ÚSTAV ZEMĚDĚLSKÝCH A POTRAVINÁŘSKÝCH INFORMACÍ

POTRAVINÁŘSKÉ VĚDY

FOOD SCIENCES

5

ROČNÍK 12
PRAHA 1994
CS ISSN 0862-8653

ČESKÁ AKADEMIE ZEMĚDĚLSKÝCH VĚD
SLOVENSKÁ AKADÉMIA PÔDOHOSPODÁRSKYCH VIED

POTRAVINÁŘSKÉ VĚDY
FOOD SCIENCES

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Potrav. Vědy, *12*, 1994 (4): 337–420

Food Research Institute Prague introduces itself

It becomes a good tradition to devote some issues of the journal Potravinářské Vědy to single theme. This time we submit you with the selection of the studies of the Food Research Institute Prague.

Despite some restrictions and problems faced to our research sphere in the present time, the Food Research Institute Prague is a complex workplace which solves the targets of cross-sectional and applied research in the field of chemistry, biochemistry, technology of foods, nutrition and in the food engineering and preservation of stocks against pests. Except workplaces within the Institute in Prague–Hostivař, the Institute has a developmental base of food technology at Hrušovany nad Jevišovkou. This workplace is concentrated on the development, design and production of food industry, packaging and handling technologies as requested by customers.

At the present time, the Institute solves in total 16 sector research projects, of which two will be completed according to the plan this year, remaining projects in 1995. Expenses of these projects are paid from the grant of the Ministry of Agriculture of the Czech Republic.

In the future another marked source of finance for research should become the Grant Agency of the Czech Republic. At the end of 1993 the Institute received so its own grant, as the participation in solving the grants of other institutions - the Institute of Chemical Technology, Czech Technical University and the university of Agriculture in Prague.

The Institute undergoes continuously some specific - the so-called permanent or expert activities, e.g. maintenance and supplementation of collections of mites and insects, microorganisms, data base of physical properties of foods, partial monitoring of contaminants, expert and consulting activities.

A significant place in activities of the Institute is taken by foreign cooperation to which we have paid more attention in recent time. Cooperation with foreign partners is carried out in several lines. The Czech Republic is represented through our research institute in several commissions, e.g. in the International Organization for Cereal Sciences and Technologies ICC and the International Organization for Biological and Integrated Pest Control. A number of specialists of the Institute is in a

direct contact with outstanding scientific institutions and universities, such as the University in Piacenza (Italy), the national Health Institute for Pest Control in Berlin (Germany), and the University in Alberta (Canada). Cooperation in solution of some research problems is also important. Here can be mentioned the cooperation with the Silsoe Research Institute in Bedford (Great Britain) in solving mathematical models of cooling of liquid foods, participation in the solution of the programme PECO (Mathetical Modelling of Food Properties during Production and Storage) and cooperation with NIZO (Netherlands) on the project of EC countries concentrated on the problems of Central and Eastern Europe countries.

Following the change of the character of the Research Institute into contributional organization, only a part of its budget, in concrete terms, contribution for solution of sector targets and permanent activities, is paid by its founder (the Ministry of Agriculture). In 1993 its share amounted to two-thirds of the Institute's budget. The remaining part is to be provided by other activities. A rising activity and promotion of works and services for payment and as ordered from business sphere is connected with it. Services are rendered practically by all departments of institutes within their sphere activities.

Ing. Jiří Celba, CSc.
Director of the Food Research Institute Prague

Potrav. Vědy, *12*, 1994 (5) : 339-350

TANDEM COLUMN BIOAFFINITY SEPARATION OF AMYLASES, AGGLUTININ AND CHYMOTRYPSIN INHIBITOR OF RYE

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Rye albumin was prepared by salt extraction of rye flour followed by dialysis, ammonium sulphate precipitation, and dialysis of the precipitate suspended in minimum-volume of water. The four columns with biospecific sorbents were used in tandem in the following order: 1. DEAE-Sephadex, 2. chitin, 3. β-cyclodextrin-agarose, and 4. chymotrypsin-agarose. The sample was applied to 1 M NaCl, 1 mM calcium chloride in 50 mM acetate pH 5.5. 4.18% of protein was adsorbed on DEAE-Sephadex column, 44.4% protein was not adsorbed on any column. 80.2 % of lectin activity applied was eluted from chitin column using 50 mM HCl as eluent, 95.6% of amylase applied was eluted from cyclodextrin column using 10 mM cyclodextrin solution as well as 79.2% of β-amylase, and 85.9% chymotrypsin inhibitor activity was eluted from chymotrypsin-agarose column using 6 M urea pH 2.0. Four active proteins were separated by this way in the single step separation by the method described.

rye albumin; protein purification; amylase inhibitor; trypsin inhibitor; agglutinin; biospecific affinity chromatography; tandem columns

In the early days of this century the mapping of the cereal proteins content was based on selective extraction followed by the precipitation procedures (Osborne, 1907). Recently, as a consequence of the introduction of modern affinity chromatography, additional more sophisticated methods have become available, thus making much more penetrating studies possible. Extracts obtained by the Osborne procedures, in various modified forms, still frequently serve as starting materials for chromatography. In our first exploratory study, we have kept to this tradition.

The albumin fraction of cereal extracts, obtained by application of such fractionation schemes, is composed of the mixture of greatly varied proteins. In the past, major attention was paid to the wheat albumin, wheat being the

most important cereal for human alimentation (Salomonsson, 1986; Buonocore et al., 1977; Contagalli et al., 1971; Feillet, Bourdet, 1966; Kasarda et al., 1976; Kato et al., 1974). A number of enzymes and enzyme inhibitors as well as other proteins have been isolated and partially characterized (Buonocore et al., 1977, Kato et al., 1974; Preston, Kruger, 1976). For example, agglutinin (LeVine et al., 1972; Allen et al., 1973; Lotan et al., 1973; Bloch, Burger, 1974; Shaper et al., 1973) and β-amylase (Vretblad, 1974) have been purified by affinity chromatography. Moreover, it is worth mentioning in connection with this study that crude albumin has been covalently bound to agarose and used as affinity sorbent for insect amylase (Buonocore et al., 1975), anticipating, we presume, the development of more effective procedure along similar lines.

Rye is considered to be the second most important cereal. How surprising, therefore, that our knowledge of rye proteins is still on such a rather rudimental stage. Indeed, so far, only superficial studies have been undertaken through the agar gel electrophoresis and chromatographic size exclusion methods which are admittedly powerful but applied, separately or together on an analytical scale, do not provide enough material for permitting detailed characterization of the partially purified components.

Agar gel electrophoresis revealed existence of five amylase isoenzymes in rye (Wagemar, Lugtenborg, 1973) some of them similar to amylases isolated from other sources. Serin-protease inhibitors are known to be present in all common cereals. They are extremely stable in heat at extreme acid and alkaline pH. Thus, virtually all activity survived 30 minutes of boiling and several hours' exposure to 0.1 M HCl or 0.1 M NaOH at room temperature (Boisen, 1983). The agglutinin, also, is extremely acid-stable. Wheat agglutinin has been purified by affinity chromatography on immobilized ovomucoid, chitin and N-acetyl-glucosamine (Lotan et al., 1973; Bloch, Burger, 1974; Mikola, Kirsi, 1972).

The substances above-mentioned form a group of proteins of great interest, constituting a substantial part of an important cereal of considerable nutritional value and exhibiting unusual stability properties. These features make them excellent models for methodological studies aiming at improved aids for protein mapping and for development of large scale isolation techniques based on biospecific adsorption.

With this background we have taken the first step to develop a new strategy for protein mapping of complex plant extracts. New biospecific adsorbents have been synthetized and their capacity determined. The rye albumin fraction was then fractionated on the adsorbents arranged in a tandem order to decrease material losses and save time and labor.

Although our general strategy include some characteristic technical details, borrowed from the researchers in the reference list, such as, for example, drastic acid elution conditions for agglutinin, it also adds some new features which hopefully will inspire further development of methods of general applicability to plant biochemistry.

MATERIAL AND METHODS

The rye used for the albumin preparation was the cultivar Petkus and was a generous gift of Dr. Marta Larsson-Raznikiewicz.

Rye albumin was prepared according to the method of Osborne modified by Chen and Bushuk (1970). The albumin fraction was precipitated with 80% saturated ammonium sulphate. The precipitate was suspended in minimum volume of water and dialyzed against 400-fold volume of 1 M NaCl, 1 mM $CaCl_2$ in 50 mM acetate, pH 5.5 (48 hours at +5 °C). This process causes some loss of proteins but it removes the major portion of polysaccharides and result in a desirable very substantial decrease in viscosity, a necessary prerequisite for chromatography.

Chitin, N-acetylglucosamin, chymotrypsin and 3,5-dinitrosalicylic acid were products of SIGMA, while N-cyclohexyl-N-morpholinoethyl carbodiimide, methyl-*p*-toluen-sulphonate and β-cyclodextrin were obtained from Fluka AG. Agarose (Ultrogel, 6% matrix content) was a gift from LKB. SE-Sephadex and DEAE-Sephadex were obtained from Pharmacia.

Chymotrypsin, N-acetylglucosamin, glycine and β-cyclodextrin were coupled to divinylsulphone activated agarose (Porath, 1974). Glucosamin was immobilized to glycine agarose by condensation with N-cyclohexyl-N--(2-morpholinoethyl) carbodiimide methyl-p-toluene sulphonate (Martensson, Mosbach, 1972). The agglutinin activity was determined with rat erythrocytes as substrate (Lis et al., 1970).

Amylolytic activity was determined by the method of Meyer et al. (1947) with soluble starch as a substrate and dinitrosalicylic acid as a reagent

for reducing sugar. The α- and β-amylase were estimated by their activities at different pH (Meyer et al., 1947), although this method for rye albumin lacks precision.

Chymotrypsin inhibitor activity was determined according to Laskowski (1955) with casein as chymotrypsin substrate.

Protein concentration was estimated from optical density at 280 nm, but for balance experiments the Bio-Rad set of reagents were used with human serum albumin as standard.

All chromatographic experiments were made at room temperature. With the exception of the experiment made in 50 mM HCl, all the buffers were 1 mM $CaCl_2$ in order to ensure enzyme stabilization. Column capacities were estimated by frontal analysis of 20-fold diluted rye albumin extract.

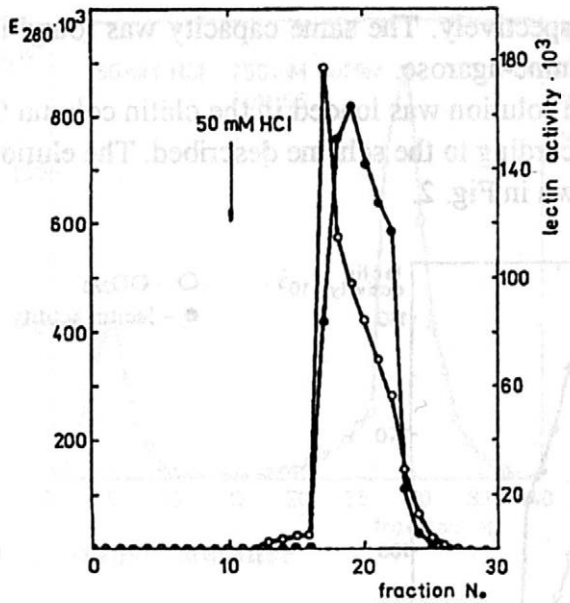
RESULTS AND DISCUSSION

Amylases

Immobilized cyclodextrins have been used for adsorption of β-amylases (Vretblad, 1974) and also to separate α-amylase from wheat albumin (Maeda, Nikumi, 1978). We have used divinylsulphone-coupled β-cyclodextrin as biospecific adsorbent for separation of α-, as well as β-amylase from rye.

The cyclodextrin-agarose bed was equilibrated with a 50 mM acetate pH 5.5, 1 M NaCl and 1 mM $CaCl_2$. After introduction of the sample, the column was washed with 10 volumes of the same buffer followed by another 10 volumes of the buffer without sodium chloride. Finally, the amylases were eluted with 10 mM β-cyclodextrin in the sodium chloride-free buffer. The material eluted in this way was distributed in a rather broad peak indicating the presence of proteins with different affinities (Fig. 1). Attempts to separate the amylase isoenzymes by use of pH- and cyclodextrin concentration gradients failed.

Using the outlined procedure the gel was found to adsorb 0.18 mg and 0.54 mg per ml of crude rye protein and prepurified (albumin), respectively. The lower yield in the former case presumably reflects the presence of polysaccharides. Thus the ammonium sulphate, in addition to lowering the viscosity of extract, increases the gel capacity with respect to amylases.



1. Chromatography of rye albumin on agarose-bound β-cyclodextrin. The sample (15 ml, OD_{280} 1.03) was applied to the column of total volume 42 ml in 1 M NaCl 50 mM acetate buffer pH 5.5 and the column was washed with the same buffer and with 50 mM acetate buffer pH 5.5 (not shown). The amylolytic enzymes were eluted with 10 mM β-cyclodextrin in the same buffer. Fraction volume 7.6 ml, flow rate 0.23 l/min

Agglutinin

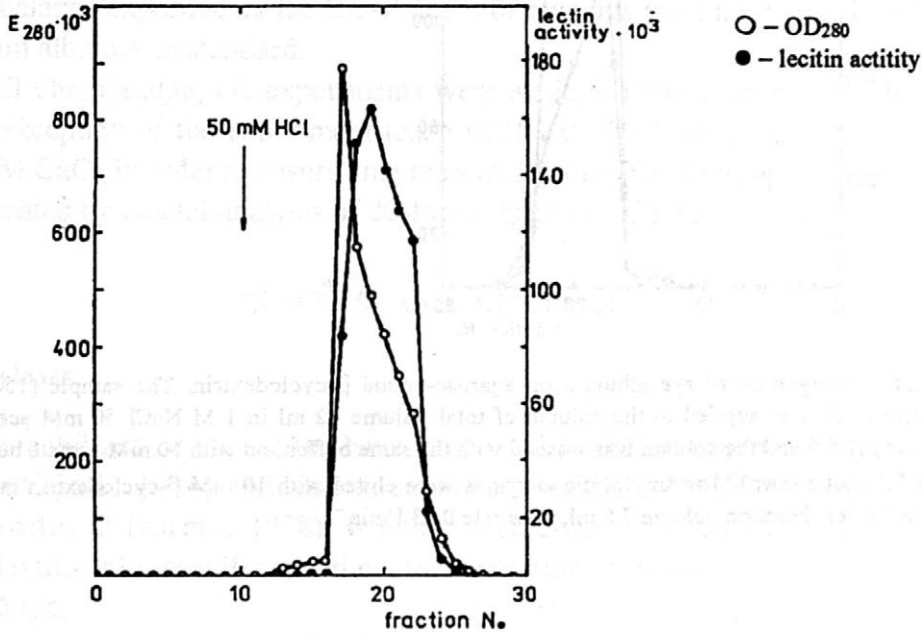
As ligand for the biospecific adsorbents we have chosen chitin, N-acetylglucosamine and N-glycylglucosamine. The latter ligand was chosen since it was believed to exhibit affinity strength to the agglutinin between that of glucosamine (too weak) and acetylglucosamine (too strong). Nevertheless, all the gels adsorbed the rye agglutinin although with different capacities.

The columns were equilibrated with the 1 M NaCl-acetate buffer as in the amylase case of former paragraph. The rye albumin solution was applied and the unadsorbed proteins were washed out with the same buffer and the column washed with 10 volumes of sodium chloride depleted buffer. The agglutinin was eluted with 50 mM HCl.

The chitin column was found to have the highest adsorption capacity: 1.03 mg /ml and 2.14 mg/ml for crude and prefractionated sample, respectively. Bound N-acetylglucosamine exhibited the corresponding capacities of

0.08 mg/ml and 0.14 mg/ml, respectively. The same capacity was found in the same case of glycylglucosamine-agarose.

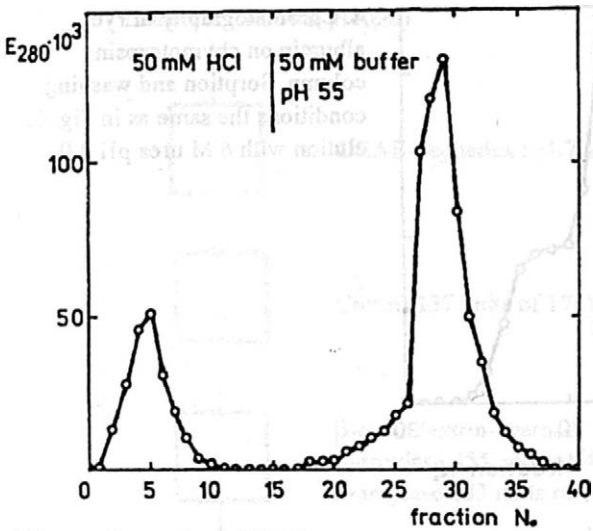
In an experiment rye albumin solution was loaded in the chitin column to half its capacity and washed according to the scheme described. The elution profile for the agglutinin is shown in Fig. 2.



