-test

▲ = trace element content significantly higher in samples treated with Wuxal (t = 0.05)

◄ = trace element content higher in this cultivar (t = 0.05)

◄ ◄ = trace element content significantly higher in this cultivar (t = 0.05)

► ► = trace element content significantly lower in this cultivar (t = 0.05)

▲ = trace element content higher in samples treated with Wuxal (t = 0.05)

► = trace element content lower in this cultivar (t = 0.05)

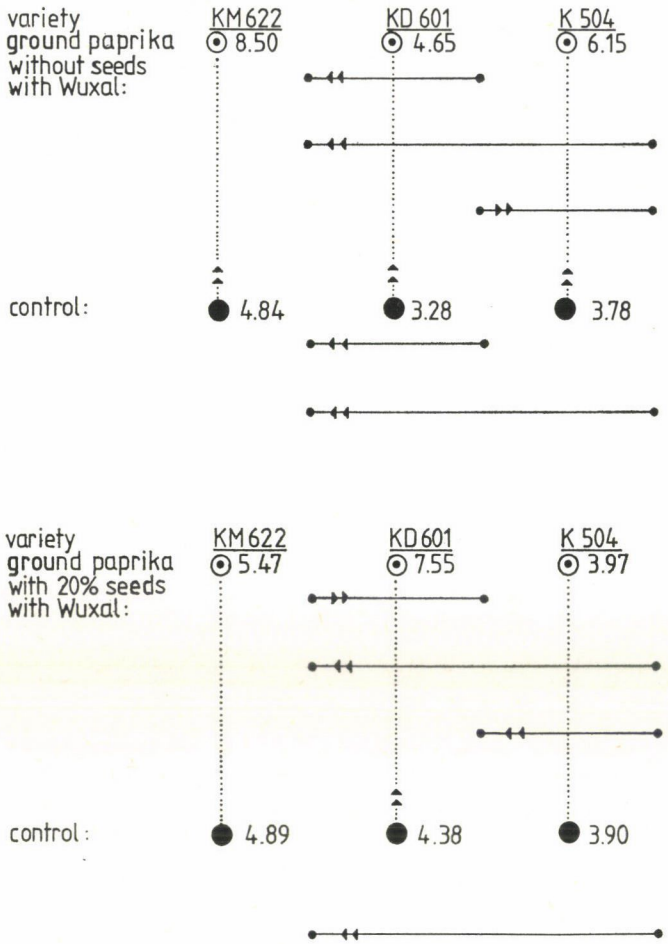

Fig. 4. Differences in Co content of powdered paprika samples calculated by *t*-test
▲ ▲ = trace element content significantly higher in samples treated with Wuxal (t = 0.05)
◄ ◄ = trace element content significantly higher in this cultivar (t = 0.05)
► ► = trace element content significantly lower in this cultivar (t = 0.05)
▲ = trace element content higher in samples treated with Wuxal (t = 0.05)
► = trace element content lower in this cultivar (t = 0.05)
◄ = trace element content higher in this cultivar (t = 0.05)

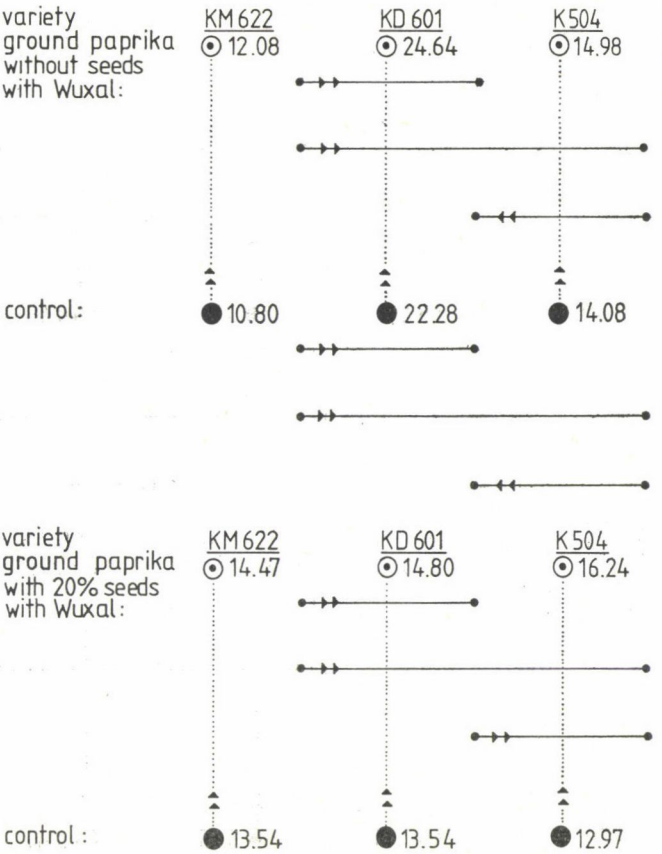

Fig. 5. Differences in Br content of powdered paprika samples calculated by *t*-test
▲ ▲ = trace element content significantly higher in samples treated with Wuxal (t = 0.05)
► ► = trace element content significantly lower in this cultivar (t = 0.05)
◄ ◄ = trace element content significantly higher in this cultivar (t = 0.05)
◆ = difference non-significant
◄ = trace element content higher in this cultivar (t = 0.05)

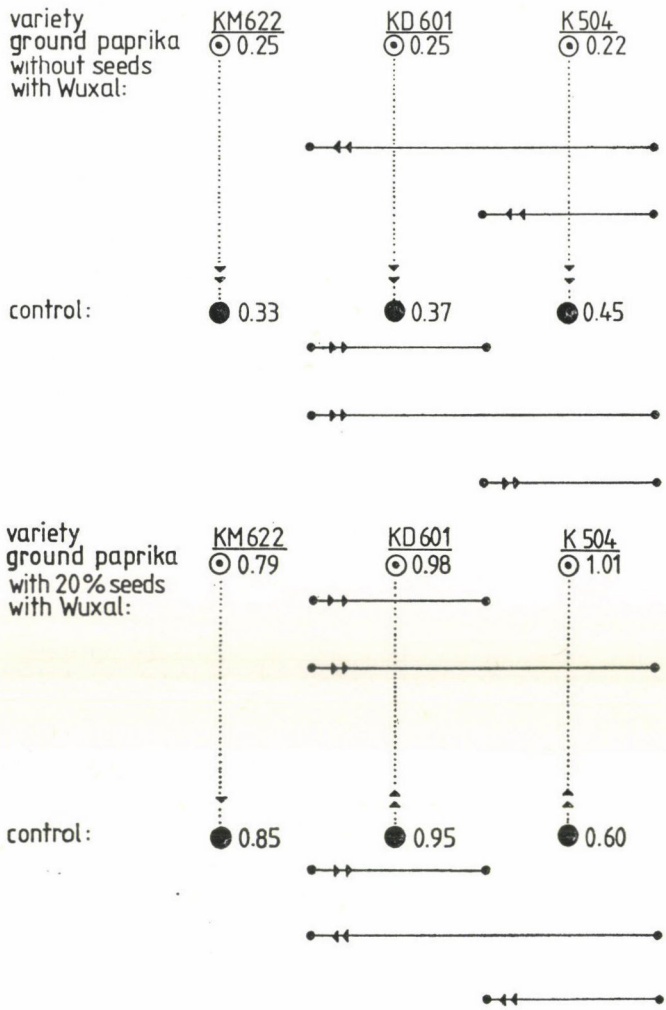

Fig. 6. Differences in As content of powdered paprika samples calculated by *t*-test

▼ ▼ = trace element content significantly lower in samples treated with Wuxal (t = 0.05)
◆ = difference non-significant
◄ ◄ = trace element content significantly higher in this cultivar (t = 0.05)
► ► = trace element content significantly lower in this cultivar (t = 0.05)
▼ = trace element content lower in samples treated with Wuxal (t = 0.05)
▲ ▲ = trace element content significantly higher in samples treated with Wuxal (t = 0.05)
► = trace element content lower in this cultivar (t = 0.05)

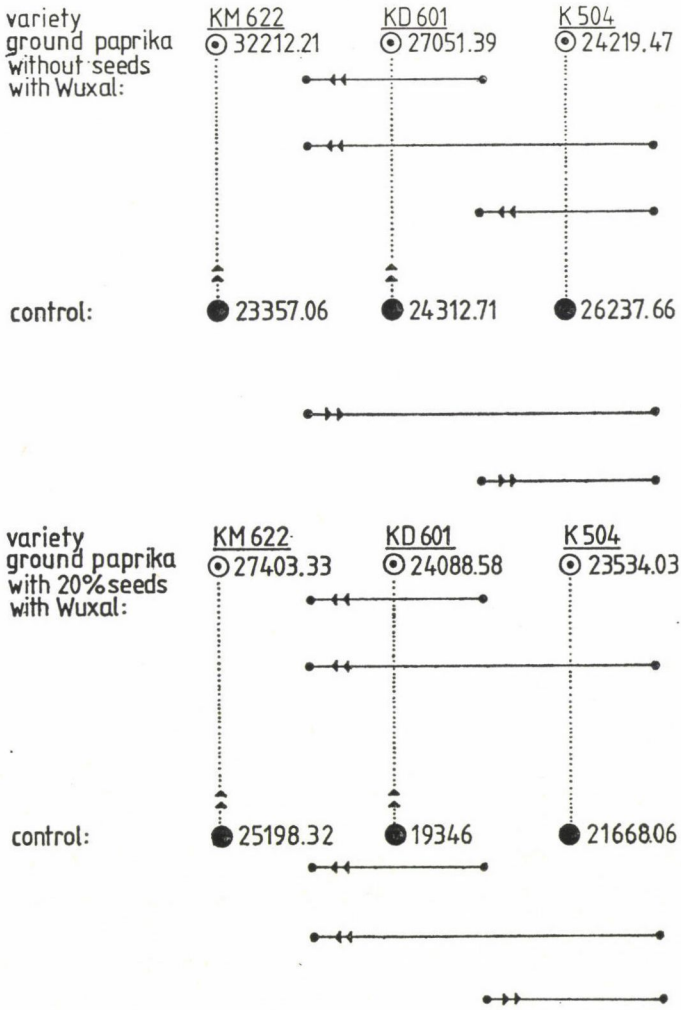

Fig. 7. Differences in K content of powdered paprika samples calculated by *t*-test

▲ ▲ = trace element content significantly higher in samples treated with Wuxal (t = 0.05)
◄ ◄ = trace element content significantly higher in this cultivar (t = 0.05)
▼ = trace element content lower in samples treated with Wuxal (t = 0.05)
► = trace element content lower in this cultivar (t = 0.05)
► ► = trace element content significantly lower in this cultivar (t = 0.05)
◄ = trace element content higher in this cultivar (t = 0.05)
▲ = trace element content higher in samples treated with Wuxal (t = 0.05)

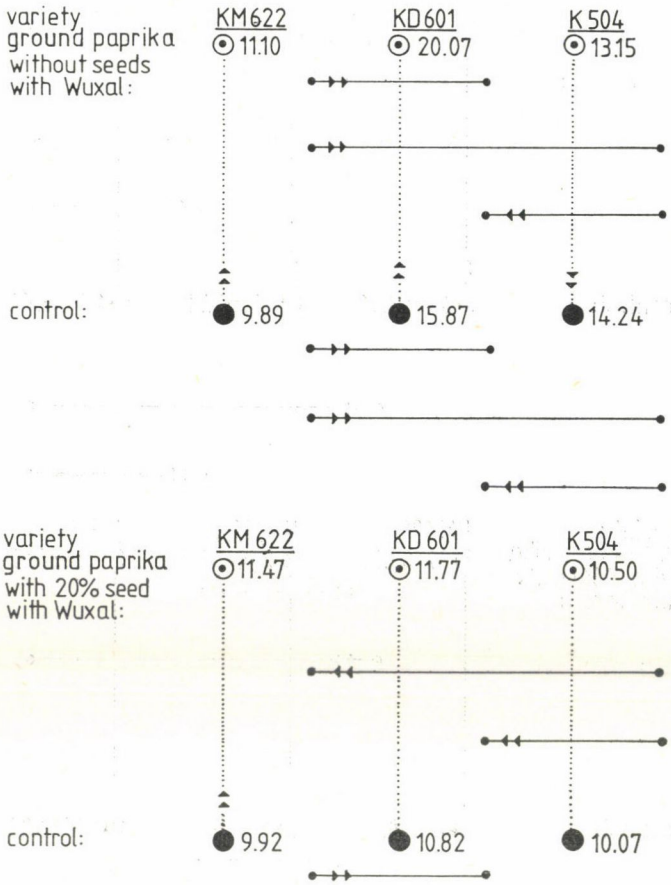

Fig. 8. Differences in Mn content of powdered paprika samples calculated by *t*-test

▲ ▲ = trace element content significantly higher in samples treated with Wuxal (t = 0.05)
► ► = trace element content significantly lower in this cultivar (t = 0.05)
◄ ◄ = trace element content significantly higher in this cultivar (t = 0.05)
▼ ▼ = trace element content significantly lower in samples treated with Wuxal (t = 0.05)
► = trace element content lower in this cultivar (t = 0.05)
▲ = trace element content higher in samples treated with Wuxal (t = 0.05)
◄ = trace element content higher in this cultivar (t = 0.05)

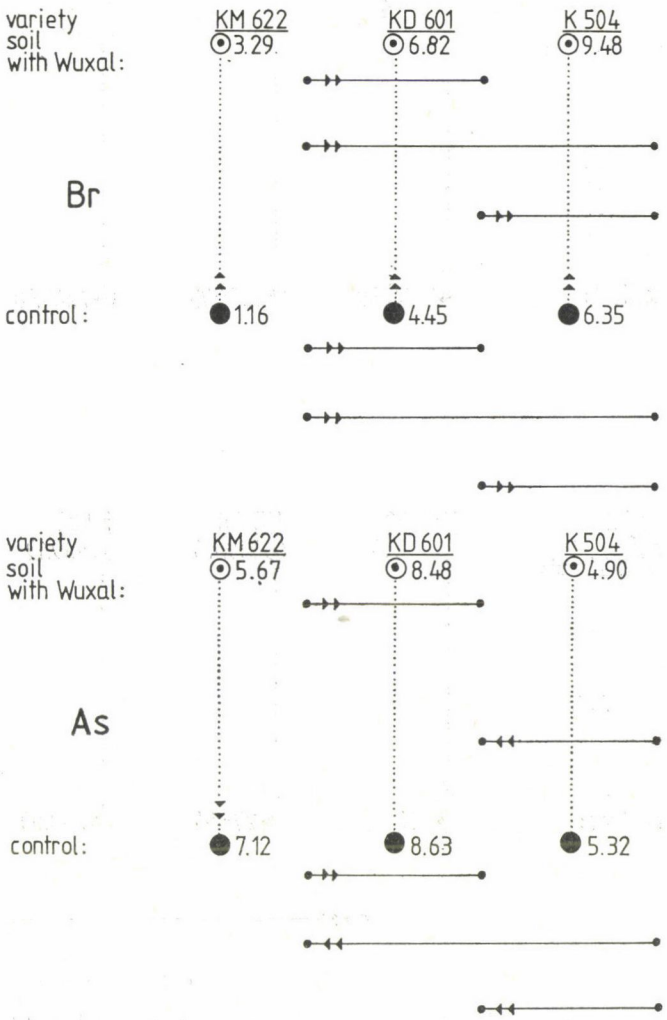

Fig. 9. Differences in Br and As contents of soil samples calculated by *t*-test

▲ ▲ = trace element content significantly higher in samples treated with Wuxal (t = 0.05)

► ► = trace element content significantly lower in this cultivar (t = 0.05)

▼ ▼ = trace element content significantly lower in samples treated with Wuxal (t = 0.05)

◄ = trace element content higher in this cultivar (t = 0.05)

▼ = trace element content lower in samples treated with Wuxal (t = 0.05)

◄ ◄ = trace element content significantly higher in this cultivar (t = 0.05)

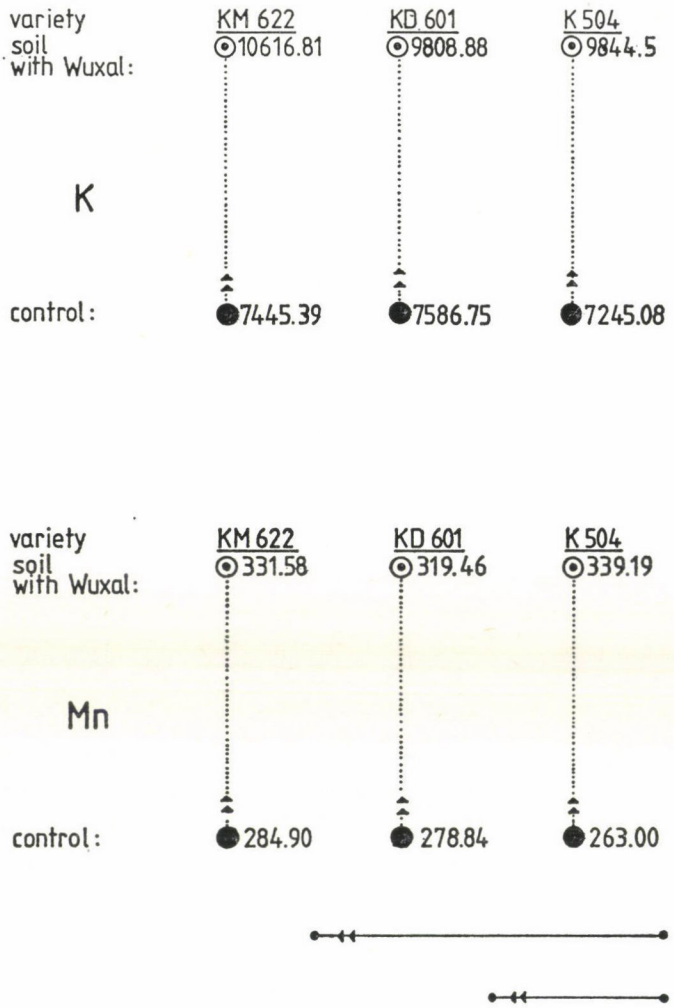

Fig. 10. Differences in K and Mn contents of soil samples calculated by *t*-test
▲ ▲ = trace element content significantly higher in samples treated with Wuxal (t = 0.05)
◄ = trace element content higher in this cultivar (t = 0.05)
► = trace element content lower in this cultivar (t = 0.05)
◄ ◄ = trace element content significantly higher in this cultivar (t = 0.05)