2. Chromatography of rye albumin on chitin column. The same conditions as in Fig. 1, total volume 16 ml. Elution of lectin was carried out with 50 mM HCl

After five cycles the capacity of the N-acetylglucosamine column had decreased to 82 % and similarly the N-glycylglucosamine had deteriorated to 81% of their respective original value. No decrease in capacity was observed for the chitin column.

The agglutinin eluted from the chitin column was introduced directly into an sulphoethyl-Sephadex column. No activity was found in the acid elution peak (Fig. 3) while 98% of the activity was recovered by elution with acetate buffer pH 5.5. The inactive fraction was adsorbed to DEAE-Sephadex at pH 5.5 even in the presence of 1 M NaCl. No other rye albumin proteins were adsorbed to the anion exchanger under these conditions.



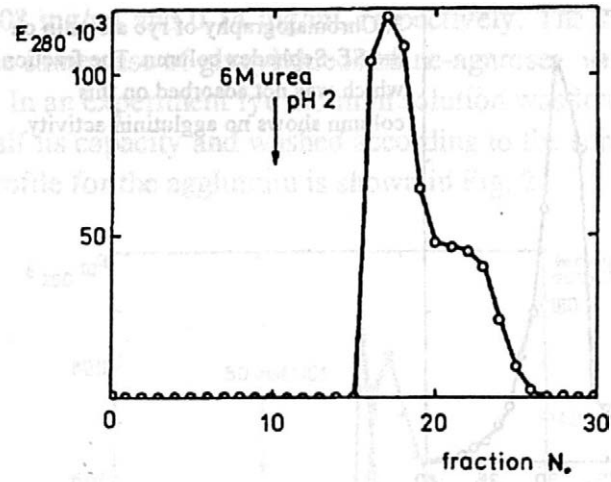
3. Chromatography of rye albumin on the SE-Sephadex column. The fraction which was not adsorbed on this column shows no agglutinin activity

Chymotrypsin inhibitor

We have used chymotrypsin fixed to agarose by divinylsulphone to purify the inhibitor in the rye bioselectively. The sorption step was the same as the one described in the two previous sections. Although pH 5.5 is far from the chymotrypsin interaction optimum for its inhibitor the adsorption was found to be very strong. Desorption was achieved upon increasing the acidity to pH 2 and including 6 M urea in the solvent. No separation of different inhibitors of serine proteases could be expected under this conditions. Presumably all inhibitors of these enzymes were collected in the displaced fraction. The chromatogram is shown in Fig. 4. The individual fractions were pooled and solvent exchange accomplished by gel filtration on Sephadex G-25 in 50 mM Tris-HCl pH 7.6 and after that the activity was determined and found to exceed 90% of the activity loaded.

Tandem column

The rye albumin was prepared from 20 g rye flour for determination of material as well as activity balances. The sample solution was prepared by saturation the rye extract with ammonium sulphate to 80% ammonium sulphate saturation, collecting the precipitate by centrifugation and dissolving it in minimum-volume of 50 mM sodium acetate, pH 5.5 and dialysis against a large excess of the same buffer and removing undisolved matter by centrifugation. The yield and specific activity are shown in Table I.

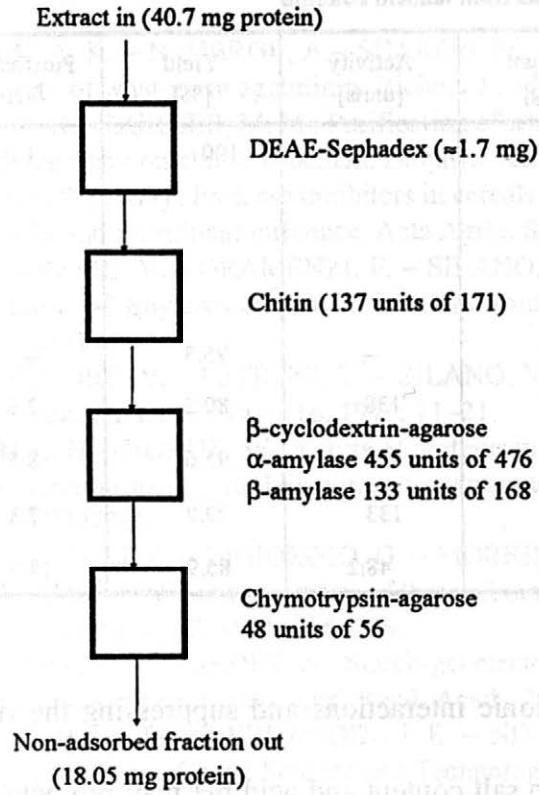


4. Chromatography of rye albumin on chymotrypsin column. Sorption and washing conditions the same as in Fig. 1, elution with 6 M urea pH 2.0

The extract thus obtained and adjusted to become 1 M in NaCl, was applied to a tandem columns of DEAE-Sephadex, chitin, β-cyclodextrin-agarose and chymotrypsin-agarose preequilibrated with the 1 M NaCl, 1 mM $CaCl_2$ in 50 mM acetate buffer pH 5.5 and arranged as shown in Fig. 5. The volumes of the individual columns were chosen to possess twice the capacities of the proteins to be adsorbed (as determined from the preliminary experiment with single bed). The columns were then washed with the NaCl-containing starting buffer until no further proteins were eluted. The column beds were disconnected and separately eluted as described previously for the single bed experiments. The yield and specific activities obtained in the chromatographic experiment are shown in Table II.

I. The yield and the specific activity of the active proteins separated

Step	Volume [ml]	Protein [mg/ml]	Amylase (α/β) [EU/ml]	Lectin [U/ml]	Chymotrypsin inhibitor [U/ml]
Extraction	290	0.34	1.76/1.78	0.92	0.39
Dialysis	347	0.23	–/–	0.72	0.26
$(NH_4)_2SO_4$ precipitation	110	0.37	4.33/1.53	1.56	0.51



5. Arrangement of tandem columns

General discussion

Tandem column arrangement in affinity chromatography has been described earlier by P o r a t h et al. (1988). Used for "all or none" adsorption--desorption process it is, in principle, applicable for specific group fractionation of small or large samples, i.e. in micro-, laboratory (as demonstrated herein), as well as in an industrial conditions. As shown the tandem bed arrangement of affinity adsorbents makes possible isolation of classes of proteins possessing biological properties simple and relatively fast.

The necessity of selecting a common equilibration buffer systems, of course, sets a limit to the applicability of tandem columns. This, however, does not detract much to the attractive features of the technique since it is always possible to select a buffer that does not differ too much from the extraction solvent, with respect to pH, electrolyte concentration, water content, etc. In the experiment herein we included 1 M sodium chloride, thus

II. Specificities and yields of fractions from tandem columns

Product	Amount [mg]	Activity [units]	Yield [%]	Purification factor
Protein at start	40.7	–	100	1.0
Protein recovered				
desorbed	20.7			
not adsorbed	18.1			
total	38.8	–	95.3	–
Lectin	13.5	138	80.2	2.6
α-amylase	4.6	455	95.6	8.5
β-amylase		133	79.2	7.3
Chymotrypsin inhibitor	2.4	48.2	85.9	14.7

counteracting protein-solute ionic interactions and suppressing the risk of bacterial growth.

On the other hand, the high salt content and acid pH may promote complexation due to hydrophobic interaction. However, we have not noticed such a complication to occur in the present case.

According to our results in this study further effective purification of isoenzymes, isoagglutinins and various enzyme inhibitors by bioaffinity methods will be easy. While we plan to extend bioaffinity purification of the rye proteins we are convicted that for the ultimate isolation procedures we have to include also the zone electrophoresis and/or high-resolving chromatography on ion exchangers (HPLC), immobilized heavy metal ions (IMAC) and electron-donor-acceptor chromatography (EDAC).

Aknowledgement

The work was carried out partially during the stay of the author in the Separation Center, Institute of Biochemistry, University of Uppsala. The author is obliged to thank to all the colleagues in Uppsala, namely to professor Jerker Porath and Dr. Makonen Belew for the helpful discussion and advises.

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Received February 25, 1994

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Separace amylas, aglutininu a inhibitoru chymotrypsinu v žitě na základě bioafinity na tandemové koloně

Žitná mouka byla extrahována v přítomnosti NaCl a $CaCl_2$ při pH 5,5. Extrakt byl dále purifikován precipitací síranem amonným (nasycení 0,35–0,8) a precipitát byl rozpuštěn v minimu vody a dialyzován proti zředěnému pufru (acetát pH 5,5). Čirý roztok byl pak veden na řadu biospecifických kolon spojených do série v tomto pořadí: 1. DEAE-Sephadex, 2. chitin, 3.β-cyklodextrin-agarosa a 4. chymotrypsin agarosa. 18 % všech vložených bílkovin bylo sorbováno na DEAE-Sephadexu, 44,4 % bílkovin nebylo sorbováno na žádné z uvedených kolon. Bílkoviny sorbované na DEAE-Sephadexu nebyly identifikovány, avšak použití tohoto sorbentu významně přispělo k dosažení kvalitních produktů z ostatních kolon. 80,2 % veškeré vlo žené lektinové aktivity bylo eluováno z chitinu 50mM HCl, 95,6% amylasové aktivity bylo eluováno z β-cyklodextrinové kolony s použitím 10mM roztoku cyklodextrinu v počátečním pufru, 79,2 % inhibitoru amylas a 85,9% trypsin inhibitoru bylo pak eluováno z příslušných kolon. Touto cestou byly izolovány čtyři aktivní bílkoviny z žitného albuminu v jediném izolačním stupni.

žitný albumin; purifikace bílkovin; inhibitor amylas; trypsin inhibitor; aglutinin; biospecifická afinitní chromatografie; tandemové kolony

Potrav. Vědy, *12*, 1994 (5) : 351-357

MICROCALORIMETRIC DETERMINATION OF UTILIZATION OF LINEAR SATURATED ALCOHOLS AND ACIDS BY *PSEUDOMONAS PUTIDA*

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Heat conduction microcalorimetry was used to study the ability of *Pseudomonas putida* bacterium to utilize glucose, linear saturated alcohols C_1–C_{14} and acids C_1–C_8. Determined total metabolic heats and calorimetric-respirometric (CR) ratios were compared with theoretical values.

microcalorimetry; calorimetric-respirometric ratios; *Pseudomonas* sp.; alcohols; monocarboxylic acids

Pseudomonas sp. bacteria are frequently used for soil and water decontaminations, especially in cases of oil pollution. The abilities of *Pseudomonas* sp. to utilize alkanes (Olsen, Arbor, 1985; Inoue, Horikoshi, 1988); cyclic and polycyclic hydrocarbons (Porits et al., 1983; Olsen, Arbor, 1985); aromatic hydrocarbons (Foght, Westlake, 1988; Abril et al., 1989); halogenated hydrocarbons (Sojka et al., 1988; Nelson et al., 1988; Dipak, 1989) and organic silicon compounds (Wasserbauer, Zadák, 1990) have been described. Conventional microbiological methods of testing the capabilities of microbial strains to utilize different substrates are usually time and labour-consuming. Microcalorimetry offers a simple and quick alternative way, with the potential for automation. In addition, hitherto unseen metabolic data may also be generated. Applications of the method to the studies of metabolism of bacteria and yeasts and characterization of microorganisms have been extensively reviewed (Brown, 1969; Beezer, 1980; Kemp, Schön, 1990).

MATERIAL AND METHODS

Microorganism and cultivation

Pseudomonas putida culture, collection number B3, from the collection of microorganisms of the Department of Microbial Products, Food Research Institute, Prague, was grown on MPA plates and transferred into five-litre flasks under sterile conditions. The culture medium contained the following substances (per litre): NH_4Cl, 5 g; $Na_2HPO_4 . 12H_2O$, 3.4 g; KH_2PO_4, 1.6 g; NaCl, 0.5 g; $MgCl_2$, 0.5 g; molasses, 10 g; urea, 1g; ethanol, 1 ml; and 5 ml of Solution I. Solution I contained the following substances (per litre): 85% H_3PO_4, 35 ml; KOH, 30 g; $MgSO_4 .7H_2O$, 32 g; NaCl, 12.2 g; $ZnSO_4 . . 7H_2O$, 0.5 g; $CuSO_4 . 5H_2O$, 0.02 g; $MnCl_2 . 4H_2O$, 0.05 g. The cultivation was done on a rotary shaker for 48 h at 150 rpm, 30 °C, and pH 7.0. 1 ml of ethanol per litre of medium was added after each 8 hours. Cells were harvested by centrifugation (15 min at 59.10^3 m/s^2) at 4 °C, washed with an excess amount of physiological solution and centrifuged again. The obtained bacterial paste (26% of dry material) was stored at 4 °C and used for microcalorimetric experiments (determination of CR-ratios) or freeze-dried after dilution with physiological solution (determination of total metabolic heats of substrates). No cryopreserving agents were added during the freeze-drying process. The freeze-dried sample contained 5.10^8 of viable cells per gram, as determined by the conventional microbiological method on MPA plates.

Apparatus and operating conditions

Microcalorimetric experiments were performed using the LKB 2277 Thermal Activity Monitor (LKB-Pharmacia, Sweden), a free-standing multichannel microcalorimeter. Continuous heat flow measurements are conducted isothermally. The instrument is equipped with the LKB 2132 MicroPerpex Pump. A single circuit for flow-through and a double circuit for flow-mix experiments are available. Flow lines of the flow-through or the flow-mix system, resp., including measuring cell, have volume approx. 1.2 ml, 1.5 ml, resp. Effective volume of the measuring cell is approx. 0.6 ml. All measurements were performed at 30+/–0.0002 °C .

Total metabolic heats of substrates were determined using the flow-through measuring mode. The freeze-dried bacterial inoculum was suspended in 0.2 M

phosphate buffer, pH 6.0, to obtain 5.10^6 of viable cells per ml of the medium. 100 ml of the culture medium was prepared 90 min before measuring and stirred in a jacketed glass fermentor (total volume 300 ml) with aeration and a propeller stirrer. The starveling bacterial culture was then pumped through the measuring cell of the microcalorimeter with flow rate of 40 ml/h. When a stable heat flow signal generated by endogenic metabolism was recorded, diluted solutions of linear saturated monohydric alcohols C_1–C_4 and C_6–C_{14} were pipetted into the medium to obtain concentrations in the range 1.10^{-4}–1.10^{-1} mol/l. Total reaction heats were determined by integration of the peaks recorded after addition of the substrates on the stable background of endogenic metabolism. To obtain the real enthalpy changes in substrate utilization, the total heat changes obtained were corrected by subtraction of dilution heats of individual alcohols.

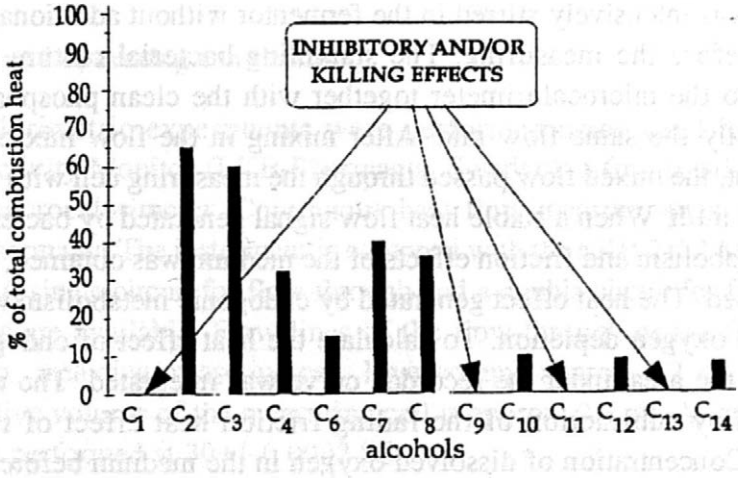
Oxycalorimetric equivalents of substrates were determined using the flow-mix-stop measuring mode. The oxycalorimetric equivalent is the enthalpy of aerobic catabolic reactions per 1 mol of O_2 consumed ranging from –430 to –480 kJ per 1 mol of O_2. This is the expected ratio of calorimetrically measured heat flux and respirometric oxygen flux (CR ratio [kJ per 1 mol of O_2]) under conditions when no work is done and when the reaction system is fully described by the aerobic catabolic half cycle (Gnaiger, 1990).

The bacterial paste was suspended in 100 ml of 0.2M phosphate buffer, pH 6.0, to obtain 1.10^9 of viable cells per ml of the medium. The culture medium was intensively stirred in the fermentor without additional aeration 90 min before the measuring. The starveling bacterial culture was then pumped to the microcalorimeter together with the clean phosphate buffer with exactly the same flow rate. After mixing in the flow mix cell of the instrument, the mixed flow passed through the measuring cell with total flow rate of 40 ml/h. When a stable heat flow signal generated by bacterial endogenic metabolism and friction effects of the medium was obtained, the pump was stopped. The heat effect generated by endogenic metabolism was measured until oxygen depletion. To calculate the heat effect of endogenic metabolism, the area under the recorded curve was integrated. The result was corrected by subtraction of the fading friction heat effect of the buffer (Fig. 3). Concentration of dissolved oxygen in the medium before the calorimetric measurement was determined using an oxygen electrode. Therefore,

oxygen consumption rate by endogenic metabolism was estimated from the results. The enthalpy changes in substrate utilization were measured in the same way, after mixing the starveling bacterial culture with substrate solutions, instead of the clean buffer. Total generated heat was corrected by subtraction of the endogenic metabolism heat effect (Fig. 3). To obtain the oxygen consumption related to the extracellular substrate oxidation, the oxygen consumption by endogenic metabolism was subtracted from the total oxygen consumption. To calculate the CR-ratios of individual substrates, the enthalpy changes of substrate utilization were divided by the oxygen consumption related to the extracellular substrate oxidation.

RESULTS AND DISCUSSION

Fig. 1 shows results of determination of total metabolic heats of linear saturated monohydric alcohols by the flow-through measuring technique. A high peak of heat production generated by endogenic metabolism was recorded immediately after suspension of the freeze-dried inoculum in the buffer. After 60–90 min the heat production decreased and stabilized. The stable level of endogenic metabolism was kept for several hours at least. After the peaks due to additions of the extracellular substrates, the heat flow

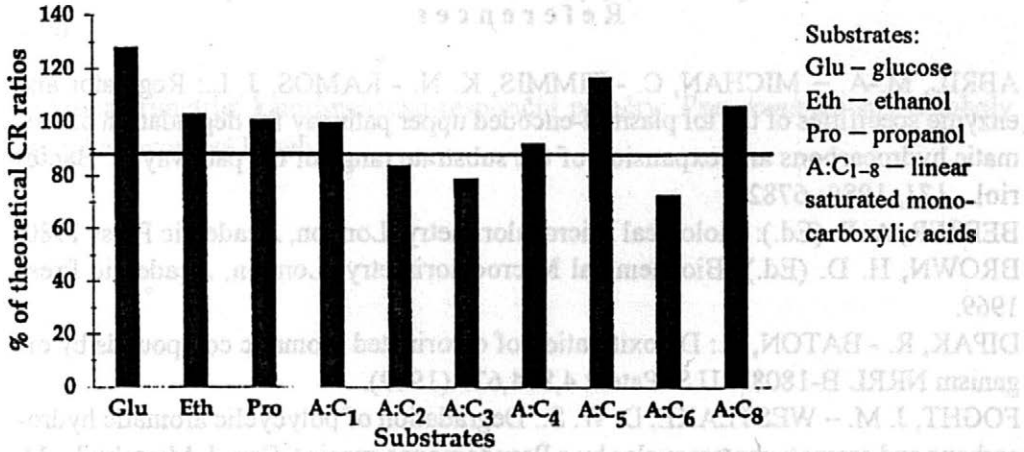


1. Determination of total metabolic heats of linear saturated monohydric alcohols by flow--through technique

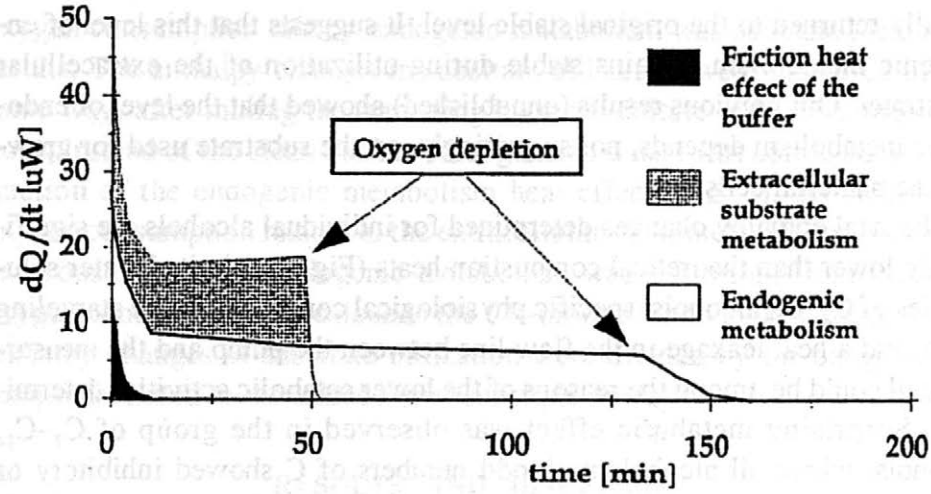
usually returned to the original stable level. It suggests that this level of endogenic metabolism remains stable during utilization of the extracellular substrates. Our previous results (unpublished) showed that the level of endogenic metabolism depends, not surprisingly, on the substrate used for growing the bacterial cells.

The total enthalpy changes determined for individual alcohols are significantly lower than theoretical combustion heats (Fig. 1). Limited water solubilities of C_4–C_{16} alcohols, specific physiological conditions of the starveling cells, and a heat leakage in the flow line between the pump and the measuring cell could be among the reasons of the lower catabolic activities determined. Surprising metabolic effect was observed in the group of C_7–C_{14} alcohols, where all alcohols with odd numbers of C showed inhibitory or killing effects. These effects have been confirmed by the conventional microbiological method on MPA plates, when no growth has been observed with C_1, C_9, C_{11} and C_{13} alcohols as C-substrates (results not shown).

Fig. 2 shows results of determination of CR-ratios of different substrates by the flow-mix-stop measuring technique. Acceptable correspondence of the CR—ratios determined with theoretical oxycalorimetric equivalents was found for all the substrates. During these experiments 35–70% of total consumed oxygen were probably used for oxidation of the endogenic substrate(s) (Fig. 3). CR-ratio of 398 kJ per 1 mol of O_2 was found for the endogenic substrate, acceptably close to theoretical oxycalorimetric equivalents of carbonaceous substrates. The results suggest totally aerobic metabolism of the bacterial



2. Determination of CR-ratios by flow-mix-stop technique



3. Typical example of determination of CR-ratio by flow-mix-stop technique. Substrate: glucose

strain. Utilization of all the tested linear monocarboxylic acids in the range C_1–C_8 has been observed. No special adaptation period was necessary for utilization of all the substrates tested.

The presented method could facilitate identification of microbial strains by determination of substrate spectrum. Selection of a proper microorganism and optimal conditions for biodegradation of different pollutants is another possibility to apply the method.

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Received July 8, 1994

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Mikrokalorimetrické stanovení utilizace lineárních nasycených alkoholů a kyselin bakterií *Pseudomonas putida*

Schopnost bakterie *Pseudomonas putida* utilizovat glukózu, lineární nasycené alkoholy C_1–C_{14} a monokarboxylové kyseliny C_1–C_8 byla studována metodou tepelně vodivostní mikrokalorimetrie. Stanovené kalorimetricko-respirační poměry a celková metabolická tepla individuálních substrátů byly porovnány s teoretickými hodnotami.

mikrokalorimetrie; kalorimetricko-respirační poměry; *Pseudomonas* sp.; alkoholy; monokarboxylové kyseliny

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Potrav. Vědy, *12*, 1994 (5) : 359-370

HONEY AND POLLEN AS BIOINDICATORS OF ENVIRONMENTAL CONTAMINATION

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Five regions of the Czech Republic differing in the classification of the total environmental load were selected. In each of them, samples of honey and pollen were taken from 3 to 8 sites between May and August 1992. The contents of certain chlorinated hydrocarbon insecticides, polychlorinated biphenyls, heavy metals (Pb, Cd, Zn, Cu) and nitrates were determined. The correlation between the pollutant values found and the environmental load of the respective regions is discussed.

honey; pollen; bioindicator; chlorinated hydrocarbon insecticides; polychlorinated biphenyls; heavy metals; nitrates; environmental pollution

It is known from the literature that bee products (honey, pollen, beeswax and propolis), or bees and their larvae, are frequently used as bioindicators of the level of environmental contamination, due to the property of bees to collect nectar, honeydew and pollen within several kilometres' radius. The foraging bees get into contact with the residues of pesticides, the dust adhering to plant surface, and water from varied natural sources. In the beehive the contaminants cumulate in bee products, and thus the analysis of these products may be a good basis for evaluating the level and nature of environmental pollution by these contaminants within the territory in question.

As for the content of particular pollutants in bees and bee products, and its bioindicative potential, it can be concluded from the papers published in this country and elsewhere that inorganic pollutants (primarily metals) are investigated more extensively than organic pollutants, mainly polychlorinated biphenyls (PCBs) and chlorinated hydrocarbon insecticides (CHI). Thus e.g. T o n g et al. (1975) studied the correlation between the level of 47 toxic and non-toxic metals contained in honey, and the sites of its origin in New York State, as early as in 1975. They showed that the pollutant content

was correlated with the environmental pollution level of the area where the samples were taken. Similarly, bees and honey were used by Shabanov and Ibrashimov (1975) for the monitoring of Cu, Zn and Pb occurrence in agricultural regions, and by Bromenshenk (1976) in a study for a mining project in Montana. The Italian authors Cimino et al. (1977) analyzed honey from the environs of Etna for heavy metals and found that this area of volcanic fallout resembles the environment of an industrial complex.

Estep et al. (1977), who measured the residues of CHs in honey and beeswax in Tennessee, found non-significant differences in CHI content between the eastern, central and western parts of this state. Certain residues were even found in bee product samples taken from the hives located at the sites with no history of pesticide application. Similar results were obtained by Ogata and Bevenue (1973) analysing honey from California and Hawaii. The content of PCBs in bees and bee products from various districts of the U.S.A. was examined by Morse et al. (1987). They also failed in an attempt to associate the PCBs concentrations found with any massive sources of such a contamination. They pointed out, however, that much higher amounts of PCBs occurred in beeswax. They ascribed this fact to the cumulative role of these markedly lipophilic substances. The same opinion is also expressed by Gayger and Dustmann (1985), who recommend the use of non-recycled beeswax for the monitoring of PCBs and CHIs in the environment. Of much interest are the papers by Gilbert, Lisk (1978) and Tonelli et al. (1980), who used bees and bee products as bioindicators of radionuclide environmental load.

In this country the xenobiotic substances have been systematically monitored for a long time with the aim to evaluate the public health safety of the food consumption basket. The studies utilizing bees and their products as bioindicators are limited to inorganic, mainly metal, pollutants. Thus Bacílek and Karvánek (1982) stated that the high concentration of industrial plants in the Kladno region appeared to be correlated with the increase of Fe content, whereas the level of Zn, Ni, Cu and Pb did not exceed the accepted public health standards and was comparable to reference samples from the areas not suffering from excessive environmental pollution. On the other hand, Toporčák et al. (1992) found that Hg concentrations in honey samples from an pollution-exposed area exceeded those from an unloaded environment about fifty times.

The papers by Hřivna, Hlušek (1989), Starý et al. (1988) and particularly the unpublished data contained in the Ph.D. thesis by Mondspiegel (1992) are of particular interest from the viewpoint of the monitoring. These authors investigated bees, or (Mondspiegel, 1992) bees and their products, as bioindicators of the occurrence of heavy metals in model areas located in the districts of the Czech Republic differing in the intensity of the environmental load. A conclusion can be drawn from their results that the distribution of heavy metals in bee colonies is mostly in no corresopondence with the maps of specific solid-emission load of the particular districts. Thus e.g. whereas Teplice v Čechách belong to the most exposed and Volary to the least exposed districts, the amount of heavy metals found in bee colonies indicates a reverse situation. Pollen is recommended as a very good indicator of the occurrence of heavy metals, these being mostly contained in pollen samples in much higher amounts than elsewhere.

MATERIAL AND METHODS

Based on the map of the environmental classification of the Czech Republic (Atlas..., 1992), five regions differing in the environmental quality were chosen:

Benešov–Vlašim:	satisfactory and high-level environment;
Jičín:	satisfactory and disturbed environment;
Hradec Králové:	disturbed environment;
Davle	disturbed environment;
Teplice–Most:	extremely disturbed environment.

In each region three to eight sites were selected where samples of honey and pollen were taken from apiaries by the staff of the Apicultural Research Institute in May to August 1992. In these samples the following pollutants were determined:

Honey:	CHIs (pp'DDT, pp'DDE, op'DDD, pp'DDD, lindane, dieldrin);
	PCBs (congeners 28, 52, 101, 138, 153 and 180)
	heavy metals (Pb, Cd, Zn, Cu)
	nitrates
Pollen:	heavy metals (Pb, Cd, Zn, Cu)

Analytical methods

Determination of CHIs and PCBs

Sample preparation: Honey samples were dissolved in the mixture acetonitril–water (2:1) and the aqueous phase was extracted three times with hexane. The occasionally formed emulsion was eliminated by the addition of anhydrous sodium sulphate. The combined hexane extracts were washed three times with distilled water, filtered through anhydrous sodium sulphate and purified on a florisil column. The purified extract was vacuum evaporated and the residue was dissolved in a small amount (adjusted to the expected content of pollutants) of cyclohexane. This solution was then injected into the column of the chromatograph.

Conditions of GC: Gas chromatograph: Hewlett-Packard 5890 Series II; capillary column: DB-5, 60 m by 0.25 mm, film thickness 0.25 μm; carrier gas: N_2, flow rate 1 ml/min; temperature gradient 60–220 °C (30 °C/min), 220–280 °C (1 °C/min); detector ECD, temperature 250 °C; injection: 1 μl sample; detection limit: 10^{-3} mg/kg; used standards: congeners PCB Mix 1 (Labor Dr. Ehrenstorfer GmbH), CHI reference sample No. 10-2-05 (Czechoslovak Metrological Institute, Bratislava).

Determination of nitrates

Sample preparation: Exactly 10 g honey was dissolved in distilled water and, if necessary, clarified with Carreze reagent, then further distilled water was added up to 100 ml. The solution was filtered through a folded filter paper and then, prior to injection, through a nylon microfilter 0,45 μm.

Conditions of HPLC: High pressure liquid chromatograph: Spectra Physics, Data Jet Detector; column: Hema Q 1000 Bio, 3 by 150 mm, particle size 10 μm; mobile phase: 0.015 M/l sodium perchlorate acidified to pH 2.5 with 0.005 M phosphoric acid, flow rate 1 ml/min; detection: UV-VIS, wavelength 210 nm; injection: 20 μl sample.

Determination of heavy metals

Sample preparation: A homogenized 1 to 2 g sample was mineralized on an electric hotplate in five 1-hr intervals (110–360 °C), then heated up in a muffle kiln from 350 to 500 °C (in 1-hr intervals) and kept at the latter temperature for 16 hrs. After cooling down, 1 ml concentrated HNO_3 was added and the sample was heated again at 110 °C until the acid evaporated. Finally,

it was heated again in a muffle kiln at 500 °C for 1 hr. The ash obtained was dissolved in 0.1 M HNO_3. The ash content of Cu, Cd and Pb was determined directly in the sample; for Zn determination, 10 ml sample was mixed with 0.05 ml concentrated water solution NH_3 and 0.1 ml 1M sodium acetate.

Conditions of polarography: Computer-controlled ECO-Tribo-Polarograph (manufactured by Polaro-Sensors); sample bubbling by nitrogen before determination for 10 min; electrode potentials:

Element	Potential [V]
Cd	–0.60
Cu	0.01
Pb	–0.40
Zn	–0.10

Detection limits [mg/kg]: Cd, 0.001; Cu, 0.1; Pb, 0.01; Zn, 0.3; used standards: Czechoslovak Metrological Institute, Bratislava.

All chemicals used were of suprapure grade.

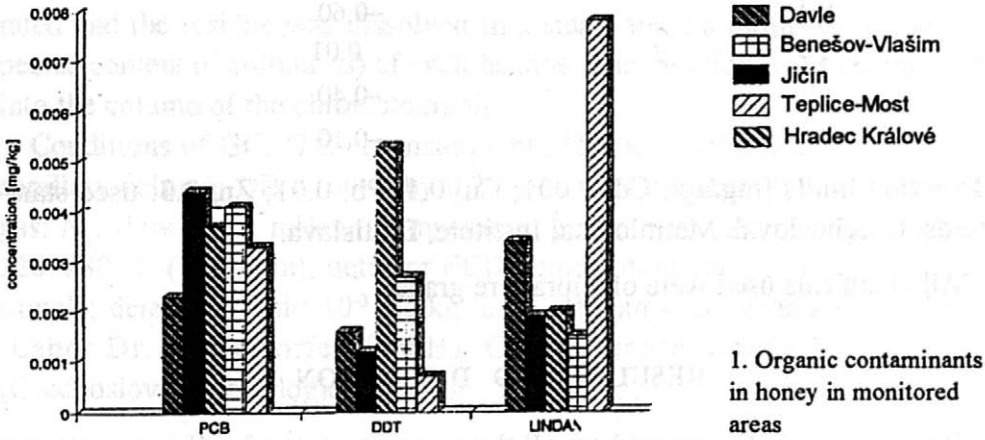
RESULTS AND DISCUSSION

It is impossible to present here all the measurements of pollutant contents in any sample of honey or pollen from all respective sampling sites. The results are available at the authors' laboratory.