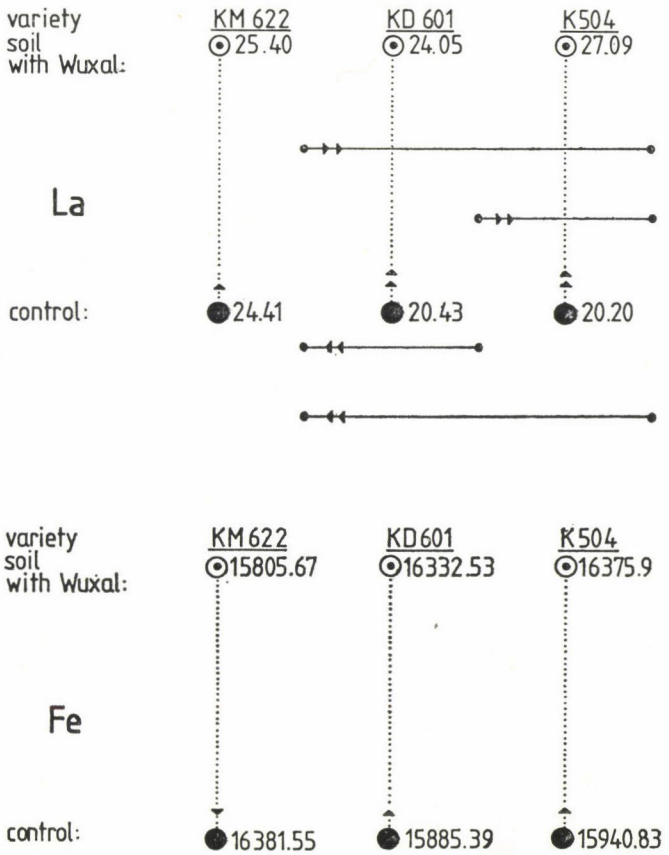

Fig. 11. Differences in La and Fe contents of soil samples calculated by *t*-test
▲ = trace element content higher in samples treated with Wuxal (t = 0.05)
◄ = trace element content higher in this cultivar (t = 0.05)
►► = trace element content significantly lower in this cultivar (t = 0.05)
▲▲ = trace element content significantly higher in samples treated with Wuxal (t = 0.05)
◄◄ = trace element content significantly higher in this cultivar (t = 0.05)
▼ = trace element content lower in samples treated with Wuxal (t = 0.05)
► = trace element content lower in this cultivar (t = 0.05)

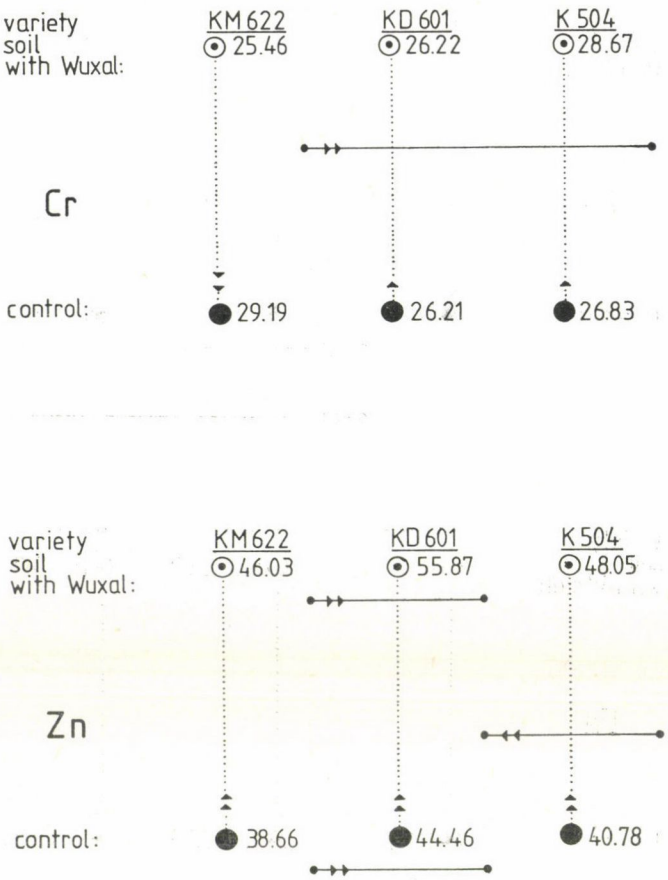

Fig. 12. Differences in Cr and Zn contents of soil samples calculated by *t*-test

▼ = trace element content significantly lower in samples treated with Wuxal (t = 0.05)

► = trace element content lower in this cultivar (t = 0.05)

►► = trace element content significantly lower in this cultivar (t = 0.05)

▲ = trace element content higher in samples treated with Wuxal (t = 0.05)

◄ = trace element content higher in this cultivar (t = 0.05)

▲ = trace element content significantly higher in samples treated with Wuxal (t = 0.05)

◄◄ = trace element content significantly higher in this cultivar (t = 0.05)

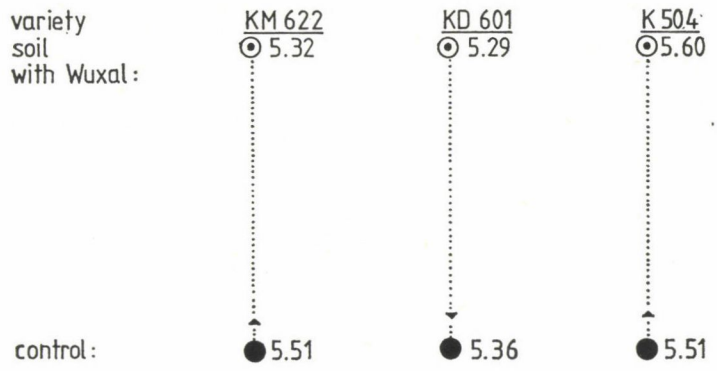

Fig. 13. Differences in Co content of soil samples calculated by *t*-test
▲ = trace element content higher in samples treated with Wuxal (t = 0.05)
◄ = trace element content higher in this cultivar (t = 0.05)
► = trace element content lower in this cultivar (t = 0.05)
▼ = trace element content lower in samples treated with Wuxal (t = 0.05)
◆ = difference non-significant

Table 8

Effect of treatment on the content of 9 trace elements in soil

Trace element	Varieties			No. of cultivars in the "higher" or "lower" or "not differing" categories			Total
	KM 622	KD 601	K 504	►	◄	◆	
Br	►	►	►	3	0	0	3
As	◄	◆	◆	0	1	2	3
K	►	►	►	3	0	0	3
Mn	►	►	►	3	0	0	3
La	◆	►	►	2	0	1	3
Fe	◆	◆	◆	0	0	3	3
Cr	◄	◆	◆	0	1	2	3
Zn	►	►	►	3	0	0	3
Co	◆	◆	◆	0	0	3	3
No. of trace elements in the "higher" ►	4	5	5	14			
or "lower" or "not differing" ◄	2	0	0		2		
categories ◆	3	4	4			11	
Total	9	9	9				27

► = indicates higher trace element content in samples treated with Wuxal
◄ = indicates lower trace element content in samples treated with Wuxal
◆ = indicates statistically non-significant difference

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EXTENDING THE STORAGE LIFE OF GARLIC BY GAMMA-IRRADIATION

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Gamma irradiation (0.03 kGy) effects on garlic bulbs from local cultivars were studied. The treatment proved to be effective in reducing weight loss and spoilage percentage of the bulbs. After 10 months of storage the weight loss was observed to be reduced by 37% in irradiated garlic. Irradiation reduced sprouting of the fresh bulbs but did not affect the rotting process.

A great part of the total garlic crops (*Allium sativum* L.) is wasted annually due to sprouting and rotting, which are usually followed by a loss of quality in the product.

Gamma irradiation has proved to be effective for sprout inhibition in root, bulb and tuber crops, as reported by EL-OKSH and co-workers (1971). Many other investigators have reported preliminary studies on sprout inhibition in garlic bulbs (MATHUR, 1963; ABDEL-AL, 1967). Relatively low doses, from 0.05 to 0.1 kGy inhibit development of the vegetative meristem within garlic bulblets. This inhibition results in a total and irreversible stop in sprouting and the bulbs can therefore be stored in permanent dormancy (MATHUR, 1968). MICHIELS (1967) found that the irradiated material shows some inhibition in its metabolism, with decrease in its respiratory activity and lowering of the weight losses.

It was reported that when irradiation is carried out within 30–60 days after harvest the response to irradiation of garlic bulbs is better than in those irradiated 90 or more days postharvest, mainly due to the great resistance of developed sprout to radiation (BRUNELET & VIDAL, 1960).

Many investigators have reported studies on sprout inhibition in garlic bulbs with doses from 0.075 to 0.15 kGy (EL-OKSH et al., 1971; ABDEL-AL, 1967; MATHUR, 1968; BRUNELET & VIDAL, 1960). These investigations have shown that an average dose of 0.12 kGy was most effective in sprout inhibition and in reducing the percentage of empty cloves. However, some other research studies showed that a lower dose level of about 0.03–0.05 kGy reduces weight losses and sprouting decay in garlic bulbs (MATHUR, 1963).

The difference of dose levels for achieving a good inhibitory effect of internal and external sprout may have some explanation in the fact that each investigator has carried out his experiment with a particular variety of garlic (with their own cultivar characteristics and storage requirements), different from the others.

In previous experiments with garlic a wide range of doses and different postharvest periods were tried. It was concluded that a 0.03 kGy dose after 30 days of harvest would inhibit the sprout development in garlic. The experiment reported here shows the effect of gamma radiation on total losses, weight loss and storage life of garlic bulbs under common storage conditions.

1. Materials and methods

Garlic was harvested during early December and was cured naturally in the fields during the first 10 days before packaging for treatment. Garlic of the variety "Red" was used in these studies.

A laboratory scale experiment was carried out, measuring the weight loss of samples and the total losses due to sprouting and rotting.

For loss of weight studies at laboratory scale 20 samples of 1 000 $\pm$ 100 g were taken and packaged in perforated paper bags. The total loss experiment was carried out with 20 samples of 50 bulbs each also in perforated bags. Ten of each sample were irradiated and the remaining were left for control.

Garlic bags were packaged in carton boxes of $28 \times 30 \times 35$ cm in size, equal to the capacity of the irradiation chamber. Irradiation was carried out at the facilities of Comisión Nacional de Energía Atómica in Ezeiza Atomic Center, in the Mobable Irradiator IMO-I. This irradiation unit was supplied with a ^{60}Co source of 0.44 PBq (12 000 Ci) and gave, at the time of these experiments, a dose rate of 1.82 kGy h^{-1} with a maximum value of the D_{max} per D_{min} ratio of 1.25.

The samples were irradiated 30 days after having been harvested, with a dose of 0.03 kGy. The irradiated and control samples were stored at the Laboratorio de Radioisótopos of the Universidad Nacional del Sur during 10 months, at a room temperature ranging from 15 to 18 °C and a relative humidity of 70–80%.

Records of the weight of samples and sprouting were taken monthly. Bulbs were carefully observed to detect symptoms of rotting or external sprout; in case of rotting or sprouting the bulbs were immediately discarded. The weight of each sample was taken with a Bosch P115 monoplate balance.

2. Results

The development of the cumulative weight loss during the 300-days storage period for both control and irradiated garlic bulbs is shown in Fig. 1. While control samples suffered an average decrease of 60% of their weight, the irradiated ones lost only 23%.

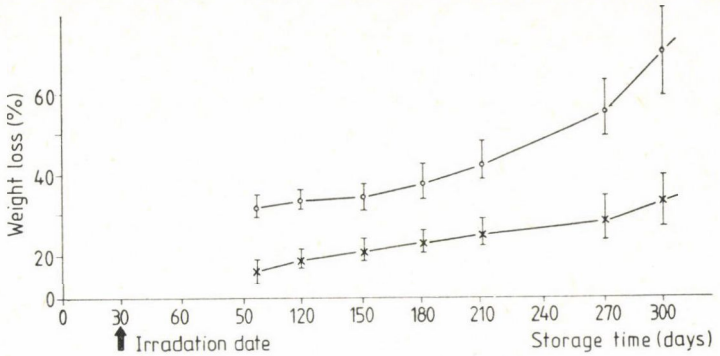

Fig. 1. Loss of weight in irradiated (—x—) and control (—o—) garlic bulbs during 300-days storage at 15–18 °C (R. H. 70–80%)

Table 1

Number of garlic bulbs discarded stored at 15—18 °C temperature and 70—80% relative humidity

Treatment	Number of bulbs	Days of storage						
		90	120	150	180	210	240	270
		Number of discarded bulbs						
Control	50	4.5±1.7	4.5±1.7	4.5±1.7	5.5±2.0	7.0±3.0	7.5±3.0	8.5±3.3
Irradiated	50	2.0±2.0	3.0±2.0	3.0±2.0	3.0±1.7	3.0±1.7	4.0±1.7	5.0±2.0

The spoilage percentage of bulbs due to sprouting and rotting in the irradiated samples was lower than that of the control samples (Table 1). This tendency was observed to be more accented after 180 days of storage.

3. Discussion

The lower weight loss in irradiated samples may have some explanation in the inhibitory effect of radiation on the respiratory activity of bulbs (MICHIELS, 1967), further in the absence of sprouting and because γ-rays inhibit the action of certain oxidases (EL-OKSH et al., 1971) and other enzymes. The shrivelling of cloves during storage is also one of the most important factors influencing weight loss.

Greater differences between control and irradiated spoilage percentages towards the end of the storage period were due to sprout development in control samples after the sixth month.

It can be concluded that in this variety of garlic the major effect of radiation was observed in weight loss reduction (37% lower in irradiated than in control); this was caused mainly by radioinduced sprout inhibition, having little effect on spoilage by rotting.

*

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QUALITY DESCRIPTION OF SHREDDED ENDIVE I. PRELIMINARY RESEARCH AND STATISTICAL APPROACH

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The purpose of this experiment was to define relevant parameters, to describe the external sensory quality of prepacked shredded endive within the scope of quality control. In the first part of this research 36 samples were used to evaluate sensory as well as physical and physiological variables.

A stepwise regression analysis revealed that the sensory variables could be characterized sufficiently by only one physical variable: with Hunter colour values a/b of the adherent water correlation coefficients of 0.78–0.88 were achieved. When the quotient a/b was more negative than −0.219 the sample was acceptable on account of its colour. Comparison of this classification with the sensory one revealed only 4 discrepant classifications.

On account of the numbers of discrepant classifications the next best describing variables were respectively the CO_2- and O_2-content in the package. However, in the light of sampling, analysis, possible adulteration and the relatively low correlations with the sensory variables, these variables are not preferable. Although total viable count of bacteria had an unacceptable number of discrepant classifications, rejection of this variable is not justified because of its relatively high correlations with the sensory variables.

The sale of prepacked shredded endive, ready for cooking, has increased in the Netherlands in recent years. Whereas unpacked whole endive heads can be stored for about 2 weeks at 0–1 °C and 90–95% R. H., and even longer by inclusion of 1% CO in the atmosphere (BOARINI & BUONOCORE, 1973), shredded, washed, centrifuged and prepacked endive can be held for only 3 days under these storage conditions (data from SPRENGER INSTITUTE, 1973). LANGERAK (1975) found that shredded and prepacked endive after irradiation with 1 kGy had a storage life of 4 days, compared to 2 days for non-irradiated endive, both at 12 °C. It is clear that prepacked shredded endive requires rigid control of quality throughout distribution, since improper storage conditions quickly lead to an unacceptable loss of quality from the consumers' point of view.

Although sensory quality is commonly considered as a property of vegetables important to the consumer, in recent literature there is little, if any research reported, devoted to the description of the quality of shredded endive. The purpose of the experiment, reported here, is to define physical parameters which can be used to describe the external sensory quality of any sample of prepacked shredded endive, irrespective of its previous history (storage period, storage temperature, origin, etc.).

Deterioration in quality of prepacked shredded vegetables becomes evident through discolouration and microbiological spoilage. Brown discolouration of leafy vegetables after handling and shredding can be caused by oxidation of phenolic substances, catalyzed by enzymes, and also by non-enzymatic reactions (MATHEW & PARPIA, 1971). WINTER (1958) found that a red pigment, a complex quinone (intybin), was formed in endive leaves after injury. Therefore the colour of endive may be taken as a parameter of quality.

A second variable could be the total viable count of bacteria which is related to the amount of decay (BOARINI & BUONOCORE, 1973; LANGERAK, 1975). Rate of decay may be influenced by the quantity of adherent water, which becomes the third physical variable.

Decay can be retarded by cooling. Rapid cooling can be achieved by vacuum-cooling (GREIDANUS, 1969), provided that sufficient perforations are present in the package. In non-perforated prepacks a relatively low O_2 content retards respiration which is favourable, but at a too low O_2 level decay and souring will result. On account of these considerations the O_2 and CO_2-concentrations within the package can be tested as the fourth and fifth physical variables (DE MAAKER, 1979).

The first part of this research is focused on the selection of the best physical variables from the 4 variables mentioned above. In the second part of this experiment the result from the first part will be confirmed by testing the selected variables with 252 new samples of endive.

1. Materials and methods

1.1. Sampling

Samples (36) of prepacked shredded endive, each of 1 kg, were bought from various retailers in and near Wageningen over a period of one week. The endive had been cut mechanically into strips of 5—8 mm width and packaged in non-perforated polythene bags of 0.02 mm thickness. After purchase the product was stored at 1, 8, 14 or 20 °C during 0, 24 or 48 hours. In this way a range of samples having sharply divergent qualities was created.

Measurements and sensory evaluation took place during the last 3 days of this week in 12 series of 3 samples, selected at random from samples left. In each series the samples for gas-chromatographic determination were taken first and after that odour was evaluated by sensory methods. Subsamples for the other measurements were taken next.

1.2. Gas-chromatographic determination of CO_2 and O_2

Samples were taken with a hypobaric syringe of 0.5 cm^3 (Pressure Lok, type A) after strengthening the spot of perforation with a piece of adhesive tape (Scotch). Two gas samples were taken per bag. The gas chromatograph

was an Intersmat, type 112M, using a catharometer detector (temperature 90 °C, bridge-current 150 mA). Two columns were used in parallel at a temperature of 85 °C (carrier gas H_2, 10 $cm^3\,min^{-1}$). The 200 cm column with Porapak Q, 50–80 mesh, separated CO_2 from O_2—N_2; the 400 cm column with Molsieve 5A, 60–80 mesh, separated O_2 from N_2. Concentrations were calculated by comparing peak areas of the samples with those of a mixture of known composition.

1.3. Sensory evaluation

After sampling for CO_2 and O_2, bags of 3 samples were opened and the odour was assessed by 3 panelists, experts on raw vegetables. After assessing odour, each sample was assessed in triplicate for stain/rot, browning and general impression of the raw product. The panelists used one sample for each parameter, randomly presented for parameter and sample. After this evaluation for the external quality, the endive was cooked and assessed in the same way. The endive was not eaten for reasons of health. The panelists judged by scoring on a line anchored at the ends with "least" and "most" for the sensory variables mentioned above.

1.4. Determination of total viable count of bacteria

Immediately after assessment of odour, samples were taken for determination of total viable count and adherent water. For determination of total viable count a suspension of a 100 g subsample was homogenized with 300 g physiological solution of salt for 3 minutes in a sterile polythene bag using a Colworth Stomacher 400. Six dilutions were made in the series 1 : 10, 1 : 10^2, . . ., 1 : 10^6 by means of the Colworth Droplette. One cm^3 of each dilution was mixed with 10 cm^3 of plate count agar (Oxoid CM325) in a Petri dish; this was done in duplicate. After solidification the Petri dishes were incubated at 30 °C for 3 days. In those dishes with 50–150 colonies counting was performed by means of a colony counter.

1.5. Determination of adherent water

A quantity of 150 g of sample was weighed in duplicate and centrifuged for 5 minutes at a speed of 3000 r.p.m. (Braun household juice-centrifuge, type MP50); decrease in weight (D_w) was measured. The removed water was preserved for the determination of colour. From D_w adherent water content H in % was calculated using the formula $H = 100 \times (D_w - 1.44)/(D_w + 70.4)$. (A conversion according to an experimentally determined calibration line.)

1.6. Determination of the colour of adherent water

A sample of endive with little adherent water will give a darker colour than a sample of the same quality with much adherent water. In order to correct for this, a specific dilution was made for each sample: $D_w/2$ cm^3 (D_w = decrease in weight, see above) of the removed water was diluted to a volume of $(150 - D_w)/4$ cm^3 ($150 - D_w$ is the weight of the centrifuged endive). Colour of the diluted adherent water was measured in a cuvette with 10 mm light-path by transmittance. Colour was expressed in L-, a- and b-values with the Hunterlab colour difference meter type D25D2 Optical Head (arrangement I).