The data obtained indicate that the low-chlorinated PCB congeners 28 (mean concentration 0.0013 mg/kg, range 0.000–0.008 mg/kg), 52 (0.0011; 0.000–0.008) and 101 (0.0010; 0.000–0.006), pp'DDT (0.0017; 0.000 to 0.040) and lindane (mean 0.0019) are the major organic pollutants. The high-chlorinated PCBs (congeners 138, 151 and 180), DDT analogues (i.e. pp'DDE, op'DDD and pp'DDD) and dieldrin were found only as traces. The mean contents of total PCBs (0.0035 mg/kg), total DDT (0.0023 mg/kg) and lindane (0.0019 mg/kg) were relatively stable in most sampling sites, being of the order 10^{-3} mg/kg. The exceptions were the sampling sites Běleč nad Orlicí (Hradec Králové region) with the mean DDT content 0.0157 mg/kg, and Třebušice and Rvenice (Teplice–Most region) with the mean lindane contents 0.010 and 0.015 mg/kg, respectively.

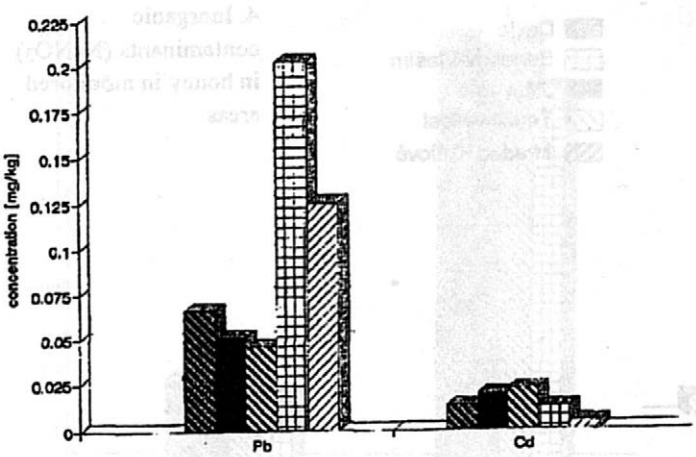
No significant differences in the mean contents of total PCBs and total DDT and in the mean content of lindane among the regions investigated

were found, either. Fig. 1 shows that the level of PCBs was relatively stable. As for DDT and lindane, approximately threefold values were recorded in the regions Hradec Králové and Teplice–Most than in the four remaining regions. A general statement can be made that the level of the strongly lipophilic organic contaminants in honey is very low, nearly reaching the determination limits. In that case it would apparently be advisable to use non-recycled beeswax instead of honey or pollen, as is recommended in the literature (Morse et al., 1987; Gayger, Dustmann, 1985).



1. Organic contaminants in honey in monitored areas

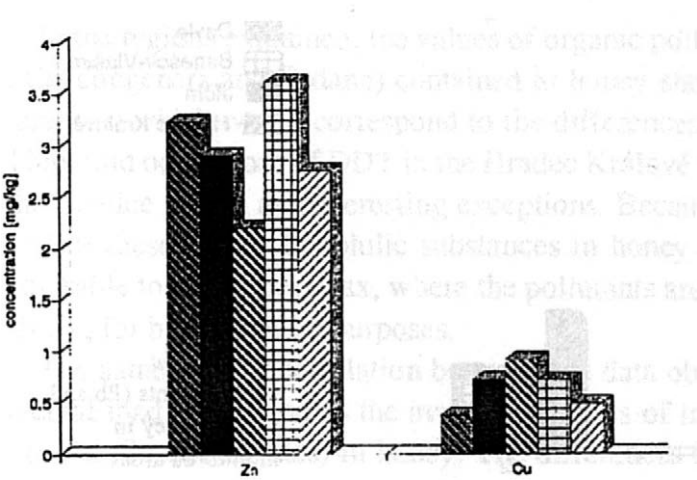
The inorganic contaminants, i.e. heavy metals and nitrates, were found in all honey samples tested in detectable amounts. The means and the ranges of concentrations found were as follows: Pb 0.113 mg/kg (0.020–1.64 mg/kg); Cd 0.013 (0.001–0.064); Cu 0.636 (0.146–2.996); Zn 2.949 (0.587–13.078); nitrates 41.65 (11.1–172.0). Even though high current concentrations of these pollutans were sporadically recorded at some sites of the regions Benešov–Vlašim (e.g. Pb 1.641 mg/kg – Třetužel; Cd 0.064 mg/kg – Horní Borek; Zn 12.054 mg/kg – Mrkvová Lhota and 13.078 mg/kg – Nová Střezimíř), and Hradec Králové (Cu 2.996 mg/kg – Běleč nad Orlicí), it is possible to say that their levels corresponded to the average concentrations in those regions. A certain trend of a slight increase in heavy metal levels was also observed in late summer. Figs. 2 and 3 show that higher Pb levels were found in the regions Benešov–Vlašim and Teplice–Most, i.e. 0.202 and 0.125 mg/kg respectively. The levels of Cu reached 0.918, 0.712 and 0.706 mg per kg in the regions Hradec Králové, Jičín and Benešov–Vlašim.



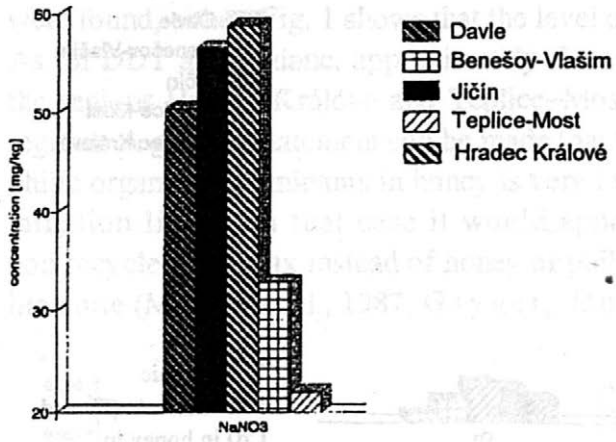
2. Inorganic contaminants (Pb and Cd) in honey in monitored areas

As far as the levels of Zn and Cd are concerned, their values were rather stable, with the exception of the region Teplice–Most, where the lowest Cd level, i.e. 0.004 mg/kg, was found. Similarly, the lowest levels of nitrates (Fig. 4) were found in the regions Benešov–Vlašim (32.8 mg/kg) and Teplice–Most (22.0 mg/kg).

Pollen was used as another bioindicator for the monitoring of heavy metals. Their concentrations found in pollen were 7 to 16 times higher than those in honey, particularly in Cd, Zn and Cu. The values found were: Pb mean 0.252 mg/kg (range 0.001–1.159 mg/kg); Cd 0.094 (0.001–0.873); Cu 8.59 (0.36–14.39); Zn 46.69 (25.55–117.71). No correlation between the contents of these pollutants in honey and in pollen was found. The average content of heavy metals in pollen in the regions examined (Figs. 5 and 6) was



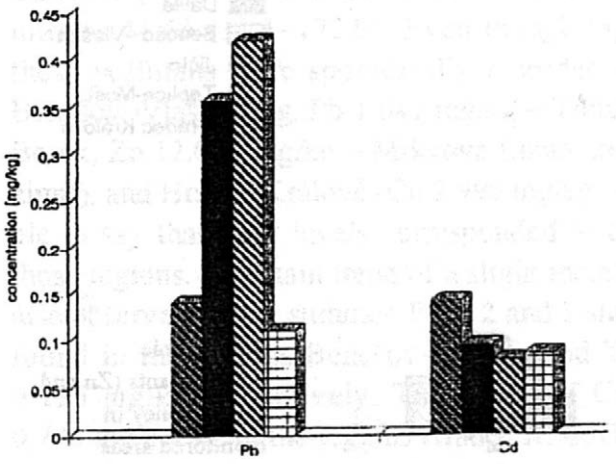
3. Inorganic contaminants (Zn and Cu) in honey in monitored areas



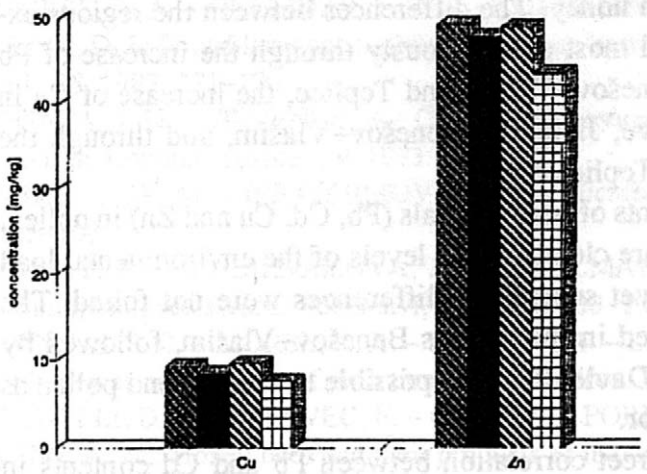
4. Inorganic contaminants ($NaNO_3$) in honey in monitored areas

rather stable in Cd (0.080–0.143 mg/kg), Cu (7.61–9.62 mg/kg) and Zn (43.68–49.34 mg/kg) and, in spite of small differences, showed better correspondence with the environmental classification of the regions in question. In Pb, however, the values found in the regions Hradec Králové and Jičín (0.420 and 0.358 mg/kg respectively) were about twice as high as in the regions Davle and Benešov–Vlašim (0.111 and 0.142 mg/kg). On the basis of the present results and the data of Montspiegel (1992) and Bičík, Kašpar (1986) it is possible to recommend pollen for the bioindication of heavy metals.

The results and conclusions presented here are in very good concordance with the data presented by other authors examining the possibility of the utilization of bees and bee products as the bioindicators of environment quality. We consider a highly qualified interpretation of the experimental



5. Inorganic contaminants (Pb and Cd) in honey in monitored areas



6. Inorganic contaminants (Pu and Zn) in honey in monitored areas

data, respecting specific features of the whole process of pollutant transfer from the particular components of the environment into bees and their products, as one of the most essential conditions for their future use in monitoring systems. It is necessary to take into consideration that these bioindicators can only reflect a real pattern of environmental contamination within a rather small area, delimited by the foraging radius of bees. It may be very difficult to associate these specific data with the environmental load of larger regions without a sufficient number of reference sites, and without the knowledge of pollutant transfer in nature.

CONCLUSIONS

In the regions examined, the values of organic pollutants (total DDT, total PCB congeners and lindane) contained in honey showed no significant differences, which would correspond to the differences in environmental load. Threefold occurrence of DDT in the Hradec Králové region and of lindane in the Teplice region are interesting exceptions. Because of the very low content of these highly lipophilic substances in honey it would apparently be advisable to prefer beeswax, where the pollutants are cumulated more intensively, for bioindication purposes.

The same lack of correlation between the data obtained and the environmental load was found in the average contents of inorganic pollutants (Pb, Cd, Zn, Cu and nitrates) in honey. The differences between the regions ex-

Cd, Zn, Cu and nitrates) in honey. The differences between the regions examined were demonstrated most conspicuously through the increase of Pb contents in the regions Benešov–Vlašim and Teplice, the increase of Cu in the regions Hradec Králové, Jičín and Benešov–Vlašim, and through the very low Cd values in the Teplice region.

As for the average contents of heavy metals (Pb, Cd, Cu and Zn) in pollen, their values correspond more clearly to the levels of the environmental load in the regions examined, yet substantial differences were not found. The lowest values were recorded in the regions Benešov–Vlašim, followed by Jičín, Hradec Králové and Davle. It is thus possible to recommend pollen as another suitable bioindicator.

We failed to prove a direct correlation between Pb and Cd contents in honey and pollen. This might be scrutinized by further research, when the influence of particular environmental components should be taken into consideration when deciding about the sampling sites and timing.

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Atlas životního prostředí a zdraví obyvatelstva ČSFR. Brno, Geografický ústav a FVŽP 1992.

Received June 6, 1994

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Med a pyl jako bioindikátory znečištění životního prostředí

Podle mapy stavu životního prostředí ČR bylo zvoleno pět oblastí s různou třídou úrovně životního prostředí:

Benešov–Vlašim: vyhovující a vysoká úroveň prostředí,
Jičín: vyhovující a narušené prostředí,
Hradec Králové: narušené prostředí,
Davle: narušené prostředí,
Teplice–Most: extrémně a silně narušené prostředí.

V každé oblasti pak bylo určeno 3 až 8 konkrétních stanovišť, na nichž byly v průběhu května až srpna 1992 odebírány od jednotlivých producentů vzorky medů a pylů.

Ve vzorcích byly stanovovány:

med – organochlorové insekticidy (pp'DDT, pp'DDE, op'DDD pp'DDD, lindan a dieldrin), polychlorované bifenyly (kongenery 28, 52, 101, 138, 153 a 180), těžké kovy (Pb, Cd, Zn, Cu), dusičnany;

pyl – těžké kovy (Pb, Cd, Zn, Cu).

V průměrném obsahu organických cizorodých látek (suma DDT, suma kongenerů PCB a lindan) v medu nebyly u sledovaných oblastí nalezeny signifikantní rozdíly, které by korespondovaly s úrovní jejich životního prostředí. Zajímavá jsou pouze trojnásobná zvýšení obsahů DDT v oblasti Hradec Králové a lindanu v oblasti Teplice. Vzhledem k velmi nízkému obsahu těchto výrazně lipofilních látek v medu by bylo zřejmě lepší používat zde jako bioindikátor včelí vosk, v němž dochází k jejich vyšší kumulaci.

Totéž platí co do korespondence s úrovní životního prostředí sledovaných oblastí i pro průměrné obsahy anorganických látek v medu (Pb, Cd, Zn, Cu a dusičnany). Rozdíly mezi jednotlivými oblastmi se nejvíce projevily zvýšením obsahu Pb v oblastech Benešov–Vlašim a Teplice, Cu v oblastech Hradec Králové a Jičín a Benešov–Vlašim a v naopak nízkém obsahu Cd v oblasti Teplice.

Pokud se týká průměrných obsahů těžkých kovů (Pb, Cd, Cu a Zn) v pylu, koresponduje zde jejich množství s úrovní životního prostředí ve sledovaných oblastech lépe, i když výrazné (např. řádové) rozdíly nebyly zaznamenány. Nejméně jich bylo nalezeno v oblasti Benešov-Vlašim, dále pak následovaly oblasti Jičín, Hradec Králové a Davle. Tento materiál lze proto též doporučit jako vhodný bioindikátor.

Přímou závislost mezi obsahy Pb a Cd v medu a pylu se při našich pokusech nepodařilo prokázat. Mohly by však být záležitostí dalšího sledování, při kterém by bylo třeba vzít v úvahu i vliv jednotlivých složek životního prostředí při výběru stanovišť a termínech odběrů.

Námi prezentované výsledky i z nich vyvozované závěry jsou ve velmi dobré shodě s poznatky našich i zahraničních autorů, kteří se zabývají problematikou využití včel a včelích produktů jako bioindikátorů životního prostředí. Za jeden z nejdůležitějších prvků pro jejich budoucí využití v monitorovacích systémech pak pokládáme fundovaný přístup k interpretaci naměřených dat, které musí respektovat specifika celého procesu přestupu polutantů z jednotlivých složek životního prostředí do včel a jejich produktů. Zde je třeba si především uvědomit, že tyto bioindikátory mohou podat věrohodný obraz o stávající reálné úrovni znečištění pouze v relativně malé lokalitě, omezené doletem včel (4 až 6 km). Bez dostatečného počtu kontrolních stanovišť a znalosti transferu kontaminantů v přírodním prostředí je pak velmi obtížně tyto jednotlivé nálezy ztotožňovat se zátěží větších oblastí či regionů.

med; pyl; bioindikátor; organochlorové insekticidy; PCB; těžké kovy; dusičnany; znečištění životního prostředí

Potrav. Vědy, *12*, 1994 (5) : 371-376

ISOLATION OF ALKALINE PHOSPHATASE FROM CARP INTESTINAL MUCOSE

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The method was developed for the separation of carp intestinal alkaline phosphatase. The method is based on the extraction of intestine homogenate with the mixture of 1-butanol with Tris-HCl buffer pH 7.4 and batch-wise adsorption on DEAE-cellulose. The specific activity of the enzyme obtained was 58.6 μkat/mg and the purity was checked electrophoretically. The dye-ligand chromatography of this product on the other hand gives no higher specific activity. The enzyme is not adsorbed on Iontosorb blue as well as Iontosorb red at all.

fish; carp; alkaline phosphatase; isolation; purification; chromatography; ion exchanger; dye-ligand

Alkaline phosphatase is frequently used in medical and food analysis for the determination of organic phosphate compounds, as well as in fine organic synthesis for the preparation of organic phosphates.