1.7. Initial calculations

Sensory assessment was performed by a scoring method. To correct for the level and score-width of each panelist, the original observations have been transformed with the Z-transformation (deviations from the mean with unit standard deviation). From these transformed data the mean was calculated for each sample.

To enable the use of classical statistical methods a verification was made whether or not distributions of the observations were normal. Six variables were non-normal. Therefore these variables, mentioned in Table 1, were transformed according to the formulas given in this Table. After these transformations non-normality was no longer significant.

2. Results

Table 2 gives an impression of level and standard deviation of the values of the physical variables for the 36 samples. Logarithms of total viable count, in the range of 6.25–8.85, agree with the results of LANGERAK (1975), who found a range of about 6.2 to 8.8.

2.1. Selecting variables for describing purposes

The first step was to analyse which and how many physical variables are necessary to characterize the sensory quality. In Table 3 correlation coefficients are given for the sensory variables and the physical measurements. It can be seen that almost all variables, except the percentage of adherent water, can contribute to the description of the sensory characteristics.

In most cases the describing power can be increased by including more variables in the characterization. However, if these are mutually highly correlated, which is very likely (Table 3), the improvement in the describing power will be small. This improvement was investigated with the aid of stepwise

Table 1

Transformation of the non-normal variables
(y = transformed variable and x = original variable before transformation)

Variable	Transformation
Hunter L	$y = \log [(1 + x/90)/(1 - x/90)]$
Hunter a	$y = \log (x + 10)$
Hunter a/b	$y = 1/(x + 0.5)$
O_2 content	$y = \log x$
Browning	$y = \text{arc sin } \sqrt{(x/9)}$
General impression cooked product	$y = \log [(1 + x/8)/(1 - x/8)]$

Table 2

Mean ($\bar{x}$), standard deviation ($\pm s$) and extreme values of the measurements for the physical variables (36 samples)

Physical variable	$\bar{x}$	$\pm$s	Minimum	Maximum
Total viable count (log/g)	7.39	0.75	6.25	8.85
Adherent water (%)	20.6	7.5	3.0	40.2
Hunter L	75.9	9.8	46.8	87.9
Hunter a	—3.2	3.3	—7.2	8.3
Hunter b	21.0	5.1	7.3	30.8
Hunter a/b	—0.17	0.15	—0.35	0.30
CO_2 content (%)	10.8	6.7	1.2	28.6
O_2 content (%)	7.9	6.7	1.2	20.2

Table 3

Correlation coefficients between sensory and physical variables, together with the variables resulting from the stepwise regression analysis (SRA), with multiple correlation coefficient

Sensory variable	Physical variable								Variables resulting from SRA	Multiple correlation coeff.
	1 total viable count	2 adherent water	3 L[a]	4 a[a]	5 b	6 a/b[a]	7 CO_2	8 O_2		
Odour	—.80	—.27	.57	—.65	—.63	.80	—.75	.75	1+6+8	.87
Stain/rotten	—.85	—.24	.77	—.74	—.74	.88	—.71	.60	1+5+6	.94
Browning[a]	—.63	—.40	.70	—.65	—.62	.78	—.50	.57	6+8	.82
Gen. impr. raw product	—.87	—.30	.72	—.68	—.71	.88	—.77	.75	1+4+6+8	.95
Cooked[a]	—.82	—.21	.73	—.78	—.65	.83	—.75	.66	1+6	.87

[a] These variables have been transformed according to Table 1

regression analysis (DRAPER & SMITH, 1966). In this case the variable to be characterized was a sensory one; the physiological and physical measurements were treated as the explanatory variables. With this method the first step is selection of the explanatory variable having the highest correlation with the sensory variable to be explained.

The next step is to choose the variable giving most improvement in explanation of the sensory variable. This process proceeds untill the improvement is no longer statistically significant. This analysis has been performed with a Wang-2200 T6 computer with the program Stepwise Regression Analysis of the Regression Analysis Package (WANG, 1975). The results are presented in the last two columns of Table 3. From this table it can be deduced that improvement achieved by using 2, 3 or even 4 explanatory variables is only 4 to 7% compared to using only the best variable, i.e. Hunter a/b.

Although improvement gained by incorporating additional variables is statistically significant, it seems inadvisable to use more than one variable. For describing purposes an improvement of the correlation of 5% is very small in comparison to the work for extra measurements.

2.2. Describing power

The samples were divided into two groups, sensorially acceptable and sensorially unacceptable, on basis of the overall mean (M) of the 4 sensory variables for the raw product. For each explanatory physical variable the

Table 4

Survey of the mean ($\bar{x}$) and standard deviation ($\pm s$) of the physical variables for the sensorially acceptable and unacceptable samples, together with the criterion and the number of discrepant classifications, calculated with this criterion

Physical variable	Sensorially acceptable samples		Sensorially unacceptable samples		Criterion	Number of discrepant classifications
	$\bar{x}$	$\pm$s	$\bar{x}$	$\pm$s		
Total viable count (log/g)	6.88	0.48	7.89	0.61	7.38	9
Adhering water (%)	19.10	6.18	22.10	8.64	20.60	18
Hunter L[a]	1.31	0.21	0.98	0.25	1.14	9
Hunter a[a]	0.68	0.13	0.90	0.16	0.79	8
Hunter b	17.90	4.40	24.00	3.68	20.98	8
Hunter a/b[a]	4.54	0.81	2.57	0.76	3.56	4
CO_2-content (%)	6.49	3.69	15.20	6.35	10.82	6
O_2-content[a] (%)	0.99	0.31	0.40	0.38	0.70	7

[a] Transformed; recalculated to the original values the criteria are respectively 78, — 3.83, —0.219 and 5.01

mean of each group was calculated. The middle value between both means was used as the criterion for inspecting the samples. In the knowledge of the value of the relevant explanatory variable and its criterion, a classification could be made as to whether a sample should be classified as "acceptable" or "unacceptable". Comparison of this classification with the sensory one gives the number of discrepant classifications, shown in Table 4.

In aid of further comparison, Table 5 gives the results of sensory evaluation and physical measurements for those samples, which demonstrate a discrepancy between sensory evaluation and physical measurements. The samples

Table 5

Values from sensory evaluation and physical measurements for the samples with discrepancy between these values

Sample No.	Sensory evaluation S[c]	Total viable count (log/g)	Adherent water (%)	Hunter L	Hunter a	Hunter b	Hunter a/b	CO_2 (%)	O_2 (%)
1	73	6.40	20.6[b]	85.6	−3.2[b]	10.8	−0.30	6.3	10.3
6	61	7.17	8.1	72.6[b]	−3.1[b]	15.1	−0.21[b]	4.3	16.8
7[a]	−43	8.22[b]	18.1	79.2	−4.5	21.7[b]	−0.21[b]	15.2[b]	1.4[b]
8	9	7.74[b]	18.2	76.2[b]	−6.5	24.6[b]	−0.26	4.8	17.3
10	47	7.46[b]	25.9[b]	81.9	−5.2	18.5	−0.28	12.2[b]	1.5[b]
11	69	7.72[b]	27.0[b]	80.0	−6.1	21.2[b]	−0.29	4.4	15.2
13[a]	−38	7.72[b]	20.6[b]	79.5	−3.7[b]	20.8	−0.18[b]	15.1[b]	1.9[b]
15	76	6.66	23.3[b]	79.1	−7.2	22.2[b]	−0.32	5.6	11.9
19[a]	−33	7.05	18.4	80.7	−3.3[b]	17.3	−0.19[b]	4.7	16.3
20[a]	−30	7.33	19.4	76.6[b]	−4.3	22.6[b]	−0.19[b]	12.8[b]	1.7[b]
21[a]	−6	7.09	28.9[b]	79.3	−3.8[b]	20.0	−0.19[b]	12.8[b]	1.6[b]
23	56	6.62	14.3	84.7	−2.8[b]	13.2	−0.21[b]	9.4	10.6
24[a]	−16	7.02	10.6	71.3[b]	−2.3[b]	25.3[b]	−0.09[b]	7.2	10.9
28	14	6.97	22.3[b]	75.6[b]	−6.8	24.5[b]	−0.28	1.3	20.2
29[a]	−27	7.15	22.1[b]	80.0	−4.6	20.9	−0.22	11.2[b]	1.8[b]
30	4	7.42[b]	23.9[b]	80.8	−5.2	20.6	−0.25	8.3	5.0[b]
31	9	6.71	18.4	81.5	−4.3	18.9	−0.23	11.1[b]	3.8[b]
34[a]	−30	7.40[b]	19.5	79.1	−5.0	22.1[b]	−0.23	4.4	16.6
36	55	7.14	3.0	87.9	−1.8[b]	7.3	−0.25	14.3[b]	4.0[b]
Criterion	0	7.38	20.60	78.0	−3.83	20.98	−0.219	10.82	5.01

[a] Rejected on account of sensory evaluation
[b] Rejected on account of physical measurements
[c] For explanation see para. 2.2 "describing power"

without discrepancy (except for adherent water) were evaluated sensorially better than 43 or worse than −33. These values (S) were calculated according to the formula:

$$s_i = \frac{v_i - M}{v_{max} - M} \times 100,$$

where

v_i = sensory evaluation of sample i
v_{max} = sensory evaluation of the best sample (No. 17)
M = overall mean.

The quotient of the Hunter a- and b-value of the adherent water seems to be the best describing variable, with only 4 discrepant classifications. However, it must be emphasized that testing a criterion with material from which it is derived, gives a biassed estimate of the real number of discrepant classifications. Two of these discrepant classifications refer to the sensorially good samples Nos. and 23 (Table 5) and also to good values for total viable count, CO_2 and O_2; thus these two can be labelled as real misclassifications. For the other two (samples 29 and 34) the situation is less evident.

The next best describing variables are respectively the CO_2 and O_2 content in the package, but these are not preferable in the light of sampling, analysis, possible adulteration and their relatively low correlations with the sensory variables (Table 3). Although total viable count has an unacceptable number of 9 discrepant classifications, the rejection of this variable is not yet justified because of its relatively high correlations (Table 3). The describing power of the adherent water is of no value.

3. Conclusions

When the Hunter a/b-value of the adherent water at standard dilution of prepacked shredded endive is more negative than −0.219, the sensory quality of the sample may be acceptable. This experiment has been continued in order to confirm these results, see Part II.

*

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QUALITY DESCRIPTION OF SHREDDED ENDIVE II. MAIN RESEARCH AND CONFIRMATION

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In order to confirm the results concerning the two best parameters (from the first part of this experiment) to describe the external sensory quality of prepacked shredded endive, 252 samples were collected from various retailers. These were used to evaluate sensory as well as colour and microbiological variables.

The best describing variable was the Hunter a-value, measuring colour at 30 mm light-path of the standardized adherent water. When this value is negative, the sensory quality of the sample may be acceptable, but is likely to be unacceptable in the case of a positive value. With this criterion 38 (15%) discrepant classifications occurred.

Next best describing variable was the total viable count with 55 (22%) discrepant classifications; this variable has the advantage of being connected directly with aspects of health.

Total count of the *Coli* group and total count of the *Pseudomonas* group had unacceptable number (29 and 35% resp.) of discrepant classifications, but they may be useful to reject samples for reasons of health, irrespective of the sensory quality or the Hunter a-value.

In the first part of this experiment with 36 samples of prepacked shredded endive, HILHORST and co-workers (1983) found that colour of the adherent water, as quantified by the quotient of the Hunter a- and b-values, seemed to be the best parameter to act as a substitute for sensory quality. Total viable count proved to have nearly as high correlations with sensory variables as the quotient, mentioned above, but it had much more discrepant classifications.

The purpose of the second part of this experiment is to confirm the results concerning Hunter a/b and total viable count. In addition to total viable count, also the total count of bacteria from the *Coli* group and those from the *Pseudomonas* group were measured for reasons of health, as a side-aspect of this research.

1. Materials and methods

1.1. Sampling

Evenly spread over the year, 252 samples of prepacked shredded endive were examined: 72 samples in October 1978 (period 1), 36 (mainly imported endive) in February (2), 72 in June (3) and 72 in October 1979 (4). Each sample was divided into subsamples (for microbiological, colour, and sensory evaluation). For further details, see HILHORST and co-workers (1983).

1.2. Determination of the numbers of bacteria

The suspension, homogenized as described by HILHORST and co-workers (1983) remained during 1 hour at room temperature and then a sample of it was used for determination with the Spiral Plate Machine. Some suspensions had to be diluted by means of the Colworth Droplette up to $1 : 10^3$.

For determination of total viable count plate count agar (Oxoid CM325) and for the total count of the Coli-group Violet Red Bile agar (Oxoid CM107) was used. For determination of the *Pseudomonas* group the medium as described by MOSSEL and TAMMINGA (1973) was chosen. The Petri dishes were incubated 2 days at 30 °C, except those for the *Pseudomonas* group; these were incubated 5 days at 17 °C. The bacteria colonies were counted with a counter belonging to the Spiral Plate System.

1.3. Determination of the colour of adherent water

The method, as described by HILHORST and co-workers (1983) proved inapplicable to dry or nearly dry samples of endive. So a new method, universally applicable to dry and wet samples, was developed.

First the decrease in weight (D_w) after centrifuging was measured in duplicate, as described by HILHORST and co-workers (1983). From the remainder of the original sample $3 \cdot 10^4$ per (150-D_w) g of endive was weighed in a polyethylene bag, followed by addition of water up to a total weight of 300 g; thus each bag contained 200 g dry endive and 100 g water (adherent + added water). The bag was closed and allowed to stay for 1 hour at ambient temperature, followed by centrifuging of the wet endive in two parts during 3 minutes each. The removed water was poured over Schleicher and Schüll filter paper no. 520 b, and its colour was measured by transmittance with the Hunterlab meter, arrangement III, in cuvettes with 10 mm, 20 mm and 30 mm light-path and the sums, being 40 and 50 mm.

1.4. Sensory evaluation

The general impression of the raw product was assessed by 3 panelists, experts on raw vegetables. Each panelist judged every sample for smell and general impression 4 times in random order: twice according to a scoring method (see HILHORST et al., 1983), and twice with a judgement "acceptable" or "unacceptable", on a forced choice base.

1.5. Initial calculations

The original sensory observations of the scoring method have been transformed with the Z-transformation, as described by HILHORST and co-workers (1983). From the remaining variables only bacterial counts were transformed to logarithms.

2. Results

Results of colour measurements in the first period (October 1978) revealed that measurements with 30 mm light-path differentiated most between the extreme samples for the Hunter L-, a- and b-values, and that for the a-value the number of discrepant classifications tended to decrease with longer light--paths. Thus during periods 2, 3 and 4 colour measurements were continued with only 30 mm and 50 mm light-path. In three of the four periods the measurements at 30 mm and 50 mm light-path appeared to reveal about the same results, in one period however the 30 mm light-path gave much better results. In this period 6 very bad samples occurred, which produced such dark brown and turbid solutions, that the a-values at 50 mm light-path became low positive or even negative, and so in the area of good quality, whereas the a-values at 30 mm light-path remained ample positive. These phenomena are shown in Fig. 1. In practice the measurement at 30 mm light-path, which never gave a negative a-value for a very bad sample, is preferable. On account of these findings only the results of the measurements with 30 mm light-path are presented here.

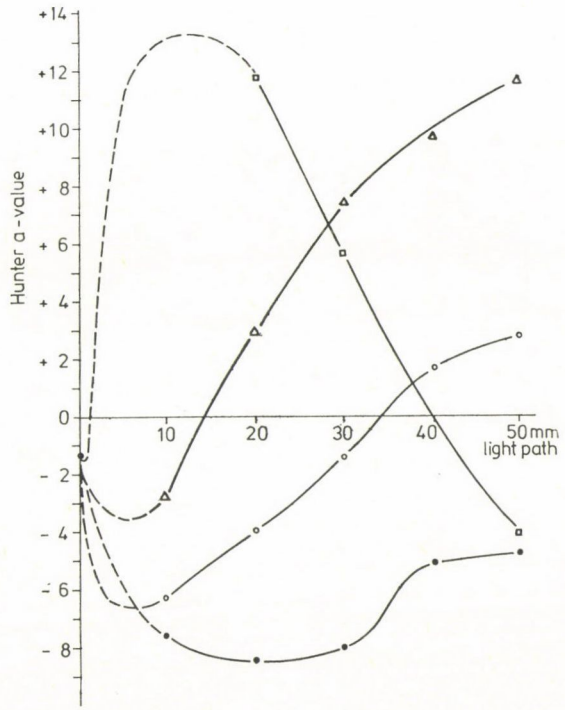

Fig. 1. Relationship of the Hunter a-value to the length of light-path for the adherent water of endive samples of good (●), moderate (○) bad (△), and extremely bad (□) quality

Table 1

Mean ($\bar{x}$) and standard deviation ($\pm s$) of the physical variables for all samples, sensorially acceptable and unacceptable, together with the criterion

Objective variable		Sensorially acceptable samples			Sensorially unacceptable samples			Criterion
		number	$\bar{x}$	$\pm s$	number	$\bar{x}$	$\pm s$	
Hunter values at 30 mm light-path	L	130	56.8	13.15	122	40.9	15.66	48.8
	a	130	−2.66	3.21	122	5.55	5.78	1.45
	b	130	25.8	4.56	122	23.2	7.47	24.5
	a/b	130	−0.10	0.12	122	0.41	0.71	0.15
	L × a/b	130	−6.38	6.95	122	9.04	9.46	1.33
log total viable count per g		130	7.17	0.56	122	7.84	0.45	7.50
log *Coli* group per g		130	6.49	0.76	122	7.29	0.64	6.89
log *Pseudomonas* group per g		94	6.28	0.69	86	6.89	0.73	6.58

Table 1 gives a survey of the physical variables for all samples, sensorially acceptable and unacceptable. The difference between the acceptable and the unacceptable samples is for Hunter a-value much larger than for Hunter b-value, in accordance with the results of BOLIN and co-workers (1977) with shredded lettuce. For all periods together the criteria are calculated for differentiating acceptable and unacceptable samples as described by HILHORST and co-workers (1983) in para 2.2.

With these criteria, given in Table 1, the percentage discrepant classifications are calculated for the sensorially acceptable, for the sensorially unacceptable samples, and for all samples. The results differentiated for each period apart and for all periods together, are given in Table 2.

The results from Table 2 show that Hunter a-value and the combination L × a/b are the best variables, followed by total viable count. These two Hunter values are much better than the quotient of the Hunter a- and b-value, which was the best variable in the preliminary research with 36 samples by HILHORST and co-workers (1983). The second period, during which endive mainly imported (from Italy) was examined, gave the worst results for the Hunter values; this may be due to the somewhat less green colour.