The enzyme is usually prepared from calf intestinal mucose (Kirchberger, Kopperschläger, 1982), less frequently from intestinal mucose of other warm-blooded animals. Alkaline phosphatase was proved to be present in intestinal mucose of all phylogenetic classes (Komoda et al., 1986) and was detected in carp intestinal mucose as well (Kuzima, 1984). Nevertheless, yet this enzyme has not been prepared from the latter source.

The consumption of alkaline phosphatase, namely for analytical purposes, is steadily growing. The traditional sources, on the other hand, are limited.

In the present work we have studied the way of preparation of alkaline phosphatase from carp intestinal mucose, as these sources have not been utilized and are available in relatively large quantities.

MATERIAL AND METHODS

The carp viscera were obtained from the State Fishery Lahovice. The intestine was separated from other parts, disintegrated and frozen and kept at –20 °C. Homogenate was thawn before use at +5 °C overnight.

Iontosorb DEAE, Iontosorb blue and Iontosorb red were the products of Iontosorb works, Ústí n. Labem. Fibrous DEAE-cellulose Whatman DE 11 was used for the batch-wise adsorption. All other reagents were the products of Lachema Brno, reagent grade quality.

Protein concentration was determined according to Hartree (1974). The activity of alkaline phosphatase was determined with the aid of reagent set Bio-La-Test, Lachema Brno. The determination is based on the hydrolysis of p-nitrophenyl phosphate. All the chromatographic experiments were carried out with the use of FPLC model 250, Pharmacia Uppsala.

The ultrafiltration was carried out with the use of Amicon model 2000 equipment with the membrane PM10 (exclusion limit 10 kDa).

Electrophoretic experiments were performed in 7.5% polyacrylamide gel at pH 8.5 and the proteins were detected by the usual way using Coomasie blue. The Multiphor II equipment, Pharmacia-LKB Uppsala was used throughout.

The centrifuge Janetzki T24 was used in smaller scale and MLW Janetzki K 70D for large scale separation (2000 g, 5 °C).

The extraction of alkaline phosphatase from carp intestinal mucose was carried out by the following way:

1 kg of homogenate was suspended in 1000 ml of 10 mM phosphate buffer pH 7.4 containing 2 mM $MgCl_2$. The suspension was stirred for three hours at 5 °C. After this time 700 ml of 1-butanol was added to the mixture (approx. 1980 ml) and stirred overnight at the same temperature. Extract was then separated by centrifugation (60 min., 2000 g). The upper layer containing butanol and fats was separated and the water phase was used for the separation of the enzyme. The sediment was reextracted by the same way and both extracts was combined. The pH of the extract was adjusted to 5.0 by the addition of appropriate amount of 2M acetate pH 4.5 and centrifuged once more.

For the first orientative experiments the extract was precipitated with ammonium sulphate, but for the preparative separation the extract was used without precipitation.

The precipitation with ammonium sulphate was performed at pH 7.0 in the range of saturation 35–65%. Sediment was dissolved after separation in minimal volume of 10 mM Tris-HCl pH 8.0 containing 2 mM $MgCl_2$. Then the solution was dialyzed against distilled water overnight, clarified by centrifugation and the pH and concentration of $MgCl_2$ was adjusted to the starting value by the addition of solid salts.

In the experiments in large scale, when the precipitation was not used, the extract was dialyzed against distilled water and after centrifugation the concentration of $MgCl_2$ was adjusted by the addition of solid salt. The pH was adjusted by the addition of appropriate amount of concentrated buffer. The final volume was adjusted to 1400 ml by ultrafiltration.

RESULTS AND DISCUSSION

Ionexchange chromatography

The optimum conditions for the adsorption were determined with FPLC on MONO Q and MONO S sorbents in the column of total volume 1 ml. The chromatography was carried out in the range of pH from 4.5 to 7.5 with MONO S and from 5.5 to 8.5 for MONO Q. 10 mM buffers were used (acetate for acidic pH, Tris for alkaline one) containing 2 mM $MgCl_2$. The proteins were eluted with increasing ionic strength up to 0.5 M NaCl in the same buffer. 1 ml of the ammonium precipitated extract was injected to the column. The column was washed first with 5 ml of starting buffer and then the adsorbed proteins eluted with the NaCl concentration gradient with the concentration changes 10 mM/ml. This slow changes (recommended maximum rate of ionic strength change for this salt and sorbent is 17 mM/ml) make sure that the optimum pH will be determined with the precision appropriate to the selection of the conditions for batch-wise separation. The cationexchanger was found to be less efficient in all the range of pH. Optimum condition for anionexchanger adsorption was found to be 10 mM Tris-HCl buffer pH 8.0. Under this conditions alkaline phosphatase is divided into two fractions, the first is eluted by the increase in ionic strength to 0.1 M NaCl, the second to 0.3 M NaCl. The both fractions are different electrophoretically and both contain alkaline phosphatase activity.

The chromatography was then repeated in larger scale with the use of sorbent Iontosorb DEAE with the same results. Optimum conditions found by this way were used for the batch-wise separation.

30 g of DEAE-cellulose (Whatman DE 11) was equilibrated with the starting buffer (10 mM Tris pH 8.0 containig 2 mM $MgCl_2$) and added to 1400 ml of crude extract of carp intestinal homogenate. The suspension was stirred for one hour at 5 °C, DEAE cellulose was filtered off, washed with the starting buffer (5 x 250 ml) and resuspended in 250 ml of the same buffer containing 0.1 M NaCl. The first fraction with phosphatase activity was eluted by this way. The second elution was effectuated with the same buffer but contained 0.3 M NaCl and the second fraction was prepared in the similar way. The results are summarized in the Table I.

In the batch-wise preparation the alkaline phospatase is obtained in the both fractions in accordance with the preliminary chromatographic results.

I. Batchwise adsorption of alkaline phosphatase from carp intestinal homogenate on DEAE--cellulose

Step	Volume [ml]	Protein [mg/ml]	AP activity [μkat/l]	Specific activit [μkat/mg]
Extract	153	9.3	121.9	13.1
Breakthrough fraction	190	0.081	0.06	0.74
Fraction 1 (0.1 M NaCl)	170	0.35	20.5	58.6
Fraction 2 (0.3 M NaCl)	125	1.54	26.06	16.9

No activity was detected in the break-through fraction. In the first fraction, eluted with 0.1 M NaCl, 18.7% of total activity was obtained, in the second one (0.3 M NaCl) 17.5% of total activity was found. The total yield of the enzyme was 35.9%. Specific activity increases in the first fraction by the factor of 4.5, but in the second fraction by the factor 1.3 only. The both fractions from the electrophoretic pattern seem to be composed of two isoenzymes in different ratio. Similar situation was described by Magiea and Krull (1992) in the study of aggregation of alkaline phosphatase. Then, different aggregates are supposed to be separated by this way instead of the true isoenzymes.

The yield 35.9% in the first step of isolation is comparable with that usual in the separation of the same enzyme from intestinal mucose of mammals. This yield could be influenced by the fact, that all the intestine was used instead of mucosa giving higher concentration of fats and mucopolysaccharides in the extract. As the raw material is easily available waste, this yield is acceptable.

Dye-ligand chromatography

Dye-ligand sorbents, Iontosorb blue and Iontosorb red were packed to the columns of 1 cm ID and the total volume of 8.2 ml (Iontosorb blue) and 8.6 ml (Iontosorb red). The columns were equilibrated with the starting buffer, 10 mM Tris/HCl of appropriate pH containing 2 mM $MgCl_2$. In accordance with the well-known fact, that the adsorption on the dye-ligand sorbents is better at lower pH as well as the range of stability of the enzyme in the question, we have used the pH range from 6.0 to 8.0. The adsorption of the enzyme was not achieved in any pH used. Moreover, the denaturation of the enzyme takes place during the chromatography and, therefore, the lower specific activity is obtained after chromatography in comparison with the starting material. As the mammalian intestinal alkaline phosphatase is known to be very easily adsorbed on the dye-ligand sorbent and purified by the biospecific elution with phosphate (Kirchberger, Koppersläger, 1982), this result suggests that alkaline phosphatase of carp differs from that of warm-blooded animals.

Carp intestinal alkaline phosphatase separated by the batch-wise adsorption on DEAE-cellulose is simple, fast and gives acceptable yield. The specific activity obtained (58.6 μkat/mg) is comparable with that of the commercial preparation (SIGMA, SERVA, Koch-Light). The enzyme obtained is not physically homogeneous. Slight, but detectable traces of other proteins are seen in electrophoregrams.

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Received March 16, 1993

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Izolace intestinální alkalické fosfatasy kapra

Byl vypracován jednostupňový postup izolace alkalické fosfatasy ze střev kapra. Postup spočívá v extrakci homogenátu střev směsí 1-butano/Tris-HCl pufr pH 7,4 a vsádkové sorpci enzymu na DEAE-perlové celulose (Iontosorb DEAE). Čistota získaného enzymu byla ověřena stanovením specifické aktivity (58,6 μkat/mg) a elektroforeticky. Chromatografie takto získaného produktu na sorbentech s imobilizovanými barvivy (Iontosorb blue a Iontosorb red) nevedla ke zvýšení specifické aktivity.

ryby; kapr; alkalická fosfatasa; izolace; sorpce

Potrav. Vědy, *12*, 1994 (5) : 377-391

MECHANICAL PROPERTIES OF CANNED SWEET AND SOUR CHERRIES

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Mechanical properties of canned sweet and sour cherries have been determined for 23 varieties. A comparison between mechanical properties of raw and canned fruit varieties was made. Prevailing part of mechanical properties of canned sweet and sour cherries depends on the corresponding properties of the starting raw material. The canned fruits are always smaller than freshly harvested fruits. The skin of compressed canned fruits cracks at the compression strain which is comparable with that of freshly gathered fruits, provided that the compression strain of canned fruits is determined as the ratio of compression to their diameter in the state prior to canning. The compression of canned fruits initially proceeds at a lower loading of fruit. The extent of this softening depends on the concentration of substances dissolved in the cell juice. The initial power shape part of the compression curve is extended to higher deformations for canned than for freshly harvested fruits. The exponent value of the power relationship used for mathematical description of this part of the compression curve is higher for canned than for freshly gathered fruits. It means that the compression behaviour of canned fruits is more similar to the behaviour of skin ball filled with juice than to the behaviour of elastic ball body.

cherries; canning; mechanical properties

Our previous paper (Blahovec et al., 1993a) deals with mechanical properties of freshly harvested cherries with respect to their resistance to pressure damage and susceptibility to skin damage by mechanical gathering. It was found that fruit resistance to pressure damage was closely related to mechanical parameters characterizing the initial parts of the compression curve (tested between two plates), whereas skin damage was best documented in a separate free fall test. In addition to the above mentioned applications, compression testing between two plates also gives very usable data

concerning the dimensions of tested fruits. Simultaneously to the mentioned study additional experiments with cherry canned fruits were performed and the obtained results are the basis of this paper.

MATERIAL AND METHODS

In the experiments, 23 fruit varieties were used, the mechanical properties of which were investigated at harvest maturity (Blahovec et al., 1993a). Experimental material and characteristics are described by Blahovec et al., 1993a. All freshly gathered fruits were canned in the same way according to the following procedure: freshly gathered fruits were given into glass jars (volume 720 ml, Omnia lid type), bottles closed and sterilized in water bath having the temperature 20 minutes at 90 °C .

Storage temperature of canned fruit was in the range 5 ± 2 °C, while storage time was about three months from canning date. The dripped fruits of individual varieties were used for testing; their basic characteristics are supplemented with data given in Table I, where some other variables used in this paper for the description of fruits are also defined. The subscript c is systematically used for variables determined by measurement on canned fruits. Thus, the ratio d_c/d is the ratio of the mean value of diameter of canned fruits to the mean value of diameter of freshly gathered fruits (both mean values are related to the corresponding mutually different sets of selection). The dripped, non-cracked or otherwise undamaged fruits were deformed in the way described in the previous paper [quasi-static compression of the whole fruit between parallel plates, deformation rate 0.83 mm/s, measured force vs. compression strain $(x/d)_c$, e.g. so called compression curve, axis of the fruit parallel to both plates] (Blahovec et al., 1993a). Thirty fruits from each variety were tested by this method using the maximum compression strain (ratio x/d) value fifty percent. The experimental procedure and evaluation of variables were identical with those used earlier (Blahovec et al., 1993a). Unlike the freshly gathered fruits, the canned fruits were destemmed.

The following variables were obtained:

b_{1c} – the parameter of the regression relationship applied to the initial part of the compression curve, for $(x/d)_c$ less than 0.1,

$$F_c = a_{1c} + b_{1c}(x/d)_c \quad [1]$$

where: a_{1c} – further regression parameter that is out of our interest,

F_{ic} and $(x/d)_{ic}$ – the coordinates of the point of inflexion on the compression curve, i.e. the point where the slope of tangent to the compression curve is greatest,

b_{2c} – the parameter of the linear equation which is the tangent of the compression curve at the point of inflection,

$$F_c = a_{2c} + b_{2c}(x/d)_c \quad [2]$$

$(x/d)_{xc}$ – the compression strain at which the skin of the compressed fruits demonstrably starts to crack.

The compression curve is replaced between its origin and the respective point of inflection with the power relationship,

$$F_c = a_{3c}(x/d)_c^{k_c} \quad [3]$$

where: variables a_{3c} and k_c – determined by the least square method from the logarithmically transformed relationship [3]

variable $F_{0.1}$ – the calculated value of F_c from Eq. [3] for the compression strain $(x/d)_c = 0.1$

The cracking of fruit skin at different spots of the surface is evaluated by means of special variables which represent the frequency of crack occurrence at different places of the fruit given in percent. If damage occurs at a certain place of the fruit in more than 50% of cases, it is stated that the variety is damaged prevailingly in this way and the damages of type 1 (near the stem), type 2 (at the fruit tip) and type 3 (on fruit side) is concerned.

RESULTS

The results of experiments are summarized in Tables I–IV. The tables contain the mean values of determined variables, whereas variance of the measured values for individual variables is very similar to the variance of these variables for freshly gathered fruits (Blahovec et al., 1993a). As for the important dimension data in Table I, the ratio of average diameters of fruits before canning and of canned fruits must be included among them (in

I. Basic characteristics (mean values) of the tested varieties harvested in 1990 and/or 1989 (*)

Sort type and variety		Harvested fruits dimensions		Ratios	
		diameter [mm]	mass [g]	raw mass flesh / total	diameter canned / raw
Sweet cherries *(Prunus avium* L.*)*					
„heart" var. *juliana* L.	Karešova	20.0	6.21	0.924	–
	*	19.2	5.98	0.923	0.954
	Kaštánka	18.2	4.38	0.922	–
"semihard" (var. *duracina* L. x var. *juliana* L.)	Burlat	20.1	6.71	0.940	0.930
	Büttner	18.3	3.86	0.904	0.907
	Frühe Rote Meckenheimer	20.9	7.10	0.934	0.890
	Lambert	20.2	5.62	0.904	0.851
	Moreau*	21.2	5.95	0.904	0.943
"hard" (var. *duracina* L.)	Granát	18.3	4.99	0.932	0.929
	Kordia	19.5	5.65	0.926	0.918
	*	21.1	6.41	0.927	0.919
	Napoleonova	18.5	4.83	0.918	0.886
	*	21.1	6.44	0.929	0.924
	Starking Hardy Giant	21.3	5.02	0.898	0.897
	Schneiderova (Thurn-Taxis)	21.5	7.42	0.930	0.884
	Sam	19.4	5.47	0.914	0.897
	Stella	23.1	8.70	0.938	0.892
	Stella Compact	21.7	6.46	0.933	0.820
	Těchlovická*	20.5	6.50	0.926	0.912
	Van	19.8	5.28	0.934	0.924
Sour cherries *(Prunus cerasus* L. subsp. *eucerasus* A. GR., var. *austera* L.*)*					
	Érdi bőtermő	19.4	5.15	0.911	0.891
	Fanal	18.3	4.74	0.886	0.951
	Kőrösi	19.2	4.34	0.878	0.880
	Montmorency*	19.6	5.35	–	0.929
	Morellenfeuer	16.2	3.98	0.905	0.938
	North Star	16.4	4.22	0.917	0.927
	*	16.0	3.42	–	0.944
	Záhoračka	17.5	3.70	0.876	0.886
	*	17.4	3.16	–	0.822

II. The obtained parameters of compression testing of canned fruits between two plates at constant strain rate

Variety	Slope of the curve [N]		Inflex		Rupture strain
	initial	inflex.	force [N]	strain **	**
Sweet cherries					
Karešova*	15.9	40.0	5.5	0.243	0.497
Burlat	17.5	104.0	12.5	0.365	0.498
Büttner	20.0	78.2	10.6	0.233	0.500
Frühe Rote Mecken.	17.5	75.1	10.0	0.278	0.472
Lambert	17.5	94.3	16.3	0.390	0.444
Moreau*	17.7	61.0	8.4	0.266	0.465
Granát	15.4	55.3	7.7	0.278	0.454
Kordia	21.6	177.0	29.0	0.373	0.432
*	19.8	100.6	17.7	0.334	0.460
Napoleonova	17.1	80.9	13.1	0.309	0.477
*	17.8	70.9	10.9	0.286	0.444
Starking Hardy Giant	19.1	111.0	18.6	0.326	0.432
Schneiderova	18.2	132.0	21.3	0.379	0.420
Sam	21.2	108.0	19.5	0.353	0.501
*Stella	15.6	58.2	9.7	0.313	0.474
Stella Compact	17.5	120.0	19.8	0.348	0.491
*Těchlovická	17.5	73.8	12.5	0.335	0.470
Van	18.4	145.0	22.7	0.312	0.449
Sour cherries					
Érdi Bötermö	11.1	33.7	4.7	0.347	0.455
Fanal	12.1	43.1	5.9	0.375	0.515
Kőrösi	13.5	49.1	6.3	0.345	0.497
*Montmorency	12.6	54.4	7.7	0.355	0.436
Morellenfeuer	10.9	42.7	6.5	0.380	0.541
North Star	11.4	29.6	4.9	0.309	0.459
*	11.8	40.3	6.8	0.400	0.452
Záhoračka	11.4	43.7	6.2	0.381	0.503
*	10.9	51.2	7.6	0.425	0.490

* denotes 1989 results

** strain is defined as dimensionless ratio of deformation and initial diameter of the deformed fruit

III. Mean values of parameters in the power relationship [3] obtained by the regression analysis of the initial parts of compression curves. $F_{0.1c}$ is compression force calculated from Eq. [3] for compression strain $(x/d)_c = 0.1$ and parameters a_{3c} and k_c given in this table.

Variety	Regression parameters of eq. [3]		$F_{0.1c}$ [N]
	a_{3c} [N]	k_c	
Sweet cherries			
*Karešova	101.0	2.04	0.92
Burlat	477.7	3.30	0.24
Büttner	221.6	2.12	1.73
Frühe Rote Meecken.	228.9	2.43	0.85
Lambert	171.5	2.48	0.57
Moreau*	179.8	2.85	1.01
Granát	150.6	2.32	0.72
Kordia	328.0	2.50	1.04
*	169.8	2.06	1.48
Napoleonova	164.4	2.14	1.19
*	146.4	2.07	1.25
Starking Hardy Giant	190.8	2.08	1.59
Schneiderova	263.1	2.60	0.66
Sam	148.1	1.97	1.59
*Stella	113.4	2.13	0.84
Stella Compact	247.7	2.39	1.01
*Těchlovická	141.5	2.20	0.89
Van	309.9	2.33	1.45
Sour cherries			
Érdi Bőtermő	98.0	2.81	0.15
Fanal	139.9	3.20	0.09
Kőrösi	190.3	3.17	0.13
*Montmorency	178.5	2.97	0.19
Morellenfeuer	190.5	2.88	0.15
North Star	67.8	2.21	0.42
*	96.1	2.87	0.13
Záhoračka	141.7	3.15	0.10
*	164.6	3.47	0.06

* denotes 1989 results

IV. Localization of skin damage to canned fruit after compression (strain about 50 percent) between two plates

Variety	Fraction of fruits in %			Prevailing type	
	type 1	type 2	type 3	canned	fresh
Sweet cherries					
Karešova*	0.0	15.3	84.6	3	1
Burlat	24.4	24.4	51.2	3	3
Büttner	42.5	20.0	37.5	0	1
Frühe Rote Mecken.	23.8	16.7	59.5	3	1
Lambert	32.7	25.0	42.3	0	3
* Moreau	66.7	19.4	13.9	1	1
Granát	54.3	17.1	28.6	1	2
Kordia	52.9	5.9	41.2	1	1
*	85.7	5.7	8.6	1	1
Napoleonova	41.2	23.5	35.3	0	2
*	91.4	5.7	2.9	1	1
Starking Hardy Giant	49.2	27.1	23.7	0	1
Schneiderova	44.9	4.1	51.0	3	1
Sam	34.2	26.3	39.5	0	1
*Stella	58.8	35.8	5.9	1	0
Stella Compact	32.4	29.6	47.0	0	3
*Těchlovická	29.0	54.8	16.2	2	2
Van	63.8	27.7	8.5	1	1
Sour cherries					
Fanal	16.7	33.3	50.0	3	0
Kõrõsi	46.0	4.0	50.0	3	2
*Montmorency	96.9	0.0	3.1	1	0
Morellenfeuer	0.0	40.0	60.0	3	3
North Star	28.9	31.6	39.5	0	2
*	93.8	0.0	6.2	1	0
Záhoračka	27.3	11.4	61.4	3	3
*	93.0	0.0	7.0	1	3

Classification of the observed cracks into types: 1 – near fruit stem, 2 – near fruit tip, and 3 – on fruit side. Mark 0 is used when the prevailing type of fruit damage cannot be determined, * denotes 1989 results

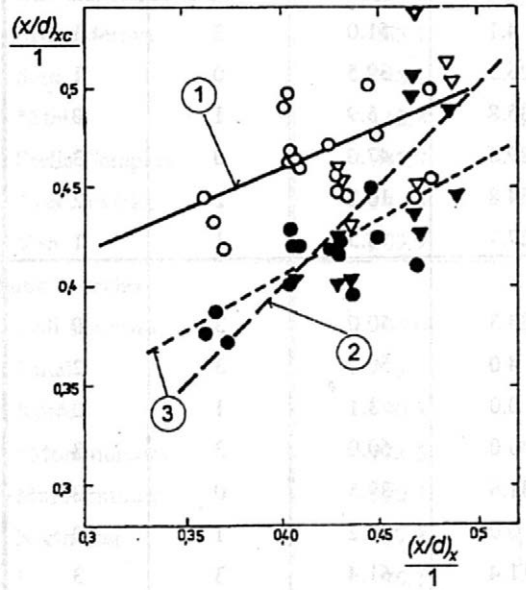
both cases different selection sets are concerned). This ratio assumes the values in the range 0.882–0.954 and shows that all the sweet cherry and sour cherry fruits shrink during canning and subsequent storage.

Table II presents the values of basic variables of the compression test and Table III contains the parameters related to the power description of compression in the first part of compression curve – see equation [3]. The data on the character of fruit skin damage in the compression test are given in Table IV.

DISCUSSION

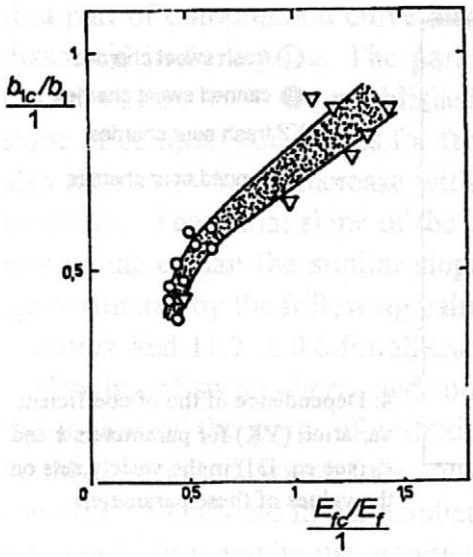
Differences between varieties as can be seen from differences between measured variables are apparent from Tables I–IV. There are large differences between some parameters and reader can find them for special varieties which are interesting for him.

But, our aim was to find general relationships between variables which can be utilized for prediction of mechanical properties of a broad family of varieties from limited number of most important mechanical parameters.



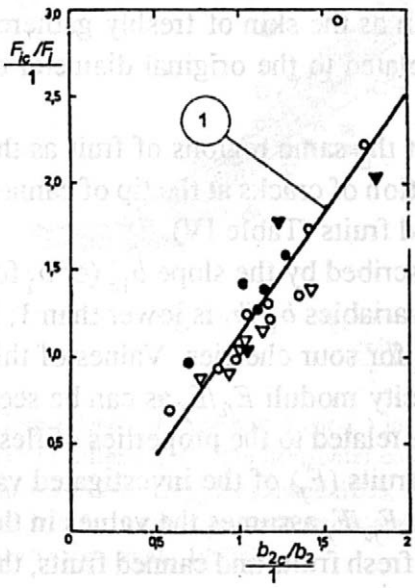
① $(x/d)_{xc} = 0.294 + 0.41(x/d)_x$
$r = 0.49$
② $(x/d)_{xc} = (x/d)_x$
③ $(x/d)_{xc} \cdot d_c/d = 0.174 + 0.584\,(x/d)_x$
$r = 0.67$

1. Dependence of compression strain of canned fruits at which the skin cracks – $(x/d)_{xc}$ on the same variable for freshly gathered fruits – $(x/d)_x$ (o – sweet cherries, ▽ – sour cherries, plotted values are the mean variety values). Filled marks denotes the following variable: $(x/d)_{xc}{}^*(d_c/d)$, where d_c is the mean diameter of canned fruits and d is the mean diameter of the gathered fruits of the same variety



2. Mutual dependence of the ratios of variables measured in canned fruits and freshly gathered fruits: the slope of initial parts of compression curves (b_{1c}/b_1) and elasticity moduli (E_{fc}/E_f) of flesh determined by penetration of a cylinder with a flat face (Blahovec et al., 1993b)

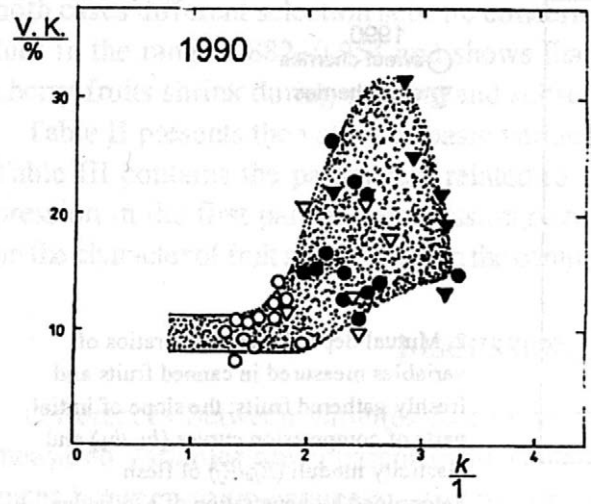
Relationships between different parameters are very close to the relationships between variables found in freshly gathered fruits (Blahovec et al, 1993a). Also the corresponding variables measured in freshly gathered fruits, and in canned fruits are closely related. The following pairs of variables exhibited especially close relationships: d–d_c, b_1–b_{1c}, b_2–b_{2c}, k –k_c, and $F_{0.1}$–$F_{0.1c}$. The diameter of canned fruits is always smaller than the diameter of freshly gathered fruits. As follows from Fig. 1, the relative com-



sweet cherries
1990
1989
sour cherries
1990
1989

① $F_{ic}/F_i = -0.287 + 1.409\ (b_{2c}/b_2)$
$r = 0.847$

3. Mutual dependence of the ratios of variables measured at compression between two plates in canned and freshly gathered fruits: force in the point of inflexion of compression curve (F_{ic}/F_i) and the slope of compression curve in the point (b_{2c}/b_2)



4. Dependence of the of coefficient variation (VK) for parameters k and k_c (see eq. [3]) in the variety sets on the values of these parameters

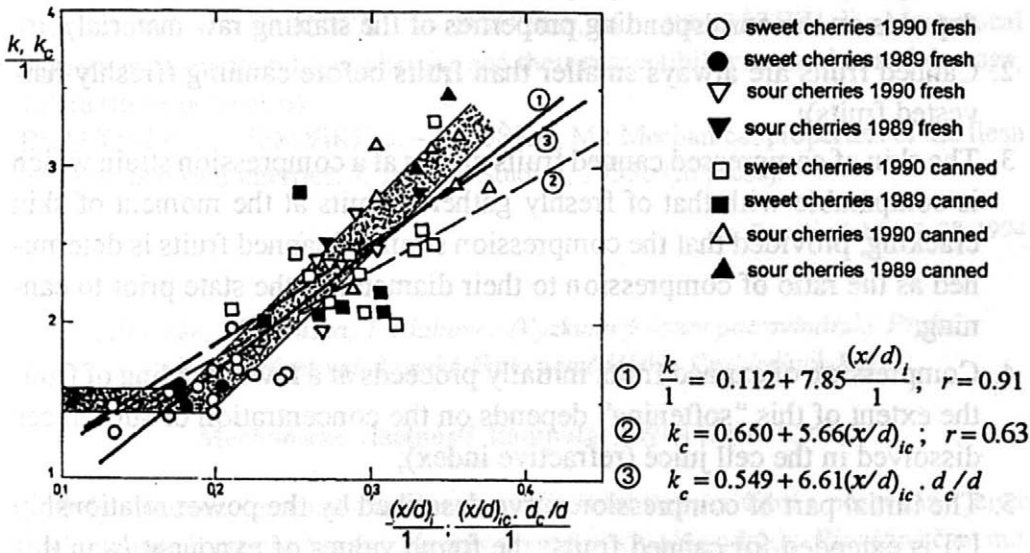
pression of canned fruits at which the skin begins to crack is higher than that of freshly gathered fruits. One of the reasons of this phenomenon may be the decreasing inner volume of fruits during canning. This decrease in fruit volume is manifested by the calculation of relative variables having the fruit diameter in the denominator – $(x/d)_{ic}$ and $(x/d)_{xc}$. By multiplying the variable $(x/d)_{xc}$ by the ratio d_c/d, we obtain an estimate of the mean value of variable x_{xc}/d (Fig. 1), which has the values very close to the corresponding values $(x/d)_x$. The values modified in this way (solid signs in Fig. 1) occur near the line $(x/d)_{xc} = (x/d)_x$. Consequently, it seems that the skin of canned fruits cracks at about the same compression strain as the skin of freshly gathered fruits provided the compression strain is related to the original diameter of fruits (at their gathering).

The skin of canned fruits cracks in about the same regions of fruit as the skin of freshly gathered fruits, only the fraction of cracks at the tip of canned fruits is smaller than that of freshly gathered fruits (Table IV).

The first part of compression curve is described by the slope b_{1c} (or b_1 for freshly gathered fruits). The ratio of these variables b_{1c}/b_1 is lower than 1; it is about 0.5 for sweet cherries and 0.7–0.9 for sour cherries. Values of this ratio depend on the ratio of fictitious elasticity moduli E_{fc}/E_f as can be seen from Fig. 2. The above elasticity moduli are related to the properties of flesh of canned fruits (E_{fc}) and freshly gathered fruits (E_f) of the investigated varieties (Blahovec et al, 1993b). The ratio E_{fc}/E_f assumes the values in the range 0.4–1.5 for these varieties. With both fresh fruits and canned fruits, the

first part of compression curve also depends on the content of components dissolved in fruit juice. The parallel study of the juice refractive index (Borovská, 1990 – unpublished) revealed that the values of the initial slope of compression curves for fresh fruits (b_1) and canned fruits (b_{1c}) and also their ratio b_{1c}/b_1 increase with the increasing refractive index of fresh fruit juice. The initial slope of the compression curve for canned cherries is less variable than the similar slope for freshly gathered fruits; b_{1c} can be approximated by the following values: 18.2 ± 1.2 for all tested sweet cherry varieties, and 11.7 ± 0.6 for all tested sour cherry varieties.

Canning of sweet cherry and sour cherry fruits mostly causes changes in the region of the point of inflexion (Fig. 3). The increase in strength is mainly concerned, which may sometimes be considerable and is obviously caused by a decrease in the number of cracks emanating from the flesh into the skin of fruit and by the growing role of skin for fruit stability.