For the Hunter-values the sensorially unacceptable samples show higher % discrepant classifications than the sensorially acceptable samples; for the bacterial counts the situation is just the reverse. This result justifies closer inspection of the number of discrepant classifications for the a-value, the combination L × a/b and for total viable count. For these variables Table 3

Table 2

Percentage discrepant classifications for the sensorially acceptable and the sensorially unacceptable samples, calculated with the criteria from Table 1 for each period apart and for all periods together

	Percentage discrepant classifications for the sensorially acceptable and unacceptable samples					Percentage discrepant classifications for the sensorially acceptable samples					Percentage discrepant classifications for the sensorially unacceptable samples				
Period no.	1	2	3	4	1—4	1	2	3	4	1—4	1	2	3	4	1—4
Number of samples	72	36	72	72	252	41	18	35	36	130	31	18	37	36	122
Hunter L	31	47	22	25	29	29	28	37	3	24	32	67	8	47	34
values a	17	25	17	18	18	2	22	26	0	11	35	28	8	36	26
at 30 mm b	65	61	22	38	44	63	50	14	17	35	68	72	30	58	54
light-path a/b	24	31	17	25	23	0	6	11	0	4	55	56	22	50	43
L×a/b	15	28	15	11	16	2	39	26	0	13	32	17	5	22	19
log total viable count/g	28	19	22	17	22	37	17	17	22	25	16	22	27	11	19
log *Coli* group/g	46	31	24	15	29	68	11	17	14	32	12	50	30	17	25
log *Pseudomonas* group/g	43	44	22	—	35[a]	44	50	26	—	38[a]	42	39	19	—	31[a]

[a] Values apply to 180 samples only

gives the numbers of discrepant classifications, calculated with regularly increasing criterion-values. The a-value shows a minimum number (37 = 15% of 252) of discrepant classifications at a criterion, which is 1.7 lower than 1.45, the criterion from Table 1. The more complicated variable L×a/b, shows almost the same pattern and gives therefore no advantages in comparison with the simple a-value. For total viable count varying the criterion gives no reduction of the number of discrepant classifications; the calculated criterion of 7.50 proves to be a criterion with minimum discrepant classifications.

Since the criterion (−0.25) with minimum number of discrepant classifications is so close to zero, it is attractive and easy for practice to choose zero as criterion for the a-value. In this case all samples with a positive Hunter a-value may be unacceptable and those with negative a-value acceptable. Among the 38 samples (15% from 252), discrepantly classified at criterion zero, 17 samples were not pronouncedly assessed by the panelists as acceptable or unacceptable; the S-values were between −14 and +13 (best sample = 100, see HILHORST et al., 1983). The remaining 21 samples with clear sensory assessment can be labelled as real misclassifications.

Table 3

Numbers of discrepant classifications, calculated with regularly increasing criterion-values, for Hunter a-value, Hunter L × a/b-value and for total viable count

Objectiv variable	Sign of samples	Criterion values									
		—0.75	—0.50	—0.25	0.00	0.25	0.50	0.75	1.00	1.25	1.50
Hunter	252 A+U	44	40	37	38	40	40	40	43	44	46
a-value	130 A	28	24	20	20	20	20	16	16	15	14
	122 U	16	16	17	18	20	20	24	27	29	32

Objectiv variable	Sign of samples	Criterion values									
		—1.5	—1.0	—0.5	0.0	0.5	1.0	1.5	2.0	2.5	3.0
Hunter	252 A+U	45	39	37	38	40	41	41	42	42	46
L × a/b-	130 A	30	23	20	20	20	20	17	15	13	12
value	122 U	15	16	17	18	20	21	24	27	29	34

Objectiv variable	Sign of samples	Criterion values									
		7.400	7.425	7.450	7.475	7.500	7.525	7.550	7.575	7.600	7.625
log total	252 A+U	65	62	58	58	55	57	55	58	62	61
viable	130 A	43	39	35	35	32	29	25	25	24	22
count	122 U	22	23	23	23	23	28	30	33	38	39

A = sensorially acceptable samples
U = sensorially unacceptable samples

Table 3 shows that total viable count with 55 (22%) discrepant classifications gives nearly the same result as during the preliminary research (25%). In comparison with Hunter a-value total viable count has the disadvantages of higher percentage discrepant classifications and of the long time (2 days) necessary for analysis. An advantage is that total viable count is connected directly with aspects of health. LANGERAK (1978) found that during storage the increase of total viable count to $10^8 \cdot g^{-1}$ resulted in decay of prepacked endive. Inspection of the original data reveals, that among the 130 sensorially acceptable samples 5 samples had a total viable count above $10^8 \cdot g^{-1}$. Only 2 of these 5 samples had a positive Hunter a-value.

The microbiological variables log *Coli* group and log *Pseudomonas* group, with 29% and 35% discrepant classifications, are less suitable to describe the sensory quality. These variables are more suitable to reject

samples for reasons of health. LANGERAK (1978) found that for *Enterobacteriaceae* the increase to $10^7 \cdot g^{-1}$ resulted in decay. The original data reveal that among the 130 (94) sensorially acceptable samples 33 (10) samples had a total count of the *Coli* group above $10^7 \cdot g^{-1}$l and 7 (0) samples $5 \cdot 10^7$ g^{-1}; the figures between brackets refer to *Pseudomonas* group. Only one of these 7 samples had a positive Hunter a-value and only 2 other samples had a total viable count above $10^8 \cdot g^{-1}$.

If norms are formed for total viable count and for these bacteria, it is possible to reject the samples beyond the norms, irrespective of sensory quality or Hunter a-value.

3. Conclusions

When the Hunter a-value at 30 mm light-path of the standardized adherent water is negative, the sensory quality of shredded endive may be considered as acceptable. Total viable count may be a second describing variable for the sensory quality, directly connected with aspects of health. The total bacterial count of the *Coli* group or of the *Pseudomonas* group can be used to reject samples for reasons of health, irrespective of the sensory quality.

If this research is to be continued, also other parameters may be tested, including the browning-factor used by LOEF (1977), the proteolytic desintegration factor (free amino acids) as suggested by SCHWERDTFEGER (1978), and the low frequency filter factor in the electrical frequency spectrum analysis applied by TATTAR and SYLVIA (1977).

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MICROFLORA IN BUTTER AND CAKES WITH BUTTER FILLING

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Recently butter consumption has been on the increase over the world. It is desirable to have a microflora in butter and butter filled cakes not deviating from the normal. An investigation of the quality and quantity of the microflora of 200 butter and 250 small cake samples with butter filling was carried out on POLÓNYI's milk agar by plating. The viable cell count of the butter samples, as established by colony formation unit (CFU) varied between 10^4 and $10^8\ g^{-1}$. The cell count of about 18% of the samples exceeded the $10^8\ g^{-1}$ order of magnitude. (Colonies were counted 48 h later than the specified period, thus, the order of magnitude was higher by one unit than otherwise.) The normal microflora consisted of *Micrococci*, lactic acid bacteria *(Str. lactis)* and yeast cells; 109 samples contained *Enterobacteria*, 107 aerobic spore formers, 89 chromogenic bacteria *(Pseudomonas, Achromobacter)* and 79 mould fungi. *Lactobacilli* were found in 40 samples, *Sarcinae* in 25, *Corynebacteria* in 21 and *Staphylococci* in 8.

A cell count lower than in butter was found in the cakes with butter filling and varied between 10^3 and $10^8\ g^{-1}$. Only 14.4% exceeded the 10^8 value, however, contaminating flora occurred more frequently and in higher numbers than in butter. *Enterobacteria* were found in 170, pigment forming in 162, *Bacilli* in 160, mould fungi in 118, *Sarcinae* in 56, *Staphylococci* in 27 samples. Differences may be ascribed to technology. Heat treatment reduces the viable cell count while spore forming bacteria and moulds are introduced in the filling with additives. *Enterobacteria*, chromogenic microorganisms and *Staphylococci* are of human origin.

In addition to the character of the sample the microflora of foods depends also on the methods applied (nutrient media, temperature and time of incubation, etc.).

Butter consumption is on the increase over the world. This is a good thing, because it contains a fair amount of valuable fatty acids and vitamins (CSERENYEY, 1974, 1975; FRAZIER, 1970; KUHN, 1964; RIGÓ, 1975; TANNER, 1956) and at the same time is useful for kitchen-technical and industrial application (VENESZ, 1973; DEN HARTOG, 1974; RIGÓ, 1975). A disadvantage of butter lies in the fact that it contains a great number of a large variety of microorganisms, which proliferate in butter itself less rapidly, but in products prepared with butter at a high rate, causing frequently the spoilage of the product (TANNER, 1956; NIKODÉMUSZ, 1958; FRAZIER, 1970; CSERENYEY, 1974). It is true, that butter and butter fillings rarely transmit food contaminations or toxicosis (NÉKÁM, 1890; AUJESZKY, 1902; MOSSEL et al., 1963; NIKODÉMUSZ, 1965; PAPAVASSILIOU, 1965) although epidemics spread by butter have been described (KUHN, 1964; SEIDEL & MUSCHTER, 1970). Damage

caused by infections, intoxications or spoilage can be prevented by the knowledge of the microflora present, therefore it is important to know the kind and density of microbial contamination.

The total viable germ count of butter and butter fillings is not limited by the Hungarian standards (POLÓNYI, 1952; EGÉSZSÉGÜGYI MINISZTÉRIUM, 1978). Limits are not decreed in the Dutch, French, English and Rumanian standards either (BUTTIAUX & MOSSEL, 1957; IENISTEA, 1959; NIKODÉMUSZ, 1961; COX, 1970; HOBBS, 1976). According to the American standard butter is of good quality (exportable) under a microbial count of 100 000 g^{-1}.

However, butter with higher viable cell count is not considered unfit for consumption unless there are pathogenic microorganisms present or it is sensorially below standard (FRAZIER, 1970). Spanish standards permit a cell count of 2 000 000 g^{-1} in butter (RODRIGUEZ, 1963) while in Australia and New Zealand a colony count of 15 000—50 000 g^{-1} is specified. However, the latter specifications do not seem to be realizable (COX, 1970) the less so since limit values have not been established either by FAO or by Comecon (EGÉSZSÉGÜGYI MINISZTÉRIUM, 1978).

As regards knowledge and opinions related to the composition of the microflora of butter they vary substantially. According to IENISTEA (1959) 86% of the flora consists of lactic acid bacteria, 13% of yeasts and 1% of moulds. The total viable cell count (number of colony formers) varies between 10^6 and 10^8 per g. DEMETER (1952) considers it necessary to determine the total cell count, the number of caseolytic bacteria coliform, mould and yeast cells and all of these have to be limited.

In Hungary an opinion prevails that the number of useful flora need not be limited (lactic acid bacteria), however the extraneous flora has to be limited, because its presence is detrimental (POLÓNYI, 1952; NIKODÉMUSZ, 1958; KUHN, 1964). Generally the different components were determined on different nutrient media (DEMETER, 1952; TANNER, 1956; NIKODÉMUSZ & SZÁNTHA, 1967; FRAZIER, 1970). Our aim was to find out the composition of the microflora on a single nutrient medium, or in other words the meaning of the concept "total viable cell count". These investigations do not affect, at least up to the present, the efforts exerted in Hungary to standardize methods of food microbiology. This is an individual search for a solution which according to VAS (1977) cannot be and is not excluded by the application of unified methods.

1. Materials and methods

During 1966 and 1967 a total of 200 butter samples and 250 butter filling samples taken of various products stored in different ways were tested for "bacterial purity" as specified (POLÓNYI, 1952). An aliquot of every test tube

of a basic dilution series (between 10^1 and 10^5) prepared from 1 g of a sample was inoculated on an appropriate nutrient medium in order to establish the total viable cell count, *Escherichia coli*, *Staphylococci*, mould and yeast fungi count in the sample (NIKODÉMUSZ, 1958). Here we give an account of the total viable cell count determinations, only. Of the 10^1–10^5 dilutions of the samples aliquots of 0.5 cm^3 were plated with milk agar according to POLÓNYI (1952). (Composition of POLÓNYI's (1952) nutrient medium: 80% of agar and 20% of skimmed milk, mixed at 50 °C.) Koch's method was used for plating. The plates were incubated for 24 h at 37 °C, then for further 24 h at room temperature. The colonies were counted. Determination was started after another 24 h while the number of colonies increased generally by 1 order of magnitude. The colonies were then determined on the basis of their morphological and biological characteristics (TANNER, 1956; NIKODÉMUSZ, 1958 and 1976; FRAZIER, 1970). The form, staining, motility of the microbes, was established (native preparation, Gram test). The characteristics of cultivation, carbohydrate and protein decomposition (sugar series, gelatin, blood agar, etc.) were tested. In the case of bacteria determination was carried to the genus, sometimes even further, however, this except for *Staphylococci*, is not shown. As regards the fungi, it was shown whether mould or yeast cells were present but further differentiation was not carried out.

Table 1 shows the microorganisms found on the milk-agar plates.

Table 1

Microorganisms found on the milk-agar plates

Spherical bacteria cocci	Gram positive	*Micrococci* clustered, catalase positive *Staphylococci* haemolysin, coagulase positive *Sarcinae* forming eights *Streptococci* forming chains, catalase negative	
	Gram negative	*Neisseria* in pairs	
Rod shaped bacteria	Gram positive	sporeformers *Bacilli* (aerobic sporeformers) *Clostridia* (anaerobs)	
		without spores lactic acid bacteria (aerobic or anaerobic rods) *Corynebacteria* (not of human origin)	
	Gram negative	with or without cilia *Enterobacteria* (saprophytes and pathogens) acetic acid bacteria	
		with or without polar cilia *Achromobacter* chromogens	
Yeasts		*Other microorganisms* circular or elliptoid, larger than bacteria	*Saccharomyces, Torula Candida, Rhodotorula*
Moulds		multicellular forms, recognized by their colonies	*Mucor, Aspergillus Penicillium*

The table shows the microorganisms growing on Polónyi's milk-agar, during 72 h. Detailed explanations are not needed, however, some notes are given as follows:

— Of the cocci only those were accepted as *Staphylococcus* which were found to react positively to the pathogenicity tests (coagulase, haemolysis, pigment formation, mannitase). The rest were considered as *Micrococci*.

— *Streptococci* are meant to be *Streptococcus lactis* or indifferent *Streptococci*, never *Streptococcus pyogenes*.

— The name *Corynebacterium* was not applied to strains originating from men, even less to *C. diphteriae*. They were Gram-positive, spore-free bacilliforms, with or without granula.

— The expression "*Enterobacteria*", relates to *Escherichia, Klebsiella* and coliforms.

— *Pseudomonas* and *Achromobacter* species were considered chromogenic bacteria, although a number of their strains does not produce pigment.

2. Results

The distribution of the colony forming units of the 200 butter samples is listed in Table 2.

As it can be seen in the Table, the lower limit of the total viable cell count of the butter samples was 10^4 g^{-1}. The colony count of 110 samples fell between 10^6 and 10^8, while that of 36 samples exceeded 10^8. The major part of the microflora was formed by lactic acid *Streptococci*, which were present in 90% of the samples. *Lactobacilli* were present in only 40% of the samples, because of the unfavourable pH of the medium. The pH of Polónyi's milk-agar is 7.0—7.2, while *Lactobacilli* favour a pH around 5. *Micrococci* were found in many of the samples, they as ubiquitous germs formed the incidental flora of the samples (IENISTEA, 1959; NIKODÉMUSZ, 1976). *Micrococci* were found in more of the samples than *Streptococci*, however, their average number was lower. Generally their presence is not harmful (MOSSEL et al., 1963). *Sarcinae* were found in 25 samples, *Staphylococci* in 8. The former are insignificant. *Staphylococci* in the number as found in these samples (below 10^5 g^{-1} order of magnitude), are not dangerous for the consumers and the prevalence of lactic acid forming microorganisms inhibits their increase.

Aerobic spore forming bacilli were found in 107 samples (in 15 samples their number was above 100 000 g^{-1}). Mainly *Bacillus subtilis* were identified and this corresponds to results obtained elsewhere (NIKODÉMUSZ, 1980). In some of the samples *B. pumilus* and *B. megaterium* were also found, while *B. cereus* occurred hardly at all.

Table 2

Microflora of butter samples (results of 200 samples)
Frequency of the occurrence of the individual components, cell count related to 1 g sample, expressed as exponent

Limit value per 1 g (exponent)	Frequency of occurrence of individual components grouped according to exponents											
	Total cell count (c.f.u.)	Streptococcus lactis	Lacto-bacteria	Micrococci	Sarcinae	Staphylococci	Aerobic spore-formers	Entero-bacteria	Chromogens	Coryne-bacteria	Yeasts	Moulds
$<10^3$	—	—	8	—	6	4	41	14	17	4	30	51
10^3-10^4	—	1	20	8	8	2	32	30	20	10	32	12
10^4-10^5	14	9	8	30	7	2	19	26	17	3	27	8
10^5-10^6	30	17	3	42	2	—	10	25	14	1	17	6
10^6-10^7	56	56	1	66	2	—	5	14	12	—	10	2
10^7-10^8	64	70	—	39	—	—	—	—	1	—	5	—
$>10^8$	36	27	—	1	—	—	—	—	—	—	3	—
Number and proportion of the microflora components related to the samples												
Number of positive samples	200	180	40	186	25	8	107	109	89	21	124	79
Percentage of samples	100	90	20	93	12.5	4	53.5	54.5	44.5	10.5	62	39.5

Enterobacteria were found in 109 and chromogenic bacteria in 89 samples and in 39 and 37, respectively, of the samples their number exceeded 100 000 g^{-1}. Of the *Enterobacteria Escherichia* and *Klebsiella* were mainly identified. Neither *Salmonella*, nor *Shigella* were found in the samples. Of the chromogens the pigment non-forming strains of *Pseudomonas* and *Achrobacter* were mainly identified. It was considered as the sign of faecal contamination, or of inefficient reduction of cell count, their presence in high number is undesirable.

Yeasts were found in 124 samples, moulds in 79 on the milk-agar nutrient. Most of the yeasts belong to the normal microflora therefore only their large number is objectionable. The presence of mould fungi is not agreeable, although their number was mostly below 10^5 g^{-1}, only in 8 samples above this number. Mould formation becomes visible to the naked eye above a cell count of 10^8 g^{-1}.

The composition of the microflora of the 250 butter filling samples is presented in Table 3.

In the course of preparing the butter fillings various ingredients are added and the mixture is exposed to different procedures. The basic butter filling is prepared according to the specifications of VENESZ (1973) as follows:

Table 3

Microflora in the butter filling of cake samples (results of 250 samples)
Frequency of occurrence of individual components, number of microorganisms in 1 g sample, expressed as exponent

Limit value per 1 g (exponent)	Frequency of occurrence of individual components grouped according to exponents											
	Total cell count (c.f.u.)	Streptococcus lactis	Lactobacteria	Micrococci	Sarcinae	Staphylococci	Aerobic spore-formers	Entero-bacteria	Chromogens	Coryne-bacteria	Yeasts	Moulds
$<10^3$	—	7	7	6	3	1	52	36	42	8	38	66
10^3-10^4	10	28	12	18	25	18	38	38	38	9	32	30
10^4-10^5	43	42	2	61	21	5	30	36	32	3	17	12
10^5-10^6	45	48	4	42	7	3	19	28	24	—	12	8
10^6-10^7	50	47	1	54	—	—	16	23	16	—	3	2
10^7-10^8	66	39	—	42	—	—	5	6	6	—	—	—
$>10^8$	36	23	—	6	—	—	—	3	4	—	—	—
Number and proportion of the microflora components related to the samples												
Number of positive samples	250	234	29	239	56	27	160	170	162	20	102	118
Percentage of samples	100	93.6	11.6	95.6	22.4	10.8	64	68	64.8	8	40.8	47.2

460 g of butter is blended with 460 g of granulated or powdered sugar and 160 cm^3 water while slightly heated. Uniform blending is achieved with powdered sugar more rapidly than with granulated sugar. The temperature applied should be between 40 and 60 °C. To this basic butter filling various flavourings are added, e.g. cocoa powder, vanilla, etc.

The various procedures to which the butter filling is exposed are reflected in the composition of the microflora in comparison with that of butter. Heat treatment reduces the viable cell count. The lowest population is 10^3 g^{-1}, while in the case of butter this was 10^4 g^{-1}. The total viable germ count of none of the samples was below this value, although the density of individual microorganisms occasionally was. Lactic *Streptococci* occurred rarely under a density value of 1000 g^{-1}. The reducing effect of heat treatment affected mostly *Streptococci*. The number of *Micrococci* in butter fillings is higher than in butter. The number of extraneous microorganisms is higher in butter fillings, probably introduced by the added ingredients. Sugar contains a great variety of microorganisms (NIKODÉMUSZ, 1980; NIKODÉMUSZ & SZÁNTHA, 1967) and the other flavouring substances are not free of microorganisms, either (POLÓNYI, 1952; TANNER, 1956).

The frequency of *Sarcinae* increases from 12.5% in butter to 22.4% in the cakes. The number of *Staphylococci* increases, too. This is due to the various procedures, or rather to the men carrying out these procedures, because these microorganisms come from the naso-pharyngeal tract of human beings. The frequency of spore formers increases moderately, from 53.5% in butter to 64% in butter filling samples, their number being substantially higher in the latter. This is evident, since the multiplication of *bacilli* is not inhibited by a high sugar content (NIKODÉMUSZ & SZÁNTHA, 1967).

Both the frequency and number of enterobacter and chromogen bacteria increases in the course of the preparation of fillings. Apathogen *Enterobacteria* are present in 54.5% of the butter samples and in 64% of the filling samples. In butter out of the 109 positive samples in 39 (in 18.5% of the total number of butter samples) the number of *Enterobacteria* exceeds the order of hundred thousand. Of the 170 filling samples containing *Enterobacteria* in 60 samples (24% of all the filling samples) is higher than the above value and in 9 samples (3.6%) their number is above 10^7 g^{-1}, which is higher than that of the human intestinal content.

As mentioned above, the frequency and number of chromogenic bacteria is also higher in butter fillings than in butter. The increase is more extensive than in the case of *Enterobacteria*. The proportion of positive samples increased from 45% in butter, to 66% in butter fillings. In 27 samples of butter (13.5%) the number of colonies exceeded hundred thousand and one sample the order of 1 million (0.5%). Of the butter filling samples 50 (20%) and 10 (4%) fell in the respective categories.

Both the *Enterobacteria* and the chromogenic bacteria are of human origin and indicate faecal contamination. They are introduced in the product by manipulation by hands and are in this case inevitable (NIKODÉMUSZ, 1958). The presence of pigment formers points to earlier contamination, because outside of the intestine they outlive *Enterobacteria*. Both bacteria are relatively resistant to the heat treatment given butter fillings.

The importance of sporeformers has already been discussed. The increased resistance of the genus Bacillus is worth mentioning. *Clostridia* were not isolated in this study.

The difference between the two products as regards the frequency and number of moulds was not as great as in the case of other microorganisms. Moulds were found in 39.5% of the butter samples and in 47% of the butter filling (79 and 118 samples, resp.). However, the 10^5 g^{-1} value was outnumbered in 8 and 10 cases, respectively, which means 4% for both kinds of sample.

As regards the frequency and number of yeasts the situation is the reverse of the above. They are found more frequently and in greater number in butter than in butter fillings. Of the butter samples 124 contained yeasts (62%) and in 35 samples their number exceeded 100 000 g^{-1}. Of the butter

filling samples 102 (40.8%) contained yeasts and out of these only 6% (15 samples) more than hundred thousand. This shows a similarity to the lactic acid *Streptococci*.

Twenty per cent of the butter samples contained *Lactobacilli*, while 11.6% of the butter filling samples. This shows that this genus favours acidic medium pH therefore the number of strains or cells growing on Polónyi's milk-agar is very low.

The reduction in the number of lactic acid *Streptococci*, *Lactobacilli* and yeasts in filling in comparison to butter is achieved by the heat treatment applied during blending (40–60 °C). The secondary contamination caused by additives, appliance and manual handling represses the propagation of the residual surviving microbial cells.

3. Conclusions

The microflora, as detected by the above methods, consists of *Streptococci*, *Bacilli*, *Micrococci*, yeasts, in addition it contains aerobic sporeformers, *Enterobacteria* and chromogenic bacteria, more seldom *Sarcinae*, *Corynnebacteria* and *Staphylococci* as extraneous microorganisms. *Sarcinae* and *Corynnebacteria* were not discussed in detail because both occur rarely and only at a low cell count and their significance is as yet unknown. It should be noted that *Corynebacteria* do not originate in men.

The number of colony forming units (viable cell count) proved to be by Polónyi's method (1952) above 10^4 g^{-1} in all the samples and in 18% of the samples above 10^8 g^{-1}. This in itself is not unwholesome, on the contrary the presence of the normal microflora in milk and milk products is desirable. Ács (1939), for instance, considers inoxious if the butter and its products do not contain indigenous microflora. Fodor (1889) and several contemporaries (Székely, 1890; Grosz, 1901) maintained that it is not useful to sterilize milk because thereby the way is opened for foreign microorganisms. Although the composition of the microflora in food has not been known to them they realized that a change in the conditions may be followed by unwanted consequences. The belief in the significance of useful bacteria increased with time (Siegert, 1933, and 1937; Polónyi, 1935). The opinion established itself that that the normal microflora in milk or in its products inhibits the proliferation of pathogens (Aujeszky, 1902; Hubay, 1933, and 1936) and this has to be kept in mind during processing (Halkó, 1934). Thus, it is evident that the total cell count in itself is not a decisive factor in judging the product in question.

The situation is different as regards the microflora of butter filled cakes. In consequence of the technology of production various changes occur. The heat treatment causes a slight decrease in the microbial count, however, a large part, moreover the less useful part survives it. On the average the num-

ber of colony forming units is lower than in butter. The selective effect of heat treatment is disadvantageous because the reduction in the number of lactic acid *Streptococci, Lactobacilli* and yeasts is greater than in that of *Micrococci*.

Blending itself may cause contamination, in addition the microflora of the additive increases the cell count. *Enterobacteria* and chromogenic bacteria are introduced by manual handling (NIKODÉMUSZ, 1958, and 1965). *Staphylococci* enter partly from human hand and partly from the rhino-pharingeal cavity into the product (PAPAVASSILIOU, 1965). Sugar, spices and flavourings are contaminated by aerobic sporeformers, moulds, *Sarcinae* (FRAZIER, 1970; DEN HARTOG, 1974; CSERENYEY, 1975; NIKODÉMUSZ & SZÁNTHA, 1967; NIKODÉMUSZ, 1980). The introduced microflora further reduces the indigenous microflora.

Staphylococci reside in milk products and probably the antagonist microflora inhibits their propagation and toxin production (MOSSEL et al., 1963; NIKODÉMUSZ, 1965; PAPAVASSILIOU, 1965).

Our findings are supported by those of NEVECSERAL (1977). If milk is inoculated with a butter culture and left ripening, its viable cell count increases by 2–3 orders of magnitude. If extraneous microflora is not present and the butter remains in its original state, the quality of the product is acceptable.

To establish the total viable cell count (CFU) in milk and milk products milk agar was found most suitable. Rumanian data (IENISTEA, 1959) show that the highest cell counts for milk products are obtained on milk agar. Lower results are obtained on casein of hydrolysed casein nutrient media and the lowest results on bouillon agar (POLÓNYI, 1952; BUTTIAUX & MOSSEL, 1957; CSERENYEY, 1975; NIKODÉMUSZ, 1976).

Lactobacilli were found to develop poorly on milk agar of pH 7.2. This findings are supported by PULAY and KRÁSZ (1980) who found *Lactobacilli* to favour media of pH 5.

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APPLICATIONS OF OVERPRESSURED THIN-LAYER CHROMATOGRAPHY

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Overpressured thin-layer chromatography (OPTLC) using a pressurized ultramicro (PUM) chamber is a new planar liquid chromatographic technique, wherein the sorbent layer is completely covered by an elastic plate put under external pressure and the mobile phase is forced to flow among the particles of the sorbent layer by means of a pump system.

The advantages of OPTLC have been applied for the efficient separation of essential oils, sugars, amino acids, poppy alkaloids, dimedone adducts of formaldehyde and other aliphatic aldehydes. These instances show that OPTLC is suitable for the separation of complex substance mixtures using fine-particle sorbent layers and optional development distances.

OPTLC is especially attractive when a large number of samples are to be investigated because the time required for analysis is small compared to HPLC separation.

For the quantitative evaluation various chromatogram spectrophotometers (CAMAG TLC/HPTLC Scanner and a microcomputer-controlled, zig-zag scanner, SHIMADZU Model CS-920) have been used.

It seems that OPTLC will be a leading technique in the field of planar liquid chromatography.

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Table

The applicability of overpressure thin-layer chromatography in some determinations

Determinations	Adsorbent	Solvent system	Detection	Pressure (kPa)	Rate of development (μl per min)	Development (min)	R_f values	Kind of food product
Preservatives (salycilic acid, benzoic acid, sorbic acid, p-hydroxybenzoic acid-methyl ester; side by side)	Kieselgel 60 F_{254}	n-butanol : ethanol : 2% ammonia : 12% ammonium carbonate (40 : 11 : 9 : 9)	UV_{254} UV_{366}	981	150	20	0.48; 0.39; 0.37; 0.71	pickled green tomatoes, natural lemon juice
Antioxidant (BHT)	Kieselgel 60 F_{254}	chloroform	solution of phosphorus molybdic acid in ethyl alcohol	981	150	17	0.94	lard
Organic acids (malic acid, tartaric acid, succinic acid, lactic acid; side by side)	Kieselgel 60 F_{254}	chloroform : methanol : formic acid (45 : 9 : 3)	bromophenol blue	981	200	18	0.20; 0.07; 0.51; 0.48	wines (e.g. Cabernet of Csongrád, Rizling of Pusztamérges)
Saccharides (glucose, fructose, saccharose, lactose; side by side)	Kieselgel 60 F_{254} (0.1 *M* H_3BO_3)	acetonitrole : water (9 : 1)	diphenylamine-aniline (in phosphoric acid)	981	150	15	0.17; 0.13; 0.08; 0.05	ground paprika (Sz-20); pericarp, seed, wines, carbonated grape juice
Natural pigments (beta-carotene, capsorubin, capsantine; side by side)	Kieselgel 60 F_{254}	petrolether : benzene : acetone : glacial acetic acid (40 : 10 : 2 : : 2.5)		981	150	24		ground paprika (Sz-20) pericarp, seed

Synthetic food colours, water soluble (Acid yellow, Tartrazine, Amarant, Neucoccine, side by side)	Kieselgel 60 F_{254}	i-propanol : 25% ammonia (4 : 1)		981	200	25	0.41; 0.25 0.21; 0.13 (over developed)	Sugar dragee, commercial rum
Vitamins (B_1, B_2, ascorbic acid, nicotinic acid)	Kieselgel 60 F_{254}	glacial acetic acid : aceton : methanol : benzene (5 : 5 : 20 : 70)	UV_{254}	981	150	15	0.15; 0.00; 0.28; 0.59	Vitamin C dragee, Polyvitaplex 8 dragee

APPLICATION OF OVERPRESSURE THIN-LAYER CHROMATOGRAPHY IN FOOD ANALYSIS

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The advantages in food analysis of overpressure thin-layer chromatography (OPTLC) are of interest. This technique permits simultaneous, rapid, reproducible analysis, efficient separation of the components, economic operation and reliable qualitative and quantitative analysis of a large number of samples.

In the chemical analysis of foods in plants and in official control institutes automated analyzers and chemical methods suitable for serial tests are of particular interest. In order to enrich the choice of methods known and used for food analysis (automated analyzers, enzymatic analyzers, etc.) the over-pressure thin-layer chromatography was adapted to food analysis.

Overpressure thin-layer chromatography is suggested to be used for the determinations as listed in the Table.

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MULTISTAGE THIN-LAYER CHROMATOGRAPHIC ANALYSIS OF VOLATILE CARBONYL COMPOUNDS IN FOODSTUFFS

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A method was developed for the extraction and separation by thin-layer chromatography, of volatile carbonyl compounds present in foods and playing part in many cases in their aroma. The carbonyl compounds were extracted by steam distillation and precipitated with 2,4-dinitrophenolhydrazone (DNPH). The precipitate was dissolved, after washing and drying, in carbonyl-free ethyl acetate. The solution containing all the carbonyl compounds was then separated into 5 groups of compounds by thin-layer chromatography (aliphatic monoketons; aliphatic monoaldehydes, unsaturated monocarbonyls; cyclic or aromatic carbonyls, dicarbonyls). Each fraction, collected from several preparative layers, was then rechromatographed in different systems to obtain

individual components. These were then identified on the basis of their R_f values and colour by comparing them to the R_f values and colour of known DNPH carbonyls.

The chromatograms obtained in the first step of thin-layer chromatography have served with useful informations. For instance the distribution beetwen groups of the carbonyl compounds was characteristic of the fruit from which it was extracted. A method was developed also for the quantitative determination of the separated carbonyl groups. Applying multistep thin-layer chromatography 39 carbonyl compounds were detected in tomato and tomato products of which 25 were identified.

The method was successfully used for the analysis of the composition of volatile carbonyls in a great number of foodstuffs.

DETECTION AND QUANTITATIVE DETERMINATION OF EMULSIFYING AGENTS IN PRESERVED BAKERY PRODUCTS BY THIN-LAYER CHROMATOGRAPHY

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A thin-layer-chromatographic method was developed to detect in bread emulsifying agents serving to inhibit staling (diacetyl tartaric acid-gliceride esters, lactylate type preparations). The method used for the detection and quantitative determination of the lactylate type emulsifying agents (fatty acid-lactic acid ester, fatty acid-dilactic acid ester) is direct thin-layer chromatography.

Dichloromethan or methanol was used to extract the emulsifying agent from bread depending on its consisting mainly of fatty acid-lactic acid ester or of fatty acid-dilactic acid ester. The extract was then separated in the appropriate solvent system. Quantitative evaluation of the samples was carried out visually. The method was worked out for the emulsifying agents Admul 1914, Lisat C (Ca-stearoyl-2-lactylate), Lisat N (Na-stearoyl-2-lactylate). Eleven to 33% of the emulsifying agents could be regained by this method. The loss occurring in the course of extraction of the agent from bread as percentage of the initial agent content was found to be a constant value. In the knowledge of this the original emulsifying agent content of the sample may be calculated on the basis of the regression curve obtained from the data of the calibration series. The inherent error of the method is ± 5–10%.

The method developed for the determination of the diacetyl tartaric acid-gliceride ester type emulsifying agents, is based on the detection and quantitative determination of the tartaric acid liberated by saponification

and acidification. After separation in the given thin-layer chromatography system of the emulsifying agent, extracted from the sample with dichloromethan : methanol (1 : 1), the tartaric acid was visually estimated. Preparation Datamuls was used in the experiments. The efficiency of extraction was found to be 25%. In the knowledge of the efficiency of extraction and the tartaric acid content of the preparation by means of the equation of the calibration curve obtained as described above, the emulsifying agent may be determined with an error of ±7%.

APPLICATION OF VIDEODENSITOMETER IN THE THIN-LAYER CHROMATOGRAPHY OF OLIGOSACCHARIDES

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Legumes with their high protein content and high biological value form an important source of nutrients. Beside the valuable nutrients they contain, however, certain compounds of inconvenient effect and therefore their consumption is limited. Compounds of this character are e.g. the α-galactosyl type oligosaccharides (raffinose, stachiose, verbascose).

In the last 10 years the use in Hungary for human consumption of soybeans has become again a subject of interest. Since soybeans are not unanimously accepted it was found desirable to develop a rapid, simple method to determine oligosaccharides in order to be able to estimate the effect of the technological processes.

The principle of the method consists in extracting with 80% ethanol the uniformly granulated and defatted sample. The extract is then filtered, washed and evaporated to a predetermined volume and separated on a cellulose thin-layer. The developed spots are evaluated with the videodensitometer. Accuracy of the method is ±5–10%.

Measurement was carried out as follows: 5 g of the prepared sample was suspended in 5 cm^3 aqueous ethanol and refluxed on a waterbath for 1 h. The digested sample was centrifuged and the supernatant filtered. The sediment was mixed with distilled water and filtered again, then washed three times. The united filtrates were then evaporated under vacuum at a temperature below 50 °C. Separation was carried out on a Polygram Cell 300 ready-made foil in the mixture of n-propanol–ethyl acetate–water (6 : 1 : 3). For detection the phosphoric acid solution of alfa-naphtol was used under heating.

Determinations were carried out in the following samples: commercial soy flour, soybeans and granulate from Iregszemcse, soybeans from Kisújszállás.

From investigations carried out so-far follows that in the defatted dry matter raffinose is below 0.5%, and stachiose about 2—4%. Simultaneously with the α-galactosyl-type oligosaccharides saccharose can also be determined. The authors found about 4—8% saccharose. It was established that the amount of inflating oligosaccharides is significantly lower in the soy granulate from Iregszemcse than in the original beans.

NEW INSTRUMENT FOR CHROMATOGRAPHY DEVELOPED BY LABOR MIM

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Within the wide field of chromatography Labor Instrument Works (MIM) is engaged in the development and manufacture of instruments for liquid chromatography. Beside the units of the well known Liquochrom Model 2010 system, out of the new developments Chrompres 10, the first apparatus developed for overpressure thin-layer chromatography (OPTLC) deserves mentioning. In this apparatus the thin-layer plate is completely covered by a membrane under high pressure and the solvent is forced to flow through the adsorbent layer.

Separation is not only enormously accelerated and made reproducible by development under the controlled flow and optimal flow rate of the solvent but the number of the theoretical plates obtainable on the thin-layer is also greatly increased. The shorter development time causes the spots to remain more dense and the diffusion does not reduce the value of separation invoked by the size and distribution of the particles in the layer.

High pressure liquid chromatography conditions are well imitated under external pressure (maximum 1.4 MPa) and forced flow.

Beside the general advantages of the apparatus its versatility in biochemistry and food analysis may be proven in many practical applications.

The automated amino acid analysers, available at present, inspite of their high price are slow, their output is 1 sample per h. Therefore, our efforts with Chrompres 10 were concentrated on the separation of amino acids and amino acid derivatives.

In a large number of analyses of screening character, using silicagel of HPTLC quality, 17 amino acids were separated in the viscous n-butanol–glacial acetic acid–water solvent (4 : 1 : 1). Overlapping was observed only in the case of Ser-Hy-Pro, Ala-Thr, Try-Ile, Leu-Phe. In traditional development, carried out in a chamber, only a few groups of amino acids can be separated and it takes 6–7 h instead of 1 h. In the separation of DNP amino acids, soluble

in ether, a 6-component solvent (CCl_4 $CHCl_3$ 2-butanol n-propyl alcohol methyl alcohol acetic acid, 30 : 30 : 20 : 30 : 15 : 2) was found of optimal effect. The mixture of 12 components was separated and suitable to evaluation. Sulfur containing amino acids were separated on a cellulose layer of fine particles in a 6-component solvent. The sample of 13 components separated in relatively uniform distribution.

For evaluation Shimadzu CS-920 High-speed TLC/HPTLC Scanner was used at 540 nm absorption. Development was carried out with ninhydrin reagent.

The apparatus is suitable for the separation of many other complexes and their derivatives (alkaloids of poppy-seed, corticosteroides, etc.) which were earlier analysed by traditional methods of thin-layer chromatography but due to the great number of components or the extremely similar structure the efficiency of separation was not satisfactory.