5. Dependence of exponent k (or k_c) in the relationship (3) on the compression strain in the point of inflexion. On the horizontal coordinate axis, the variable $(x/d)_i$ is plotted for freshly gathered fruits, $(x/d)_{ic}$ for canned fruits, and $(x/d)_{ic}.d/d_c$ for the expression plotted as number 3. The data for freshly gathered fruits and the hatched area are taken from the previous paper (Blahovec et al., 1993a)

The whole first part of compression curve of cherries can be described by expression [3]. However, the variance of obtained values of empirical parameters a_3 (a_{3c}) and k (k_c) increases with the increasing value of coefficient k or k_c, as it is shown in Fig. 4 for the second case. The coefficient of variation for variable k is in the range 8–11% for k smaller than about 1.6, whereas for k in the range 2–3 it amounts to 10–30%. This behaviour may have various causes. One of the causes can be the increase in values (x/d), and $(x/d)_{ic}$ with the increasing value of k and k_c, respectively, and vice versa - Fig. 5 and the preceding paper (Blahovec et al., 1993a). With the growing extent of compression strain for which the best agreement between experimental values and the relationship [3] should be obtained, the increase in variance of the obtained values a_3 and k, or a_{3c} and k_c, for canned fruits may occur as well as the increase of the corresponding coefficients of variation .

CONCLUSIONS

The following facts concerning the mechanical properties of canned sweet and sour cherries were ascertained by the analysis of experimental results:

1. A majority of mechanical properties of canned sweet and sour cherries depends on the corresponding properties of the starting raw material;
2. Canned fruits are always smaller than fruits before canning (freshly harvested fruits);
3. The skin of compressed canned fruits cracks at a compression strain which is comparable with that of freshly gathered fruits at the moment of skin cracking, provided that the compression strain of canned fruits is determined as the ratio of compression to their diameter in the state prior to canning;
4. Compression of canned fruits initially proceeds at a lower loading of fruit, the extent of this "softening" depends on the concentration of substances dissolved in the cell juice (refractive index);
5. The initial part of compression curve described by the power relationship [3] is extended for canned fruits; the found values of exponent k_c in this relationship are higher than in freshly gathered fruits (k);
6. The slope of compression curve near the point of inflexion is always higer for canned fruits than for freshly gathered fruits.

List of symbols

a_{1c}	parameter of the eq. [1] for canned fruit [N]
a_{2c}	parameter of the eq. [2] for canned fruit [N]
a_{3c}	parameter of the eq. [3] for canned fruit [N]
b_{1c}	parameter of the eq. [1] for canned fruit [N]
b_{2c}	parameter of the eq. [2] for canned fruit [N]
d_c	diameter of the canned fruit [mm]
d	diameter of the fresh fruit [mm]
F_c	force (here measured at compression of canned fruit) [N]
k_c	exponent in eq. [3] valid for canned fruit [–]
x	deformation [mm]

Indexes

i	at inflexion point
c	canned
x	at skin crack
0.1	at deformation 10%

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Received March 28, 1994

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Mechanické vlastnosti kompotovaných třešní a višní

Byly stanoveny mechanické vlastnosti 23 odrůd třešní a višní a porovnány jejich hodnoty naměřené pro čerstvé a kompotované plody téže odrůdy. Převážná část mechanických vlastností čerstvých plodů koreluje s hodnotami naměřenými na plodech kompotovaných. Kompotované plody jsou vždy menší než čerstvé. Prasknutí slupky stlačovaných kompotovaných plodů nastane při deformaci srovnatelné s deformací, při níž praskají plody čerstvé, za předpokladu, že vyjádříme deformaci jako poměr

skutečného stlačení plodu a jeho velikosti v čerstvém stavu. Stejné deformace lze u kompotovaných plodů dosáhnout menší silou než u plodů čerstvých. Stupeň tohoto změknutí závisí na koncentraci substancí rozpuštěných v buněčné šťávě. Náběhovou část zatěžovací křivky lze popsat mocninovou závislostí. U kompotovaných plodů platí mocninová závislost do větších deformací než u plodů čerstvých. Exponent této závislosti je větší u kompotovaných plodů než u plodů čerstvých. Z toho plyne, že se kompotované plody mnohem více blíží deformačnímu chování tenkostěnných míčů vyplněných tekutinou.

Plody byly kompotovány v 720ml skleněných lahvích s víčky typu Omnia. Plody byly umístěny do lahví a zality cukerným roztokem (1,5 kg cukru na 1 litr vody) a sterilovány ve vodní lázni o teplotě 90 °C po dobu 20 minut. Skladování probíhalo v lednici o teplotě 5 °C po dobu cca tří měsíců. Poté byly plody ponechány odkapat a měřeny jejich mechanické vlastnosti při stlačování mezi dvěma deskami přístroje Instron 1140 (rychlost desek 0,83 mm/s).

K popisu počáteční fáze zatěžovací křivky byla použita rovnice [1]. K popisu hlavní přímkové části (ukončené inflexním bodem na křivce) byl použit vztah [2]. K popisu mocninové části zatěžovací křivky od počátku do hlavní přímkové části sloužila rovnice [3].

Výsledky stanovení rozměrů plodů jsou uvedeny v tab. I, kde jsou rovněž uvedeny poměry veličin k plodům čerstvým. V tab. II jsou uvedeny vybrané parametry vztahů [1] a [2], včetně síly a deformace v inflexním bodě a deformace v době prasknutí slupky při stlačování. V tab. III jsou uvedeny parametry mocninové závislosti [3] a vypočtené hodnoty síly pro 10% deformaci. Ze všech těchto hodnot je možné činit závěry o srovnání odrůd z hlediska jejich pevnosti po kompotování.

V tab. IV jsou uvedeny údaje o procentuálním zastoupení prasklin při stlačování v různých místech plodů jak kompotovaných, tak (pro veličinu – převažující typ prasklin) čerstvých. Závislosti mezi vybranými parametry kompotovaných a čerstvých plodů jsou uvedeny v obr. 1 až 3. V obr. 4 je uveden variační koeficient exponentu k jako funkce hodnoty k. Pro rostoucí hodnoty k variační koeficient roste.

Závěry:

1. Převážná část mechanických vlastností čerstvých plodů koreluje s hodnotami naměřenými na plodech kompotovaných.
2. Kompotované plody jsou vždy menší než čerstvé.
3. Prasknutí slupky stlačovaných kompotovaných plodů nastane při deformaci srovnatelné s deformací, při níž praskají plody čerstvé za předpokladu, že vyjádříme deformaci jako poměr skutečného stlačení plodu a jeho velikosti v čerstvém stavu.
4. Stejné deformace lze u kompotovaných plodů dosáhnout menší silou než u plodů čerstvých. Stupeň tohoto změknutí závisí na koncentraci substancí rozpuštěných v buněčné šťávě.

5. Náběhovou část zatěžovací křivky lze popsat mocninovou závislostí. U kompotovaných plodů platí mocninová závislost do větších deformací než u plodů čerstvých. Exponent této závislosti je větší u kompotovaných plodů než u plodů čerstvých.

6. Sklon zatěžovací křivky v blízkosti inflexního bodu je téměř vždy vyšší u kompotovaných než u čerstvých plodů, totéž platí i o síle v inflexním bodě (obr. 3).

třešně; višně; kompotování; mechanické vlastnosti

NEKROLOG

Za ing. Miloslavem R u t e m, CSc.

Koncem července nás zastihla smutná zpráva. Uprostřed činorodé práce ve věku nedožitých 58 let zemřel na následky zranění po autonehodě ing. Miloslav R u t, CSc., vedoucí oddělení mikrobiálních produktů Výzkumného ústavu potravinářského Praha.

Ing. Miloslav R u t se narodil 19.10.1936. Po studiích na Střední průmyslové škole potravinářské technologie a Vysoké škole chemickotechnologické, obor kvasná chemie, nastoupil v roce 1961 do lihovaru v Kralupech nad Vltavou. Ve Výzkumném ústavu potravinářském byl zaměstnán od roku 1963 a pracoval na problematice výroby mikrobiálních enzymů. Díky svým odborným znalostem a organizačním schopnostem byl vybrán jako expert pro práci v zahraničí a v letech 1965 až 1967 působil na Kubě.

Ani jemu se nevyhnuly nepříjemnosti v době normalizace v 70. letech a v jejich důsledku přešel v roce 1971 do Výzkumného ústavu krmivářského průmyslu a služeb v Pečkách, kde pracoval v pražském oddělení mikrobiálních výrob na problematice výroby kvasničních bílkovin ze syntetického etanolu. Největších úspěchů dosáhl v oblasti materiálových bilancí procesu v úpravě kvasnic pro potravinářské využití a při automatizaci a regulaci potravinářských procesů.

V roce 1983 byl s celým oddělením převeden zpět do Výzkumného ústavu potravinářského v Praze, kde pokračoval v řešení rozpracované problematiky. Ing. Miloslav R u t, CSc., patřil mezi přední vědecké pracovníky v oblasti syntézy mikrobiálních bílkovin s výraznými schopnostmi aplikace získaných poznatků v průmyslové praxi.

Byl autorem a spoluautorem několika desítek odborných článků a přednášek, 14 patentů a autorských osvědčení. V poslední době vedl výzkumný projekt *Deficity stopových prvků ve výživě a jejich saturace dietetiky mikrobiálního původu* a měl velký podíl na výzkumu a zavedení výroby dietetika s organicky vázaným chromem pro prevenci cukrovky.

Odchodem ing. Miloslava R u t a, CSc., ztratil Výzkumný ústav potravinářský významného pracovníka a zaměstnanci ústavu dobrého přítele.

Spolupracovníci VÚP

Potrav. Vědy, *12*, 1994 (5) : 393-406

DISTRIBUTION AND PROFILES OF LOCAL TEMPERATURES IN HOMOGENEOUS LIQUID MATERIALS DURING MICROWAVE HEATING*

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Microwave heating has many advantages over conventional heating, but the process itself is compicated. Not only the heating rate the temperature distribution in the heated material is different in the microwave and conventional heating. The problems with temperature uniformity are known from practical using of microwave ovens. Many factors influence the temperature distribution in the food heated by microwaves including microwave field patterns in the oven cavity, food and package geometry and size, food composition and physical properties, temperature. Because of complexity of its influence, the design of optimal parameters for microwave heating presents a serious problem. The fluoroptic thermometric system LUXTRON 755 was used for monitoring of local temperatures history in four different liquid materials heated by microwaves in flat cylindrical glas containers (dia 185 mm). The household oven MOULINEX 850 W was used for experiments. The experiments were made with heating of 1 000 ml samples of potable water, solution of NaCl (6% weight), mixture of water and dried mashed potatoes (13% dry matter) and sunflower oil. The experimental transiet temperature profiles for different places inside the heated materials, the axial and radial temperature profiles in heated materials as results of this study are shown and discussed. The comparison with recent results of other autors and microwave heating theory is carried out too.

microwave heating; temperature distribution; heating uniformity; temperature profiles

* The work was supported financially by the Czech Ministry of Economy (project No Z 606) and Czech Ministry of Agriculture (project No A 0935 0120).

The temperature distribution and the rate of temperature change in materials during their heat treatment determine both the quality of the final product and the economy of the heating process.

Microwave heating differs from the classic procedures especially in the rate of temperature increase. The speed of heating that represents the largest advantage of this method is also in relation with the mechanism of this process.

The classic heat transfer from the external source through the surface of the heated material is in the microwave (MW) procedure substituted by heat generation directly in the mass of the heated substance. The heat generation and the subsequent temperature rise occur within the whole volume of the material into which the mocrowaves can penetrate.

The heat generation throughout the sample volume causes – jointly with other specific properties of MW – that the heat distribution within the substance differs from that typical of the classic methods of heating.

Whereas in the classic heating procedures maximum temperatures can be expected on the surface of the heated substance and minimum temperatures in its middle, a quite different situation can occur durring MW heating if the effects of composition, physical properties, shape and size of the heated substance are suitably adjusted. Even a completely uniform heating, which is impossible by the conventional methods, can be achieved by MW also in solid materials.

Complex geometry, dimensions, composition and also the heterogeneity of most food materials and products with the mechanisms of microwave heating and larger or smaller nonhomogeneity of the microwave field in real equipments sometimes, however, result in an opposite phenomenon, i.e. the heating is nonuniform. Such heating is considered a cause of the possible negative effect of MW on the quality and hygienic safety of foods processed in MW equipments (Ohlsson, 1990; Burfoot, Foster, 1992; Dealer et al., 1992).

Obtaining of exact data on the course and distribution of temperatures during MW heating was hindered by technical problems connected with temperature measurement in the MW (electromagnetic) medium. A significant improvement was reached after the introduction of thermometric systems applying optical fibres (Houšová, Topinka, 1993) that represent a useful tool for the investigation of the course and effect of MW heating.

This paper brings the results of the first part of experimental study during which the distribution and course of temperatures were monitored during

MW heating of different food materials and products by means of the fluoroptic measuring system LUXTRON 755. The whole investigation was performed with the financial assistence of the Ministry of Economy and Ministry of Agriculture of the Czech Republic. The results outlined here deal with MW heating of liquid materials with different viscosity and chemical composition treated in a container of cylindrical shape.

MATERIAL AND METHODS

Experimental equipment

MW oven for households MOULINEX FM 2915 Q, declared output 850 W, calibrated output 890 W (IEC 705), frequency 2 450 MHz, cavity volume 24 l, without the turntable (removable glass shelf), five basic output degrees, four intersteps.

Material

Potable water, NaCl solution in potable water (6 mass %), sunflower oil (Lukana, Manufacturer: Milo Olomouc), mixture of potable water and dried mashed potatoes (Starch Works Bohdalov) prepared by mixing of 300 g dried mashed potatoes, 1 200 ml boiling potable water and 800 ml of water with temperature of 20 °C. After adding of the mashed potatoes and mixing the temperature was adjusted to approximately +5 °C.

Chemical composition of the mashed potatoes is given in Table I, physical properties of the model materials are shown in Table II (according to literature or measurements performed).

Container for heating: glass container 185 mm inner diameter, 69 mm high, 2 mm wall thickness and mass 0,424 kg.

I. Composition of mashed potatoes according to the chemical analyses

Dry [%]	Proteins [%]	Chlorides [%]	Fats [%]	Glycides [%]
13.06	1.59	0.04	0.06	11.12

Measuring devices

Temperature: LUXTRON 755 (Luxtron Corp., California, USA), four probes type MIW 2 m, working with PC by means of a series port RS 232.

II. Physical properties of used materials

Material	Temperature [°C]	Density [kg/m^3]	Specific heat capacity [J/kgK]	Heat conductivity [W/mK]	ϵ' [1]	ϵ'' [1]	Penetration depth [m]	Source
Water	20	998	4 180	0.598	78	12.74	0.0135	1
	80	972	4 199	0.669	62.5	4.92	0.0313	1
Mashed potatoes	20	1 035	3 800	0.45	65	22.7	0.007	2, 3
	50	1 035	3 800	0.45	72	28.0	0.006	2, 4
Sunflower oil	20	916	1 930	0.166	2.49	0.18	0.170	3, 5
	80	876	2 110	0.151	2.56	0.185	0.168	5
Solution NaCl 6%	20	1 041	3 890	0.593	60.5	7.0	0.0216	1, 5

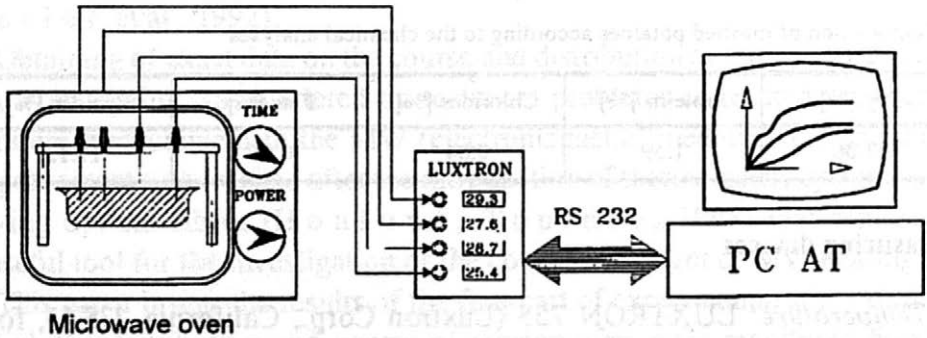
1 = Rogov, 1981; 2 = Ambros, 1993; 3 = Kent, 1987; 4 = Datta et al., 1992; 5 = Šesták et al., 1993

A plexiglass plate with holes and special devices for the fixation of probe location were used for the installation of the probes. During the major part of experiments the plate was positioned on the upper part of the container. The scheme of the measuring equipment and of the probe installation is shown in Fig. 1.

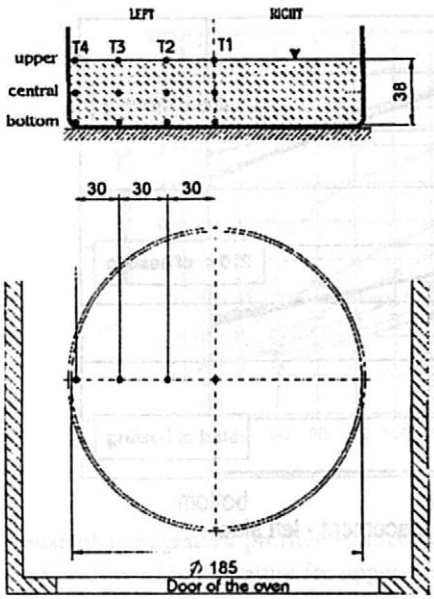
Mass: The balance OWA with upper scale with capacity up to 5 kg (OWA Labor).