ANALYSIS OF THE CARBOHYDRATES IN BEER BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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An important process of beer brewing is the decomposition of the carbohydrates in malt while forming an alcohol and extract content as well as an aroma characteristic of the given type of beer. The success of this process is decided partly in the brewing house, partly in the fermenting vat. The process is definitely hindered by the application of industrial enzyme products and various types of substitute syrups.

By the use of HPLC in the analysis of these processes it is hoped not only the detection of the carbohydrates but also the possibility of operative intervention.

The measurements were carried out on a HPLC apparatus, in its main parts manufactured by Waters with an RI-401 type detector (Waters). Essentially microBondapak/Carbohydrate column was used.

The aim of the experiments was the operative control of mashing and fermentation of the wort. The expedient sampling points of mashing were established and the composition of Hungarian brewages among which some were corrected with substitute syrup, was investigated.

The process of brewing was highly affected in the presence of a larger amount of iso-syrup substitute. Because of glucose repression the final degree

of fermentation was about 10% lower. Of the carbohydrates suitable to fermentation about 30% of the maltose content and 35% of the maltotriose content remained unfermented.

It was found that the HPLC technique permitted to follow up the utilization rate of everyone of the carbohydrates. Thus, it is possible to conduct fermentation on the basis of the parameters arrived at by chromatography.

It is an interesting feature of the carbohydrate analysis of beers that if the technology is constant, the carbohydrate distribution may be considered the finger print of the products of a given brewery. However, to prove this conclusion further measurements are required.

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF CARBOHYDRATES IN FOOD ANALYSIS

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The analysis of carbohydrates has always been a crucial point of quality control in the food industries. Because of their high time requirement, however, the traditional methods of chromatography were not adequate. The introduction of high-performance liquid chromatography (HPLC) permits of rapid analysis of not only the final product but on the line, too.

In the course of these experiments the products of the starch and distilling industries, within their frames the products of dextrose, maltose and iso-syrup manufacture, the formation of their by-products, the products of the confectionery industry, alcohol-free beverages and alcoholic drinks, were studied.

The main task the authors set themselves was the adaption of HPLC and the conditions of its use to requirements and possibilities in Hungary.

The HPLC technique proved to be very efficient in the investigation of the products and by-products of the starch industry (e.g. formation of resynthesis products), following up the conversion in iso-syrup manufacture and in the formation of psicose. The method was found also suitable for on-line operative control.

Similarly satisfactory experiences were gained in the brewing industry, too, in the following up both of mashing and of fermentation, as well as in the analysis of the final product. The method promises to be an operative tool in vine cultivation and viticulture and in the production of preparations for diabetics.

The experiments were carried out on an equipment consisting mainly of units manufactured by Waters, an RI-401 detector and microBondapak/Carbohydrate (Waters) and Spherisorb-NH_2 columns. In serial tests the variation coefficient was found to be below 3%.

DETERMINATION OF FAT-SOLUBLE VITAMINS AND ANTIOXIDANTS BY HPLC TECHNIQUE

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A method was developed and adopted to determine the vitamin A and E content and the antioxidants BHT, EMQ and XAX-M in feeds and premixes.

In premixes the vitamins A and E were determined with a coefficient of variation of 3%, while in feeds this was 10—15%.

The coefficient of variation in the determination of antioxidants was 1% for premixes, while 10% for feeds and for XAX-M in adipose tissue as residual contamination.

The changes of vitamins A and E in a feed and a premix with EMQ antioxidant and without, were followed up. No significant change was found to occur in vitamin A during a 12-week period. In the sample without antioxidant the vitamin E content decreased significantly. The reduction of the vitamin content was significant in both feed samples, however, in the sample containing EMQ the rate of reduction was 20% slower.

FOOD ANALYTICAL APPLICATION OF CAPILLARY GAS CHROMATOGRAPHY

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The general interest in the microcomponents of foods requires the introduction of analytical methods hitherto not applied in this field. By means of the newly introduced methods it becomes possible to determine components studied and known earlier by a more simple, quicker, more sensitive and reliable method and to detect substances as yet not studied because of analytical difficulties.

The first sample is taken from the field of fatty acid analysis. Whether polar (Carbowac 20M) or apolar (SE 30) partition phase is applied only 6–8 min are needed for the quantitative and qualitative determination of the essential fatty acids, in contrast to the 15–20 min required by analysis on a column pack. Experiences are particularly favourable in relation to the lipid or fatty acid composition of blood plasma. Saturated, mono-unsaturated and poly-unsaturated fatty acids gave a base-line separation. That the sensitivity of this analytical method can be improved was observed in relation to the analysis of steroids of animal and plant origin (glass capillary of 15 m length, 0.25 mm i.d. and wetted with SP-2100 agent). The sensitivity threshold for the trimethyl-silyl ether derivatives of steroids studied was found to be 0.1 mg kg^{-1}.

The technique was successfully applied in the detection of fungal mico-toxin, mainly fusariotoxin. A method was worked out for the qualitative and quantitative determination of 6–8 fusariotoxins, applying a glass capillary wetted with SE 52 agent. The sensitivity of the method was 50–100 μg kg^{-1}. The same method was adapted for use in food analysis. After the proper modification of sample preparation the method could be used in liver, kidney and meat analysis with a sensitivity of 5 μg kg^{-1}.

GAS CHROMATOGRAPHY OF SHORT CARBON CHAIN ALIPHATIC ALCOHOLS IN FOODS

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A gas-chromatographic technique was developed for the quantitative determination in foods of primary aliphatic alcohols (C_1—C_5).

Using an appropriate temperature program the best result was achieved on the stationary phase Chromosorb 103. Quantitative evaluation was carried out on the basis of the calibration curve obtained by means of an inner standard.

The method was found suitable for direct analysis of aqueous alcoholic distillates. From not distilled solutions or suspensions the alcohol components were obtained by a specific micro steam distilling apparatus.

Error of the method: for methanol $\pm 6.9\%$; for ethanol $\pm 2.6\%$; for n-propanol $\pm 5.3\%$; for sec-butanol $\pm 5.7\%$; for n-butanol $\pm 8.2\%$; for i-pentanol $\pm 3.6\%$.

The method can be used in a wide range in the food industry, it is rapid, well reproducible, suitable for serial tests. Since Chromosorb 103 stationary phase is not sensitive to water, it is suitable for the analysis of aqueous solutions and suspensions containing as little as 0.05% (v/v) alcohol (methanol, ethanol, etc.).

Under the given gas-chromatographic conditions the individual alcohol components can be separated well even if there are differences of a high order in their quantity.

The technique was successfully applied in the qualitative and quantitative analysis of methanol and higher alcohols in fruit brandies and for the separation of methanol and ethanol in diluted industrial condensed waters, in fruit aromas and fruit juices.

The highly sensitive method was successfully applied also in the study of methionine incorporation in proteins.

USE OF GAS-CHROMATOGRAPHIC ANALYTICAL METHODS IN THE DISTILLING INDUSTRY

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Methods of gas chromatography were developed for the reliable, objective, up-to-date determination of components of the raw materials, additives, semi-products and final products of the distilling industry which, hitherto, could not be separated by the traditional analytical methods (alcohol and ester homologues) while their quantity, their relative proportions are characteristic of the product. These methods are suitable for the control of technological processes, of equipments (e.g. dewatering apparatus), of theoretical calculations (e.g. composition of terner azeotrop mixtures), etc.

The most important methods developed at the Research Institute of the Distilling Industry are as follows:

- Determination of the composition of fusel oil (adaptation of the method developed at the Veszprém University of the Chemical Industry);
- Quantitative determination of methyl alcohol, ethyl acetate, propyl acetate and higher alcohols number in wine distillates, brandy, fruit brandies, refined and dewatered alcohol and various distillates, etc.;
- Detection and quantitative determination of acroleine;
- Simultaneous determination of benzene, petrol and ethyl alcohol;
- Purity test of benzene and petrol;
- Quantitative determination of aceton;
- Rapid evaluation of unknown samples based on their chromatograms;
- Detection of aroma substances in alcohol-free beverages;
- Determination of the i-amyl acetate content of liqueurs and essences.

Alcohols, esters, aceton, benzene and acrolein are separated on a Chromosorb G column wetted with 10% didecyl phtalate. Aromatic substances of alcohol-free beverages were detected on a Chromosorb W column treated with 15% ethylene glycol adipate. To measure i-amyl acetate Gas Chrom Q adsorbent was treated with 10% PEG 1540 stationary phase. All measurements were carried out under isotherm conditions with flame ionisation detector.

Identification was carried out by addition technique, quantitation by the inner standard method using normalization factors.

Samples of 1–3 μl were applied directly except those of the alcohol-free beverages. These were subjected to extraction with ether and then evaporated prior to analysis. A separation took 10–15 min.

The lower threshold of detection is 0.5–1 mg per 100 g. The average error of the methods was found to be $\pm$5–8%.

Reproducibility of each newly developed method is controlled by chromatography of model solutions.

The methods described are rapid, simple, reliable and suitable for serial tests.

EVALUATION OF SYNTHETIC OR PARTLY SYNTHETIC AROMA CONCENTRATES BY GAS CHROMATOGRAPHY

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A gas-chromatographic technique was developed to evaluate the quality of five fruit aroma concentrates (apricots, lemon, orange, strawberry and hazelnut).

After the addition of the inner standard, the samples were directly chromatographed on Dexsil 300 stationary phase under an appropriate temperature program.

The percentage proportion of the mixtures containing imported and Hungarian aroma concentrates was established by the integration of the areas under the peaks characteristic of the given aromas. In samples, where such characteristic peaks were not available the integrated values of peaks showing significant differences in the basic aromas, were utilized in the measurements.

Study of the identity of aroma samples was carried out, beyond the simple comparison of chromatograms, by determining the proportions of the more significant or characteristic peak areas.

The techniques developed are particularly suitable to serial tests because their sample size and reagent requirement is minimal and they produce reliable results within a very short period of time (1 analysis takes 10–30 min).

Sensory evaluation methods used hitherto for the qualification of the aroma preparations purchased can be replaced by the new technique and this permits the rapid and continuous control of quality. Thus it becomes possible to lodge a claim in time and to produce preparations of uniform quality.

OBJECTIVE EVALUATION OF THE BOUQUET OF WINES BY GAS CHROMATOGRAPHY

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Investigating the possibilities of the objective evaluation of sensory characteristics — subsequent to summing up the generally valid basic principles — a technique was developed for the evaluation of the bouquet of wines.

A gas-chromatographic head space analysis method was applied to study the vapour space. Based on the data obtained a so called bouquet qualifying number was defined which, taking into account the peak areas may be calculated as follows:

$$I = \frac{a\frac{\overline{T}_1}{\overline{T}_{1v}} + b\frac{\overline{T}_2}{\overline{T}_{2v}} + c\frac{\overline{T}_3}{\overline{T}_{3v}}}{d\frac{\overline{T}_4}{\overline{T}_{4v}} + e\frac{\overline{T}_5}{\overline{T}_{5v}} + f\frac{\overline{T}_6}{\overline{T}_{6v}}} \qquad (1)$$

where $\overline{T}_i (i = 1-6)$ is the average size (mm^2) of the peak area of the i-th aroma component of the wine investigated and $\overline{T}_{iv} (i = 1-6)$ of that of the wine used for comparison,

$a - f$ are constants.

$\overline{T}_{iv}$ and $a-f$ values differ according to the type of wine (e.g. for muscat wines: $a = b = d = e = 3$; $c = f = 1$; $\overline{T}_{1v} = 181.6$; $\overline{T}_{2v} = 97.7$; $\overline{T}_{3v} = 137.9$; $\overline{T}_{4v} = 33.1$; $\overline{T}_{5v} = 122.2$; $\overline{T}_{6v} = 279.4$).

To evaluate the results obtained a mathematical statistical method was developed which permits of establishing the reliability of the method or predetermining it in accordance with the given task.

Thus, the bouquet of two wines (j and j') may be considered different with P probability belonging to value t_{fp}, if the proportion of their measuring numbers $\frac{I_{j'}}{I_j}$ exceeds the numerical value of the right side of correlation (2).

That is:

$$\frac{I_{j'}}{I_j} > \frac{1 + t_{fp} \cdot s_{I_r} \sqrt{\frac{(n+m) - t_{fp}^2 \cdot s_{I_r}^2}{m \cdot n}}}{1 - \frac{t_{fp}^2 \cdot s_{I_r}^2}{m}} \qquad (2)$$

where n and m is the number, respectively, of wine samples j' and j analysed, while t_{fp} is the parameter of Student's test,

$$\left|\frac{1}{f} = \frac{1}{(n+m)^2}\left(\frac{m^2}{n-1} + \frac{n^2}{m-1}\right)\right|$$

s_{I_r} is the relative scatter of the measuring number qualifying the bouquet.

In the course of testing applicability the possibilities of refining (1) correlation or detecting an operation resulting in change of quality, e.g. "watering" were investigated.

The method provides data for the comparison of samples not available at the same time and therefore not subjectable to sensory test. The method is thus most useful in commercial evaluation, in the establishment of the possible reasons of quality deterioration.

Literature

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RECOGNITION OF THE PATTERN OF CHROMATOGRAMS IN FOOD ANALYSIS

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A group of methods under the name pattern recognition method of modern computing and applied mathematics are suitable for the indirect classification of foods or their components by quality, origin, kind, type, aroma, etc. on the basis of some characteristics established by measurement, e.g. a chromatogram.

Utilization of the pattern recognition method in food analysis has been started only a few years ago and is on spread at the moment. It has been used

to establish the quality among others of snap bean products, soy sauce, milk, whisky and wine on the basis of their chromatograms.

The pattern recognition computer program system, developed by the research team of the author was successfully used in the solution of various tasks of analytical chemistry, among others in the classification of wines.

The cases described in the literature as well as the experience of the author shows that the pattern recognition method is important, fundamental and generally applicable method of food qualification and its introduction on a wide scale is an urgent task of the food industry.

EXPERIENCES ON THE COMPARISON OF CAFFEINE DETERMINATION METHODS

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The caffeine content of various foodstuffs has been the subject of study for a long time. The methods available are of different accuracy and are suitable for use with different materials.

The aim of this study was to control, of the methods used in food analysis, the utility of chromatography and spectrophotometry. In the case of foods of different character the method has to be selected in accordance with the composition of the product. Pepsi Cola as an alcohol-free beverage, "Karavan" roasted coffee and "Golden Delicatess" powdered cocoa were used as model samples. These materials of different character represent foods containing caffeine.

In the analysis of coffee and Cola the methods used gave equally acceptable results. However, in the case of powdered cocoa of high fat content the error inherent in the methods proved to be high (CV: 5.4% and 3.8%). Thin-layer chromatography cannot be used without fat extraction and clarification. Petroleum ether was used for extraction and lead acetate for clarifying. This process was then compared with the extraction method with ammonia and chloroform as specified in the standard.

Results obtained were:

	Caffeine content (mg kg^{-1})		
	Spectrophotometry	GC	TLC
Pepsi Cola	60.15	59.54	57.8
Karavan coffee	17 987.00	18 034.00	18 100.00
Golden Delicatess cocoa	4 576.00	4 574.00	4 176.00

DETERMINATION OF CHLORINATED HYDROCARBON RESIDUES IN GROUND PAPRIKA

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The residual chlorinated hydrocarbons have regularly been controlled in industrially ground paprika since 1976. Since methods for determining the pesticide residues in ground paprika have not been available in the literature the authors have developed a method and published in 1978 (Aczél & Selmeci, 1978). The principle of the method is: chlorinated hydrocarbons extracted from the sample with acetonitrile, shaken in petroleum ether and purified on a florisyl column, are determined by gas chromatography.

Average residue in ground paprika samples during 1976—1980 was the following:

Quality of ground paprika	Average of the years 1976 to 1980			
	Lindan (mg kg^{-1})		DDT (total) (mg kg^{-1})	
	min.	max.	min.	max.
Delicacy	0.0006	0.0017	0.0025	0.0029
Nobel-sweet	0.0001	0.0017	0.0023	0.0103
Half-sweet	0.0003	0.0016	0.0011	0.1102
Rose	0.0003	0.0021	0.0089	0.590
Hot	0.0004	0.0030	0.0129	0.0665

It was found that the residual chlorinated carbohydrates in ground paprika showed a diminishing tendency. The values obtained were all below the value as specified in regulations 1/1970 EÜM-MÉM and 4/1978 EÜM-MÉM (Ministry of Health—Ministry of Agriculture and Food).

Literature

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DETERMINATION OF AMINO ACIDS AND THEIR ENANTIOMERS BY CAPILLARY GAS CHROMATOGRAPHY

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The quality and quantity of amino acids can be determined by several analytical methods. The method most widely used is ion-exchange liquid chromatography (amino acid analyzers), however, other methods, such as gas chromatography, thin-layer chromatography, high pressure liquid chromatography, are also used.

On comparing the methods mentioned above the disadvantage of ion-exchange analysis lies in the long time requirement, whereas with gas chromatography in the necessity of producing a derivative. Thin-layer chromatography is difficult to evaluate from both aspect of quality and quantity because the amino acids interfere and the quantitative evaluation is not sufficiently reliable.

In using gas chromatography the amino acid has to be transformed into an N-fluoracyl butylester derivative. The derivative is formed with 4 *N* hydrochloric acid and butanol of sequencial purity at 100 °C and with 10% dichloromethane trifluoro acetic acid antihydride at 150 °C for 15 min. The analysis does not take more time than 10–15 min on a glass capillary column wetted with SE 52 partition phase. Except for cysteine, methionine and tryptophan scatter of parallel determinations was below 3%. For the three amino acids mentioned it was above 3%.

Two methods are generally used to separate amino acid enantiomers. With the first method the optical isomers are separated by the application of a highly polar, optically active partition phase, e.g. L-lauryl-L-valine-terc. butylamid or N-TFA-L-valyl-L-valine-cyclohexyl ester, with the second the diastereosimers are separated by medium polar phases. In the second case separation is accomplished by forming a pair of diastereoisomers to enhance the physicochemical difference of the optical isomers on mildly or medium polar phase.

Due to problems related to reagents only the second method could be tested. The diastereoisomer pair was separated with N-TFA-L-prolyl chloride reagent. The diastereoisomers thus obtained separated well in the case of 11 amino acid pairs. However, the separation of dibasic acids was not successful by this method, because these form tripeptides which cannot be eluted from the column without breaking down.

QUANTITATIVE SIMULTANEOUS GAS-CHROMATOGRAPHIC DETERMINATION OF SORBIC AND PROPIONIC ACIDS SIDE BY SIDE, IN PRESERVED BAKERY PRODUCTS

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A method was developed for the quantitative, simultaneous determination of Ca-propionate and K-sorbate in preserved bakery products. The preservatives were extracted from the samples with the aid of a special steam distillation apparatus. The distillate was made alkaline and then evaporated to dryness. The residue was dissolved in ether containing 3% formic acid and this solution was then gas chromatographed. Acids were separated on Chromosorb 101 stationary phase by applying an appropriate temperature program.

For the quantitative determination a calibration curve based on butyric acid inner standard was used. It was established that loss of preservative, within the limits as used in practice, did not occur during the determination.

On examining bread samples prepared with preservatives, Ca-propionate, was found to suffer substantial, while K-sorbate a slight loss, which was explained by breaking down of the preservative, or evaporating during baking.

The Ca-propionate content could be determined by the new method with an error of $\pm 4.60\%$, while K-sorbate with an error of $\pm 2.86\%$.

The method is rapid and reproducible therefore very suitable for serial tests. It can be used for the continuous control of preserved bakery products.

APPLICATION OF CONTIFLO ANALYTICAL SYSTEM FOR FOOD ANALYSIS

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A general review is given of the theoretical background of a continuous flow automatic analytical system with special reference to its practical application.

The CONTIFLO analytical system, manufactured in Hungary is described. Possibilities and already realized techniques of its application in food analysis are discussed.

The most important application appears to be the determination of macro-constituents.

Application and some data of the automated CONTIFLO analytical system for food analysis

Studied foodstuffs and components	Analytical modul	Principle of the method	Wavelength (nm)	Concentration range	Relative standard deviation (±%)
Milk					
— protein	OL-731	Binding of dye (Orange G)	480	0.5 – 4.5%	±1.0
— lactose	OL-733	Neocuproine-Cu^{+} complex	460	0.6 – 6.0%	±0.2
— fat	OL-732	Turbidimetry	660	0.2 – 5.0%	±1.5
— alkaline phosphatase	individual	4-amino-antipyrine + phenol complex	510	1 – 100 mg kg^{-1} phenol	±0.3
— pyruvate	individual	NADH → NAD transformation (fluorometry)	Stimulation: 366 Measurement: 463	1 – 20 mg dm^{-3}	±3.2
— lactate	individual	NAD → NADH transformation (UV photometry)	Stimulation: 366 Measurement: 463	10 g – 200 mg dm^{-3}	±3.5
Protein					
— protein	individual	Folin-Lowry molybdenate reaction	670	0.5 – 1000 mg kg^{-1}	±0.5
— NH_3	OL-786	Na-salicyl-indolate (Bertholet's method)	670	1 – 25% protein	±0.25
Carbohydrates					
— total sugar	OL-734	Maceration in sulfuric acid medium, reaction with resorcinol	400	1 – 7% sugar	±0.5
— total reducing sugar	individual	TING method (K-ferricyanide reduction, phosphorus molybdenate reaction)	660	0.1 – 1%	±0.5
— starch	individual	Iodine binding method (in buffered medium)	540	1 – 200 mg kg^{-1}	±0.5
Inorganic components					
— $P_2O_4^{3-}$	OL-784	Ammonium-metavanadate-phosphorus poly acid	420	1 – 100 mg kg^{-1}	±0.2
— Cl^{-}	OL-782	Hg-rhodanid → Fe rhodanid	460	0.1 – 8% NaCl	±0.1
— NO_2^{-}	OL-783	Gries-Ilosvay or Salzmann modified method	520	1 – 200 mg kg^{-1}	±1.0
— NO_3^{-}	individual	reduction with hydrazin sulfate Gries-Ilosvay reaction	520	0.5 – 100 mg kg^{-1}	±2.5

The continuous system may be used for protein determination based on the biuret reaction, or colour binding or on ammonium determination in the digested sample.

For the determination of carbohydrates many methods can be carried out on the continuous system, e.g. K-ferricyanide, neocuproine, TING-(K-ferricyanide-ammonium phosphorus molybdate) method, based on the determination of derivatives obtained by sulfuric acid maceration and iodide binding capacity of polysaccharides.

The fat content can be determined in an acetic acid containing medium (fat in milk) by turbidimetry or a modified version of this method.

The continuous flow system can also be applied for the determination of some vitamins, certain metals and some organic compounds or elements (e.g. P_2O_5, NO_2^-, Cl^-).

AUTOMATION ON CONTIFLO OF WINE ANALYSIS

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The growth of requirements on quality and quantity increase the necessity of the automation of quality control. Automated equipments are widely used in food analysis and their utilization expands.

The automated analyzer CONTIFLO was adapted in the field of wine analysis to the determination of reducing sugars, iron, tartaric acid content and colour intensity.

Reducing sugars are determined by the measurement of the direct reducing capacity with potassium-ferricyanide. The equation of the two-variable linear regression line is the following:

$$y = 0.979x - 0.416$$

$$r = 0.9980$$

The total iron and tartaric acid contents are important in view of the stability of wines, both are measured on the same modul. To determine the iron content the Bathophenanthroline method, as applied in clinical practice, was used. Equation of the two-variable linear regression line:

$$y = 1.068x - 0.160$$

$$r = 0.9953$$

Tartaric acid was determined by Rebelein's ammonium-metavanadium technique.

The measurement of colour intensity in red wines is important in view of uniform quality. The optical density is established in the wine diluted with buffer or distilled water and this is in direct proportion to colour intensity.

Forty to sixty measurements can be made in an hour.

APPLICATION OF THE AUTOMATED CONTIFLO ANALYZER IN THE DISTILLING INDUSTRY

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The grading of molasses, corn, wheat, potatoes, fruit and fruit juices, the control of alcohol, vinegar, yeast fermentation and the determination of the composition of the products of the industry require the carrying out of a large number of analyses. In order to increase the efficiency of the analyses the automated CONTIFLO analyser (produced by Labor MIM, Hungary) has been in use.

The most frequent analyses to be carried out are the determinations of nitrogen, phosphorus and sugar contents. A method based on a very sensitive colour reaction was adapted and an analytical modul for the required region of measurement was developed.

To determine the total ammonium nitrogen the Na-salycilate method is applied. In the 0 to 80 ppm region the correlation between concentration and colour intensity is linear. In the analysis of acetic acid inorganic nitrogen can be determined directly, without any preparation, while the organic nitrogen subsequent to sulfuric acid maceration accelerated by the addition of H_2O_2. Kjeldahl method was used for comparison and the results showed good agreement.

To determine the phosphorus content in culture media, cola drinks and in starch—phosphate esters the ammonium metavanadate technique was applied and a modul which gave linear correlation in the 1 to 60 ppm region. The samples were macerated with sulfuric acid.

The method adapted to determine total reducing sugar and the proportion of glucose and fructose was based on the neocuproine reaction (2.9 dimethyl-1.0 phenentroline). The fructose content of iso-sugar was determined by thermostating at 55 °C for 5 min accounting for the higher reaction capacity of fructose. The results were compared to the fructose contents as determined by HPLC, polarimetry, Lane-Eynon and Willstätter methods. The

results obtained on the CONTIFLO apparatus complied to practical requirements. Compared with results obtained by HPLC the deviation was found to be +0.1–+0.6%.

DETERMINATION OF MALIC ACID IN FRUIT AND VEGETABLES ON THE CONTIFLO SYSTEM

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Malic acid is an important component of vegetables, fruit, must and wine. The malic acid content undergoes a characteristic change during the ripening of fruit and vegetables, in wines as a function of the variety of grape and of vintage and technological processes affect it, too.

In these experiments malic acid was determined by the application of malic acid-dehydrogenase enzyme (L-malate: NAD, oxydoreductase, A.C.1.1.1.37) in the following reaction:

$$\text{L-malate} + \text{NAD}^+ \xrightleftharpoons{\text{malate dehydrogenase}} \text{oxalacetic acid} + \text{NADH} + \text{H}^+$$

The reaction occurs in a hydrazin-glycin medium of pH 9.5. The concentration of NADH, formed in a quantity equivalent to that of malic acid can be measured by spectrophotometry at 340 nm in the UV range.

This method of spectrophotometry was adapted to the CONTIFLO system. Vegetables were macerated with NaCl and centrifuged to obtain a clear extract. Wines can be studied in the original state. Malic acid is determined as follows:

The sample taken from the sample holder is diluted with sodium citrate buffer (5% Na-citrate, 3% Brij 35) and passed through a dialyzer (of 5 cm ∅). The receiving buffer in the dialyzer is 1 mol dm^{-3} hydrazin-glycine buffer of pH 9.5. To this is added NAD (10 g dm^{-3}) dissolved in 0.2 mol dm TRIS-hydrochloric acid buffer of pH 9.6 and the L-malate-dehydrogenase (2.10^5 unit dm^{-3}). The mixture is passed through a reactor in an UV detector measuring at 340 nm. A compensograph is connected to the detector. The calibration curve plotted with the modul is linear in the 0–8 g dm^{-3} range ($r = 0.998$, $s = 3.5\%$).

Correlation of the results obtained by spectrophotometry and CONTIFLO system, respectively, was established in red and white wine samples and was

Table

Malic acid content of some vegetables and apple

Stored produce	Storage period (day)	Malic acid content (mg per 100 g)		
		at start	stored at 4 °C	stored at 22 °C
Paprika (cultivar Kalocsa)	11	976	983	521
Eggplant (c. Bolgarski 12)	11	935	831	638
Patisson (c. Byelig)	11	1730	1413	720
Cauliflower (c. Igloó)	40	202	113	680
Onion (c. Alsógöd)	80	300	250	210
Apple (c. Golden Delicious)	60	900	700	300

found to be:

in white wine $C = 0.997$, $Sp = 0.0509$, $r = 0.9991$
in red wine $C = 0.994$, $Sp = 0.0508$, $r = 0.9990$

C is the malic acid content as measured on CONTIFLO, Sp is the same measured with spectrophotometer.

The malic acid content of some vegetables (bell paprika, patisson, eggplant, cauliflower, onion) and apple (Golden Delicious) was measured under different storage conditions. Results are shown in Table 1. The Q_{10} value, calculated for the breakdown of malic acid at different temperatures, varied between 1.3 and 2.1.

DETERMINATION WITH AN AUTOMATED ANALYSER OF FOOD COLOURS SOLUBLE IN ORGANIC SOLVENTS

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Many methods are known for the determination of food-colours. These methods are circumstantial, the vapour of organic solvents imply health hazards, the work is periodical, a significant amount of solvent is used.

A variant of the CONTIFLO automated analyser, manufactured by Labor Instrument Works, was developed which can be used for the chemical analysis of substances dissolved in organic solvents.

The CONTIFLO-ORG variant can be operated with hydrocarbons, alcohols, ketons and chlorinated organic solvents alike. For instance, when investigating the total colouring matter or the red-yellow pigment content and proportion in ground paprika, the following results were obtained:

Table

Determination of paprika colours by different methods

Quality of ground paprika	Standard methods[a]		Automated methods	
	$\bar{x}$	$\pm s$	$\bar{x}$	$\pm s$
Delicacy	3.99	0.018	3.99	0.026
Nobel-sweet	3.10	0.031	3.09	0.038
Half-sweet	2.21	0.037	2.19	0.024
Rose	1.19	0.019	1.21	0.025

[a] MSZ 9681/5—76 (Hungarian Standard on determination of colour in ground paprika) Number of parallels (n) = 5

DETERMINATION OF FOOD PROTEINS BY SPECTROPHOTOMETRY IN THE UV RANGE

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The light absorption of protein solutions as measured in the UV range between 300 and 250 nm depends on the character of the components. Eleven amino acids were tested and it was found that under the given conditions of spectrophotometry (1 cm^3 amino acid solution + 8 cm^3 of 97% acetic acid 1 cm^3 dichloromethan) only two of the amino acids gave a characteristic absorption maximum: the maximum of DL-tryptophan (0.02 mg dissolved in 100 cm^3 0.1 *N* NaOH), was 0.590 at 282 nm, while that of L-tyrosine in similar solution 0.200 at 278 nm. The optical density of DL-phenylalanine in the above concentration was 0.014, $\lambda_{max} = 258$, that of DL-histidine 0.020 at 254 nm. The optical density values of the known mixture of tryptophan and tyrosine followed the ratio of the two components in the mixture.

Thus, the optical density values of protein solutions in the above UV range is primarily determined by the tryptophan and tyrosine present.

The values obtained by spectrophotometry in protein solutions gained from fresh and freeze-dried pork and beef and some products of the meat industry were compared with values as determined by Kjeldahl's method. A known quantity of the samples was suspended in 0.1 *N* NaOH at 55 °C. An aliquot of this suspension was added to 97% acetic acid and dichloromethan.

The solution thus obtained was filtered to remove insoluble residues and measured at 273 nm. The optical density values were compared with the values obtained according to Kjeldahl. The points of measurement approximated the same line the regression equation of which was

$$y = 0.937x - 0.004$$

where y is the optical density and x is the protein content (mg). The correlation coefficient $r = 0.999$, showing a close correlation between the data pairs. In the knowledge of the weighed-in sample and that of the dilutions the protein content of the sample studied can be computed from the optical density value.

Thus, with the aid of a calibration curve based on measurements and calculations or with the help of the regression equation the protein content of a wide range of meat products may be determined. It is sufficient to carry out the calibration measurements only once under the same conditions of spectrophotometry. Routin tests take not more than 10 to 15 min. Thus the method can be used for on-line determinations and the values used for adjusting protein content.

POSSIBILITIES OF DETERMINING BIOLOGICALLY ACTIVE SUBSTANCES BY FLUOROMETRY

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The development of interdisciplinary sciences throws more and more light on the mechanism of effect of the vitamins in the living organism, therefore the quantitative determination of vitamins gains importance beside food science in human and veterinary therapy as a means of diagnostics. Vitamins, however, occur in natural substances in low concentration, frequently bound to other substances, accompanied by materials which may reduce the safety and accuracy of their determination. At the Department of Biochemistry and Food Technology many up-to-date methods of vitamin determination were developed in recent years. At present the development of a method of fluorometry is elaborated, because fluorescent spectroscopy satisfies the requirement of sensitivity and specificity.

Method has been developed for the determination by fluorometry of vitamins A, E, D, B_1, B_2, B_6 and C and the oxidized and reduced form of glutathion of biological importance as well as of selenium. Some of the vita-

mins show a natural fluorescence, while others easily transform into compounds of fluorescence. After proper extraction and purification the vitamins may be determined on an appropriate fluorometer (e.g. Perkin-Elmer 3000) to a concentration of 0.01 $\mu g\ cm^{-3}$. Inherent error of the method is about $\pm 5\%$.

APPLICATION OF SPECTROPHOTOMETER IN THE ANALYSIS OF ALCOHOL-FREE BEVERAGES

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Spectrophotometer is used for the derermination of the following substances at the Institute:

Spectral range	Components studied
Ultraviolet	quinine, caffeine, benzoic acid, sorbic acid
Visible	pigments after preparation of the sample, without preparation of the sample, pectin, saccharin, phosphoric acid, HMF, caffeine
Infrared	breakdown process of quinine

The change of some components important in view of quality as a function of storage conditions and storage period was investigated. It was established that the quinine content of the carbonated beverage "tonic" broke down during the 5 months storage in daylight at ambient temperature. The decomposition process was studied under infrared and UV spectroscopy. The results permit of assuming that the decomposition of quinine in acid medium leads to quinic acid and meroquinene. The decomposition process is catalyzed by daylight. When stored in the dark either at room temperature or refrigerated, the breakdown of quinine is negligible.

Changes in the colour during storage of alcohol-free cola, raspberry and orange beverages was subjected to investigation, too. In the case of cola and the raspberry beverage the absorption curve of the samples was established and the optical density at the absorption maximum was measured. The absorption maximum of the cola drink was found at 350 nm, while that of the raspberry beverage at 510 nm. The values measured at the maximum change with the conditions of storage and with the storage period. In cola samples stored at daylight and ambient temperature the optical density measured at 350 nm diminished only slightly during 1 year storage, but that of the raspberry drink, stored under similar conditions diminished completely in 5 months time and the beverage faded.

The pigments in the orange beverage, the carotenoids were first extracted from the samples and the colour intensity of the extracts was measured.

The study of storage has shown the carotenoid content of the samples, stored in daylight at ambient temperature, to change according to an exponential equation ($\log y = 2.632 - 0.006x$) and the carotenoid content stored in the dark either at room temperature or refrigerated according to a linear equation ($Y_{\text{in dark}} = 0.493 - 0.001x$; $Y_{\text{refrigerated}} = 0.509 - 0.007x$).

USING INFRARED SPECTROSCOPY FOR THE DETERMINATION OF THE COMPOSITION OF MILK

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Various methods have been used in practice for measuring the most important milk components such as fat, protein, lactose and water content. It is well-known that—within the infrared lightspectrum—the wavelengths of fat, protein and lactose are characteristically different in giving the adsorption maximum, and by this means the water content of the sample is also measurable if using suitable corrections. Utilizing this principle, a Danish company, the Foss Electric' developed its apparatus of Milko-scan type 104, suitable for the rapid determination of fat, protein, lactose and water contents of milk.

In testing this type of apparatus, operated as the first in our country, it was found that its capacity of reproduction was better in relation to fat, protein and lactose than that of the standard control methods. Standard deviation of the method was below $s = \pm 0.01$ and the range of scatter $\pm 0.03\%$, respectively. The same values, in regard to water, were $s = \pm 0.07$ and $\pm 0.2\%$. The apparatus can be calibrated easily to the control methods, characterized by the following correlation coefficients:

Fat, Gerber's method	$r = 0.995$
Protein, Kjeldahl's method	$r = 0.975$
Lactose, gravimetric method	$r = 0.941$
Water, gravimetric method	$r = 0.992$

No significant differences can be found between the results of the control methods and those obtained on Milko-scan at the level of $\alpha = 0.1\%$. The stability after calibration is excellent. The apparatus requires recalibration only every 2–3 months.

The effects of various factors (e.g. preservatives, increase of titratable acidity, sample temperature, watering, skimming) that may influence results, were investigated.

DETERMINATION OF THE RATIO OF DENATURED PROTEINS IN WHEY

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Research at this Institute has proved, that the extent of heat treatment of fresh milk can be characterized by the ratio of denatured whey proteins. Further, a close correlation was detected between the critical attributes of sour milk products and the extent of the denaturation of whey proteins.

The introduction of optimum heat treatment was hitherto retarded by the lack of a simple, rapid and reliable method for the determination of the ratio of denatured whey proteins during milk processing.

In order to satisfy this requirement, after the comparative analysis of related literature, the volumetric technique of Anderson and Bell (1959) was selected and with expedient modifications adapted. The principle of this method is the coagulation with 10% acetic acid solution the denatured whey proteins and casein. The precipitate is then filtered and the non-denatured whey proteins precipitated with 10% phosphoric—tungstic acid and centrifuged under predetermined conditions. The amount of precipitate is in proportion with the extent of denaturation of whey protein.

The turbidimetric (spectrometric) method as applied in research was compared with the volumetric method and in the 10 to 100% denaturation range the correlation was found linear. Equation of the fitted regression line is:

$$DSF_v = 0.696 \cdot DSF_t - 5.457$$

where DSF_v is the value measured volumetrically,

DSF_t is the value measured by turbidimetry.

The correlation coefficient showing the closeness of the correlation is $r = 0.95$.

Literature

Anderson, H. A. & Bell, R. W. (1959): A volumetric method for estimating the undenatured serum protein in milk. *J. Dairy Sci., 42,* 441–447.

MEASUREMENT OF THE HOMOGENEITY OF THE MIXTURES OF POWDERED INGREDIENTS IN FOOD PRODUCTION

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It is an important practical task to have ingredients prior to adding them to the product, thoroughly blended. In order to measure the homogeneity of blended powders, with view to the requirements of the dairy industry

two media of different character, granulated sugar and skimmed milk powder were used as models. To these basic materials cocoa in the proportion of 1 : 1, 10 : 1, 100 : 1, and food colours in the proportion of 1000 : 1 and 10 000 : 1 were added as marker substances.

A solution of 10% (v/v) was prepared from the granulated sugar-based blend with distilled water. This was then filtered and diluted to give an optical density value between 0.1 and 0.5 and measured with spectrophotometer. The maximum value for cocoa was found at 320 nm, while for food colour at 420 nm.

From the blend based on skimmed powdered milk 1 g was mixed in 9 cm^3 40% trichloro acetic acid. The solution was allowed to stand for 30 min, then filtered. Subsequently the above procedure was applied. For the cocoa marker maximum absorption was found at 400 nm and for the red food colour at 350 nm.

The homogeneity of the powder blend was characterized with the variation coefficient ($v = s\bar{x}^{-1}$). The value $v = 0.1$ is considered in the literature as acceptable.

The optimal operation parameters of the experimental powder blending apparatus, developed by the authors (POMIXER), was determined by the above method. These were: filling level, blending period, etc. Homogeneity as a function of blending period changes according to a minimum curve, where homogeneity is optimal at the optimal blending period ($v = 0.052$) and with increasing time transforms into a saturation curve ($v = 0.8 - 0.9$).

On evaluating the results for the basic substances studied the optimum filling of the POMIXER apparatus was found to be 40 kg and the optimum blending period 40 s. The output, taking into account filling and emptying, was found to be 1000 kg h^{-1}.

The method was successfully used in the control of dry blend of the powdered ingredients of cocoa-milk and of ice powders and meat hash powders.

EXPERIENCES GAINED WITH INFRAPID 31 IN THE CEREAL INDUSTRY

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The Hungarian manufactured INFRAPID 31 apparatus based on near infrared reflection (NIR) spectroscopy was developed for the determination of the protein, fat and moisture content of cereals and oil seeds. In order to

Table

Determination of some components of cereals with INFRAPID 31

Sample and the component studied	Number of samples (n)	Range of values			
		Lower limit (%)	Upper limit (%)	r	CV (%)
Durum wheat	23				
moisture		10.41	11.10	0.50	1.15
raw protein		16.80	18.89	0.56	2.93
Wheat	74				
moisture		10.79	12.73	0.66	2.84
raw protein		12.28	16.87	0.91	2.84
Barley	22				
moisture		8.78	13.37	0.97	3.71
raw protein		9.84	13.76	0.96	2.12
Corn	29				
moisture		11.17	12.90	0.51	3.24
raw protein		8.81	11.19	0.79	3.83
Wheat flour	23				
moisture		10.73	14.63	0.94	3.05
raw protein		11.00	16.29	0.94	3.47
Oat	29				
moisture		4.11	8.13	0.93	2.15
raw protein		10.24	13.56	0.83	4.88
Sorghum	20				
moisture		8.13	14.27	0.97	3.68
raw protein		9.77	14.81	0.74	8.38
Sorghum (separately calibrated for brumsorghum and fodder sorghum)	20				
moisture		8.13	14.27	0.99	2.14
raw protein		9.77	14.81	0.99	1.87
Rye	25				
moisture		8.66	10.28	0.48	3.10
raw protein		9.74	13.45	0.92	3.43
Wheat germ	17				
moisture		6.42	11.69	0.96	5.78
raw protein		25.10	34.55	0.95	2.35
raw fat		7.5	9.5	0.90	3.15
Sunflower meal	17				
moisture		8.83	10.06	0.87	1.78
raw protein		36.18	45.06	0.96	1.50
Peas	29				
moisture		10.20	12.42	0.60	3.54
raw protein		22.42	25.28	0.73	1.77
Peas (separately calibrated for green and yellow peas)	29				
moisture		10.20	12.42	0.80	2.65
raw protein		22.42	25.28	0.71	1.84

Sample and the component studied	Number of samples (n)	Range of values			
		Lower limit (%)	Upper limit (%)	r	CV (%)
Soy meal (MSKI mill)	20				
moisture		10.00	11.78	0.84	2.70
raw protein		51.23	55.33	0.96	0.59
Soy meal (Retsch mill)	20				
moisture		10.10	12.84	0.79	3.49
raw protein		51.92	56.00	0.92	0.91
Soybeans	30				
moisture		6.26	9.83	0.95	3.32
raw protein		35.10	39.55	0.95	0.91
raw fat		20.02	23.72	0.50	3.51

r = correlation coefficient
CV = coefficient of variance

obtain accurate results the apparatus has to be calibrated for each of the materials. The protein, fat and moisture content of the samples used for calibration has to be determined by other analytical methods, too, because their quantity cannot be measured directly. The instrument was calibrated for wheat, rye, barley, oat, corn, sorghum, wheat flour, peas, durum wheat, soybeans, soy meal, sunflower seed meal and wheat germ and it was then used for unknown samples. The samples were ground on the hammer mill developed at the Institute. The median of the meals was, as determined by screening, between 90 and 230 $\cdot 10^{-6}$ and that of rye at 45 $\cdot 10^{-}$ m. Calibration and the accuracy of measurements was controlled by statistical calculation. On the basis of a great number of measurements (250 samples of wheat, 126 of corn, 79 of barley, 76 of wheat flour, 41 of oat, 30 of soybeans and 29 of pea) a good correlation was found between the results of traditional laboratory methods and those obtained with INFRAPID 31 ($r = 0.80 - 0.98$). When unknown samples were analysed the correlation occasionally diminished and new measurements had to be carried out. This was found mainly with corn. While the calibration values obtained for wheat in 1979 could be used for the samples in 1980, the wheat grown in 1981 required a new calibration. In the opinion of the author this problem would be much simplified if the stability of the instrument could be controlled with an etalon. In the case of appropriate calibration the error of protein determination was found to be between 0.6 relative % (soy meal) and 4.9 relative % (oat.) Limit values of moisture determination were between 1.2 relative % (durum wheat) and 3.7 relative % (barley). The error of fat determination for wheat was 4.6, for corn 6.3, for wheat germ 3.2 and for soybeans 3.5 relative %, respectively.

COMPLEX ENZYMATIC AND INSTRUMENTAL METHODS FOR LIPID ANALYSIS

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There are different methods for the extraction of fats and fatty substances from foods, while universally accepted method for the determination of lipid and lipoids from foods does not exist.

To determine the so called raw fraction in foods the sample is usually comminuted followed by solvent extraction. In foods the fat lipids and lipoids are present in closely bound form. Lipids thus chemically bound or enclosed in other substances are difficult to extract with solvents.

Prior to solvent extraction of the fatty substances it is expedient to liberate them by some method of cleavage. This may be carried out by the denaturing of proteins or the maceration of carbohydrates. Maceration, however may cause changes in the lipids, too. A much milder technique and complex extraction is offered by the treatment of the tissues with hydrolase enzymes (cellulase, pectinase, protease, etc.).

The authors found that by breaking down the cell walls by treatment with cellulase and pectinase a lipid yield about 20–30% higher can be achieved than by mechanical breaking down. This method of breaking down is particularly important with materials of high fibre content, e.g. carrots, the carotene content of which could not be utilized at all without the maceration of the cell walls.

The result of extraction after treatment with cellulase—pectinase enzymes differs not only in its quantity but in its quality, too. By extracting lipids bound to the cell walls or enclosed in the cell structure a material differing in its composition from that obtained by traditional methods, is gained. Thus the complex enzymatic-instrumental analysis serves not only to amplify the knowledge of food industrial raw materials, but by differentiating the fatty substances provides information on the fine structure of tissues and on the place of lipid components in the structure of the raw materials. According to the results of measurement by chromatography and spectroscopy substantially more phospho- and glycolipids were found by this method in plant tissues and new components in carotenoids, sterols and fatty acids.

CONTROL OF THE QUALITY OF CHOCOLATE FOR DIABETICS BY ENZYMIC CARBOHYDRATE ANALYSIS

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In the recent decade the number of those suffering from diabetes increased abruptly. Apart from this, up-to-date requirements of nutrition draw attention to confectionery products of low saccharose content. Therefore it seems indicated to replace on the market the imported diabetic sweets by cheaper home-made products.

The impending manufacture of diabetic chocolate based on fructose necessitates an analytical technique of high accuracy and easy to carry out by which the sugar composition of the product can be controlled.

The traditional methods of sugar determination are not suitable for this purpose, because usually a mixture of various sugars is present in the products.

Of the selective sugar determination methods the authors used the enzymic glucose–fructose UV test of the firm Boehringer and Mannheim. For the determination of saccharose glucose test was applied subsequent to hydrolysis with invertase. The coefficient of variation of fructose determination was below 1.0%. Scatter of the glucose determination was 6.3% with a glucose content of 0.5 to 1.0%.

The carbohydrate composition of milk and eating chocolate samples originating from the GFR and available on the market and similar chocolates of experimental production in Hungary, was investigated. The composition of the experimental chocolate for diabetics corresponded to that of the imported chocolate (fructose 30–40%, glucose below 0.1% or saccharose also below 1.0%).

Samples with 0.1, 0.5 and 1.0%, respectively, of added glucose or saccharose content were also studied, in order to find out whether their presence could be established in the final product during quality control.

The methods developed proved suitable to detect extraneous glucose and saccharose below 0.1% and quantitatively determine amounts of 0.1 to 1.0%. The enzymic glucose–fructose UV test is suitable for quality control, its time requirement is low, thus it can also be used for on-line test and correction.

MASS SPECTROMETRIC DETERMINATION OF SOME VOLATILE NITROSAMINES IN FOODS

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The observation and quantification of the hazardous N-nitroso compounds and their suppression to a harmless level in foods as well as in human environment is a very important task. In food products the reliable detection of very low amounts of nitrosamines forms a hard and very complex analytical problem because of the presence of numerous other components.

For extraction and purification of the volatile nitrosamine content of Hungarian meat steam distillation, followed by extraction with CH_2Cl_2 and concentration were applied on the basis of literature. (Groenen et al., 1976; Sen et al., 1976; Gough et al., 1976.) For identification and quantification of the studied nitrosamines from the concentrated extracts GC-MS-mass fragmentography (GC-MS-MF) was chosen as one of the most reliable techniques for this purpose.

In the case of certain samples the appropriate separation of nitrosamines from the interfering components could be solved by applying a capillary column of much higher efficiency than that of the packed one.

The detection limit of the developed GC-MS-MF method was found to be between 10–50 pg pure nitrosamines with a reproducibility of $\pm 5\%$ using an internal GC-standard.

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APPLICATION OF CSERENKOV ANALYTICS IN FOOD ANALYSIS

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Radioanalytics is a specific branch of chemical analysis distinguishing itself by its specificity and sensitivity. Because of these attributes it is highly suitable for use in food analysis.

The application of radiological techniques in food analysis and official food control is further indicated by the increase in the number of isotope laboratories. Also, there exists a need for the many-sided utilization of the large instruments.

This is an account of the utilization of Cserenkov photometry in food analysis. In the utilization of the technique the linear correlation between the activity of the external standard and the concentration of the colouring matter was made use of.

In the measurement of protein colour formation according to Lowry was utilized. In the case of a protein content of 0.3–1.8 mg dm^{-3} the correlation coefficient of the linear relationship between the activity of the external standard and the logarithm of protein concentration, $r = 0.9942$. On comparison of the spectrophotometric and Cserenkov's photometer measurements a close linear correlation was found of $r = 0.9823$.

To determine the glucose content colour formation with o-toluidine was used. In a concentration of 0.3–1.5 mg dm^{-3} the correlation between the activity of the external standard and the logarithm of glucose concentration was found linear ($r = 0.9007$). The coefficient of the regression equation correlating Cserenkov's photometry with spectrophotometry, $r = 0.9843$.

In vitamin C determination the colour was formed with 2,4-dinitrophenyl hydrazine. In the concentration interval of 0.3 to 1.1 mg dm^{-3} the correlation of vitamin C content and the activity of the external standard was linear ($r = 0.9729$). Coefficient of the regression line correlating spectrophotometry and Cserenkov's photometry is $r = 0.9863$.

DETERMINATION OF MATURITY IN APRICOTS BY INSTRUMENTAL MEASUREMENT OF FLESH FIRMNESS AND COLOUR

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The ripeness of apricot is determined in the factory usage by the naked eye according to colour. In these experiments several objective methods were tested to replace subjective judgement. Results relate to three cultivars: Giant of Cegléd (Ceglédi óriás), Hungarian kajszi and Borsi's late rose (Borsi-féle kései rózsa) which were studied during two seasons (1980, 1981).

During the ripening period the fruit was harvested on two-three occasions. The fruit judged by naked eye to be of 60, 70, 80 and 90% ripeness, respectively, was measured on MOMCOLOR tristimulus instrument and evaluated in the CIELAB system. The firmness of texture was measured with

the manual penetrometer developed at MEFI (Institute for Development of Agricultural Machines, Hungary) and expressed in mPa units. In the fruit juice the refractive index and acid content were determined.

The correlation between parameters was studied by methods of mathematical statistics. This study has shown the firmness of fruit flesh and a^* characteristic of the basic colour to be the most suitable to determine degree of ripeness in the three cultivars studied. (For Giant of Cegléd $r = \pm 0.83$, for Hungarian kajszi $r = \pm 0.68$ and for Borsi's late rose $r = \pm 0.81$).

On the basis of the results for each cultivar values of a^* and firmness of flesh were fitted to ranges, within which the fruit can be harvested for a definite use. (E.g. the optimum picking ripeness of Magyar kajszi is characterized by the a^* range of $+1 - +8$, while the characteristic flesh firmness range mPa is 50–73).

EXPERIENCES GAINED WITH INSTRON-1140 UNIVERSAL TEXTURE MEASURING INSTRUMENT

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This is a summary of the experiences gained in the use of the universal texture measuring instrument INSTRON-1140 during several years.

In the measurement of tenderness of sweet corn Back's extrusion, Kramer's pressing and the OTTAWA measuring cell methods proved to be of equal value while not reaching the reliability of the viscosimetric method. By the latter method a correlation coefficient, of $r = 0.84$, was obtained under industrial conditions. In the measurement of tenderness of snap beans the maximal force as measured with the Warner—Bratzler chopping head ($r = 0.92$) and Kramer's press ($r = 0.84$) gave a satisfactory correlation with the AOR %. In the measurement of the texture of sour-cherry the best results were obtained by compression and puncturing.

In pastries the general texture profile analysis method was found suitable for the characterization of the mechanical properties of potato-containing pastries as affected by changes in technology and composition. In addition the relaxation and puncturing techniques proved successful, too. With several products the measurement of adhesion gave also useful results.

The mechanical characteristics of textured Urschel meat cutlets could be easily differentiated by the compression method.

A deformation rate of at least 20 cm min^{-1} was found necessary for the measurement of texture in foods.

ANALYSIS BY POLYACRYLAMIDE GEL ELECTROPHORESIS OF THE PROTEINS IN WHEAT FLOUR AND DOUGHS

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Correlation between the baking quality and protein composition of wheat flours of different baking quality: Yubileinaya 50, Rana 1 and Sava, and of their 1 : 1 mixture was studied.

This was the first time that the flours and their mixtures were graded by the standard methods of flour and bakery product analysis (total protein according to Kjeldahl, gluten content, extension of gluten, water binding capacity, valorigraphy, baking test). Further, the protein composition was established by acetic acid fractionation. Three protein fractions were separated: proteins soluble in 1 *M* NaCl solution (albumins and globulins), gluten proteins soluble and insoluble in 0.1 *M* acetic acid. The polypeptide chain of the protein fractions separated with solvent extraction was studied by the SDS-PAGE method. The quantity and composition of protein fractions separable by the same method from the flours, from the dough immediately upon mixing and after 180 min of proofing, was established.

The quantity of gluten proteins soluble in 0.1 *M* acetic acid solution was found to increase in all the samples during mixing and fermenting of the dough, however, to a different degree. The lowest increase (7.3%) was found in the good quality flour of Yubileinaya 50 and the highest (28%) for Sava flour of poor quality. In the mixtures containing Sava flour the amount of the soluble fraction increased substantially: in the mixture of Yubileinaya and Sava by 15.07%, in that of Rana 1 and Sava by 16.85%. The change in solubility of the proteins during mixing and proofing was accompanied by the following changes in the distribution of molecular mass and structure of the gluten complex:

The distribution of molecular mass of the protein extracts was studied by the SDS-PAGE method. Separation was carried out according to LAEMMLI (1970) in Tris-glycine buffer of pH = 8.3 at 10% gel concentration.

In the polypeptide chain of the protein extracted from flour and dough with 1 *M* NaCl significant change was not observed. The composition of polypeptide chain of gluten protein extracted with 0.1 *M* acetic acid changed during mixing and proofing.

The quantity of polypeptide chains of highest molecular mass (95 500, 91 000, 79 000 Dalton, resp.), which was 12–15% in the flour, diminished to 5–6%. After proofing about 80% of the polypeptide chains in the fractions soluble in acetic acid could be detected in the molecular mass range of 44 000–30 000 Dalton.

The quantity of gluten proteins not soluble in 0.1 *M* acetic acid diminished depending on cultivar. In good quality flours the reduction approximated 10% (Yubileinaya 50: 10.5%), while in flours of poorer quality 22%. Their polypeptide chains, however, did not show change detectable by the SDS-PAGE method.

It is shown by the results, that the stability of the gluten structure formed during dough formation does not depend only on the original protein composition of the flour. The changes occurring during mixing and proofing as a result of mechanical treatment and enzyme activity, are of great significance.

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GEL ELECTROPHORESIS OF GLIADIN PROTEINS IN WHEAT

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In the course of analytical work with the aim of plant improving polymorphism was frequently observed in the gliadin pattern of wheat cultivars, new varieties. This caused difficulties mainly in the detection of chromosomal allelic gliadin blocks. It seemed necessary to carry out gel electrophoresis from one or even a half grain.

A vertical layer polyacrylamide gel electrophoretic (PAGE) technique was developed for this purpose. The apparatus needed was built by the authors. The gliadins were extracted with chloroethanol (25% v/v). In this work the observations of BUSHUK and ZILLMAN (1978) and those of MAIER and WAGNER (1980) were made use of.

A gel containing 6% acrylamide was used. The electrode buffer was an 0.188 *M* acetic acid solution and the pH was set at 3.1 with glycin. Polymerization was carried out with ammonium peroxydisulfate solution (1%). The thickness of the gel layer was 3 mm. The power used for running was 400 V (or 260) with feeding unit Labor MIM OE 41–5. The gels were dyed with Coomassie Brillant Blau R-250.

The apparatus was used to investigate wheat varieties in general cultivation (NEHÉZ & ERDEI, 1982).

The chromosomal allelic gliadin blocks were detected according to the nomenclature of SOZINOV and POPERELYA (1980). Some difficulty was caused by the fact that the nomenclature was indicated for starch gel electrophoresis.

The significance of allelic blocks need not be overestimated in relation to baking properties.

The gel electrophoresis of gliadins was successfully applied in the improvement of plants.

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ANALYSIS OF VEGETABLE PROTEINS BY POLYACRYLAMIDE GEL ELECTROPHORESIS

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In the separation of plant proteins by gel electrophoresis the samples used had been previously purified or preliminarily separated. In this study the author wished to find out whether the juice of vegetables pressed directly may be used for electrophoresis providing thereby a simple rapid technique for the distinguishing of vegetables and the following up of processes in connection with changes of the proteins.

The samples were obtained from the homogenized plant tissues by pressing on a filtercloth-lined press. Electrophoresis was carried out in an acrylamide gel of 7.5 monomer concentration, using TRIS-glycine buffer of pH 8.3 at a power of 2 mA per tube, at +5 °C in 3.5–4 h time in the average. After marking the protein fractions on the gels with amido black densitometer was used for evaluation.

Thus, the preparative separation of protein fractions was not aimed at. Fractions migrating at approximately identical speed were marked jointly or grouped around a main fraction. In the electrophoretic system thus applied soluble proteins separated into 6–10 fractions.

The results of repeated analyses have shown significant differences in the electrophoretic profiles of the protein fractions of different vegetables and their densitograms providing means to their differentiation or identification. The quantitative change in the fractions of soluble proteins in the same vegetable during storage can also be followed up.

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