Time: Digital stop-watch DS 35 (Pragotron)

Chemical analysis of the mashed potatoes: Dry matter, fat, protein, chlorides and ash were determined, the content of glycides was calculated to 100% (Krtička, 1993).



1. Temperature measurement set-up with LUXTRON 755



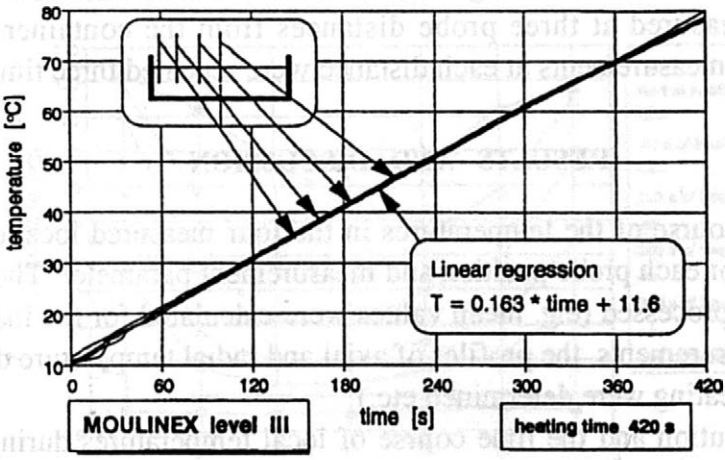
2. LUXTRON probes locations inside of materials heated in cylindrical glass container, placement of the container in the oven

Testing procedures: The respective voluminal amount of the model material (1 000, resp. 1 500 and 500 ml) was placed into the glass container for heating. The layer height of 1 000 ml was 38 mm.

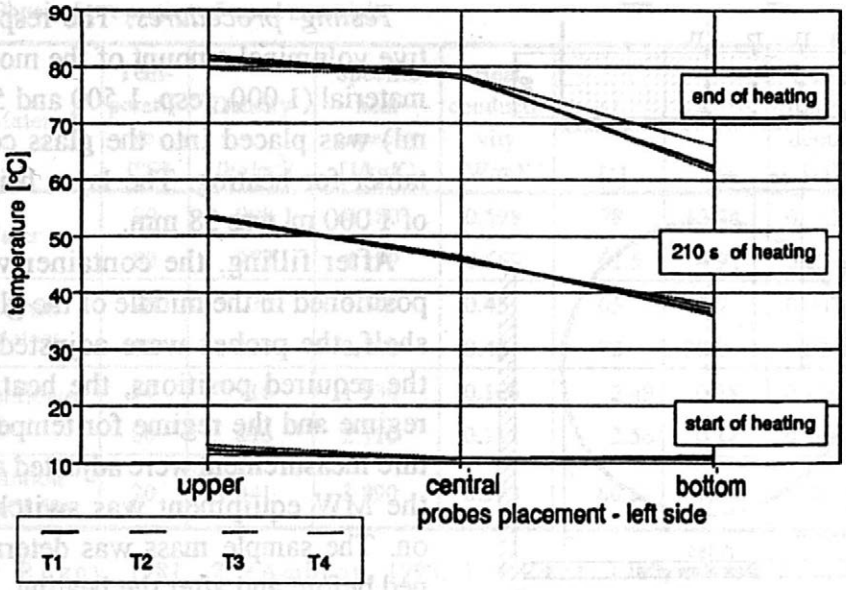
After filling, the container was positioned in the middle of the glass shelf, the probes were adjusted to the required positions, the heating regime and the regime for temperature measurement were adjusted and the MW equipment was switched on. The sample mass was determined before and after the heating.

The majority of tests was performed at the full input (850 W), some experiments were perfomed at lower input (degree I, 567 W).

The time of the heat treatment depended on the material treated and was chosen in the range 240 sec (1 000 ml of oil) to 720 sec (1 500 ml water). The highest temperatures obtained reached 70–90 °C.



3. Experimental transient local temperature profiles at central layer of water (sample 1 000 ml, probes in left side of container)



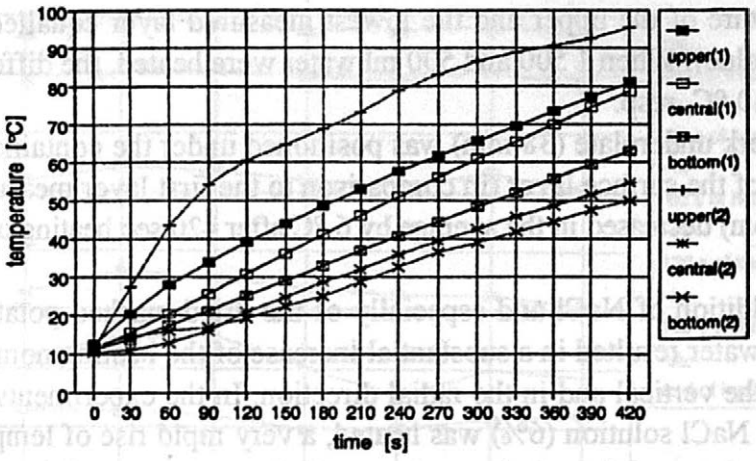
4. Axial temperature profiles during microwave heating of 1 000 ml water (probes on left side of container)

The temperature was simultaneously measured at four locations with probe ends always at the same distance from the container bottom. The holes for probe installation were located in a straight line parallel to the door plane, the probe distance was 30 mm, the distance of the marginal probes (T 4) from the container perimeter was 2,5 mm (Fig. 2). In the majority of tests the probes were installed in the left half of the container, some measurements were carried out in the right half as well. The temperatures were successively measured at three probe distances from the container bottom (Fig. 2). The measurements at each distance were repeated three times.

RESULTS AND DISCUSSION

The time course of the temperatures in the four measured locations was ascertained for each probe position and measurement parameter. The results were further processed (e.g. mean values were calculated for the individual repeated measurements, the profiles of axial and radial temperature distribution during heating were determined etc.)

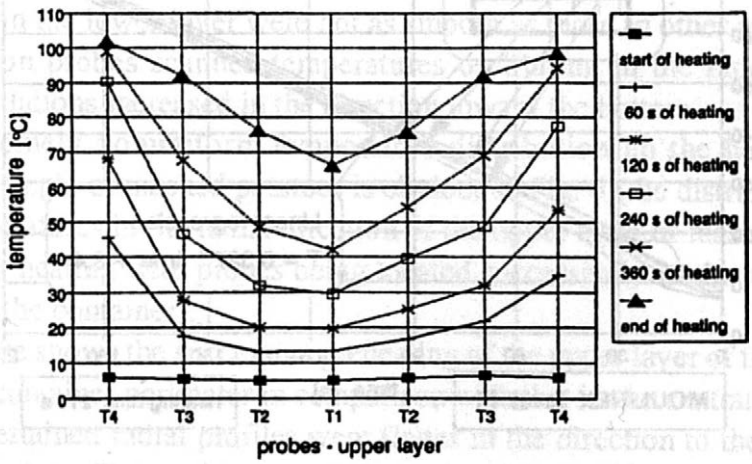
The distribution and the time course of local temperatures during water (1 000 ml) heating by MW in the flat cylindrical container is shown in Fig. 3 (the time course of the four local temperatures in the middle of the water



5. Transient temperature profiles – microwave heating of 1 000 ml water and NaCl solution (average values of temperature for upper, central and bottom layers was used)

layer in the left half of the container). The distribution of water temperatures in the vertical direction at the start of measurement, during heating and at the end of heating (7 min) is shown in Fig. 4.

The tests showed that the temperature distribution in water was fairly uniform in the radial direction (the measurements in the individual planes differed in the range 1–5 °C). An increase in water temperature in the container in the direction from the bottom to the surface was regularly repeated. After 420 sec of heating 1 000 ml water the difference between the mean



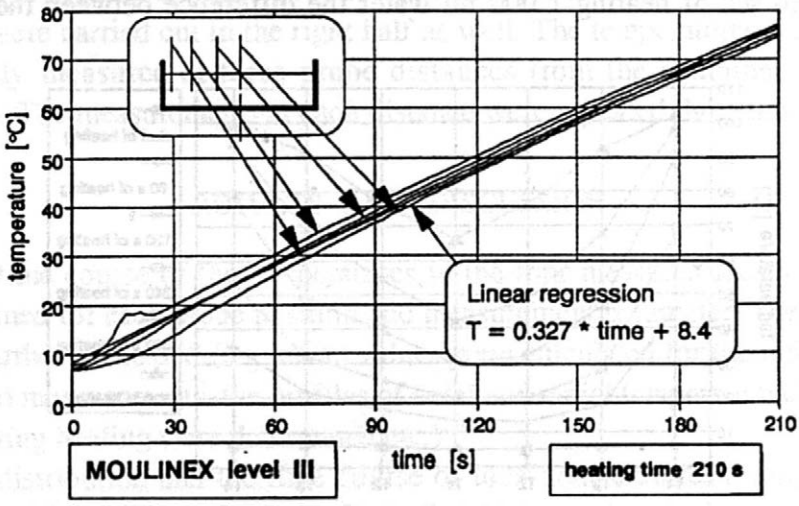
6. Radial temperature profiles – microwave heating of 1 000 ml mashed potatoes (probes in upper layers, left and right sides of container, heating time 420 s)

temperature of the upper and the lowest measured layer equalled to 20 °C (mean value). When 1 500 and 500 ml water were heated, the difference was 23 and 10 °C, resp.

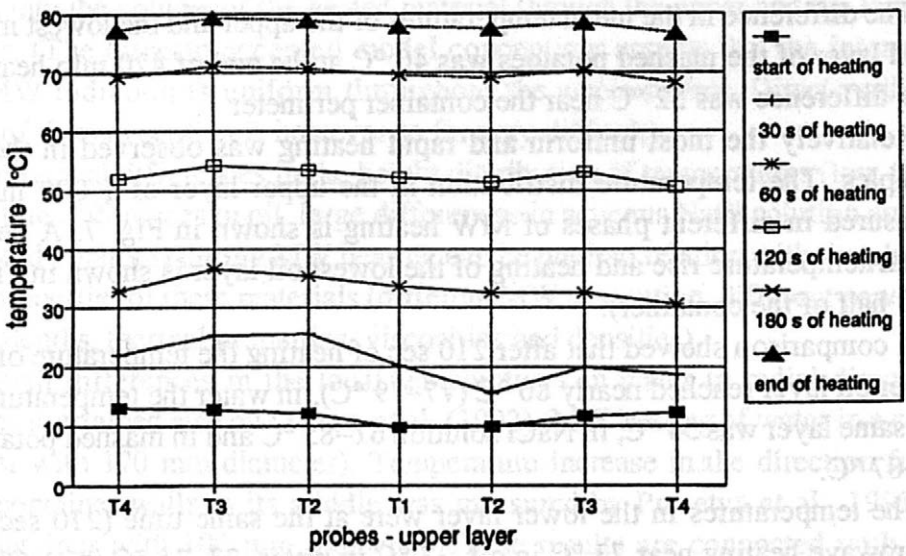
If a cork underplate (38 mm) was positioned under the container, the preheating of the surface layer (in comparison to the first layer measured above the bottom) decreased in the average by 6 °C after 420 sec heating of 1 000 ml water.

An addition of NaCl and especially of the dried mashed potatoes to the potable water resulted in a substantial increase of the heating nonuniformity both in the vertical and in the radial direction. In the experiments, in which aqueous NaCl solution (6%) was heated, a very rapid rise of temperature in the upper layer of the solution and a much slower heating of the middle and lower layers were observed.

The temperature of the upper layer of NaCl solution increased with a significantly higher rate than the temperature of the upper layer of water, i.e. the temperature of 80 °C was obtained the upper layer of aqueous NaCl solution in 240 sec, in water it took 420 sec. The central and especially the lower layer of NaCl solution were – on the contrary – heated much more slowly than the corresponding water layers (Fig. 5). A comparison shows that after 7 min of heat treatment the mean temperatures of water and NaCl solution were 63 and 50 °C, resp., in the measured layer just above the bottom.



7. Transient temperature profiles – microwave heating of sunflower oil (probes in central layer, left side, time of heating 210 s)



8. Radial temperature profiles – microwave heating of sunflower oil (probes in upper layer, left and right side, heating time 210 s)

The difference in the mean temperatures of the upper and the lowest layer of 1 000 ml NaCl solution reached 45 °C after the same time of treatment (420 sec).

The heating of NaCl solution also showed temperature differences in the radial direction, especially in the upper layer (10–20 °C). In the direction to the container bottom the differences in the radial direction diminished.

The curves expressing the profiles of local temperatures in the middle and especially in the lower layer were not as smooth as those in other materials. The Luxtron probes scanned temperatures oscillating in the range 5 °C. These oscillations increased in the direction toward the bottom.

An extremely nonuniform temperature distribution an the heating of 1 000 ml sample of mashed potatoes is obvious in Fig. 6 (the distribution of local temperatures in the radial direction of the upper layer of mashed potatoes during heating with probes being located successively in the right and the half of the container).

The figure shows the successive preheating of the upper layer of the material at the container perimeter in comparison with that in the central zone.

The ascertained radial profiles were flatter in the direction to the bottom of the container. The preheating of the layer of mashed potatoes near the container perimeter was less distinct than that in the central layer.

The difference in the mean temperatures of the upper and the lowest measured layer of the mashed potatoes was 40 °C at the end of 420 min heating. The difference was 52 °C near the container perimeter.

Relatively the most uniform and rapid heating was observed in the oil samples. The temperature distribution in the upper layer of 1 000 ml oil measured in different phases of MW heating is shown in Fig. 7. A nearly linear temperature rise and heating of the lowest oil layer is shown in Fig. 8 (left half of the container).

A comparison showed that after 210 sec of heating the temperature of the upper oil layer reached nearly 80 °C (77–79 °C), in water the temperature of the same layer was 54 °C, in NaCl solution 61–85 °C and in mashed potatoes 27–87 °C.

The temperatures in the lower layer were at the same time (210 sec) of microwave heating near 73 °C in oil, 37 °C in water, 23–35 °C in NaCl solution and 14–30 °C in mashed potatoes.

The results of the performed experimental study document both the effect of the heated material on the course of MW heating and the fact that a very nonuniform temperature distribution can occur even during the heating of completely homogeneous materials.

A series of results fully agrees both with the published data on other experiments and with the conception of the mechanism of MW heating. Some deviations observed can be explained by the differing parameters in these experiments, others will require further investigation to be perfomed. They however show the complexity of interactions occurring between materials and microwaves.

The preheating of the upper layer of the heated material in comparison with the middle layer and the layer near the container bottom that we observed to a lower or broader extent in nearly all our experiments was also published by Prosetya, Datta (1991) – heating of water and oil, Datta et al. (1992) – heating of water and Jakobsen, Miklelsen (1993) – heating of milk in bottles for infants. The course of water heating by MW is explained by spontaneous water circulation (inner circulational flow), at which the warmer water remains in the upper layer (Datta). Our experiments, carried out with water in a container with cork underplate and with highly viscous materials (mashed potatoes), where the inner circulation cannot be expected, indicate that different amounts of energy can pene-

trate into the volume of the heated material through the upper and the lowest layer. (The hitherto accepted model conceptions assume that the intensity of MW radiation is uniform throughout the whole surface. Direct verification of this conception is however so far very difficult).

Individual differences in the height distribution of temperatures (low temperature differences in oil, large differences in aqueous NaCl solution and in mashed potatoes) during MW heating can be put into relation with the physical properties of these materials (different MW absorption, different penetration depths, thermal properties, viscosities and densities).

Small differences in the local temperatures in water in radial direction were ascertained also by Datta et al. (1992) (MW heating of water in a container with 170 mm diameter). Temperature increase in the direction from the container walls to its middle was measured by Prosetya et al., 1992 in a container with 100 mm diameter. These results are connected with the 100 mm diameter of the container and with the concentrational effect of microwaves (Ohlsson, 1983).

The preheating of the layers of the material near the container walls, observed in heating of NaCl solution and mashed potatoes is in relation with the penetration depth of the two materials, and also with the additional effect of ionic conductivity in NaCl. Furthermore a significant influence of heat conductivity on the temperature profiles must be taken into account in the field of microwave absorption in the both cases.

Further measurements of mashed potato heating by MW showed that the locations of maximum temperatures occurred in the radial profiles approximately 10 mm from the wall which agrees well with the conception of the mechanism of energy transfer. The temperature of the mashed potatoes measured near the container walls is affected by the cooling effect of the convective heat transfer to the ambient air.

A substantially higher heating rate of the upper layer of NaCl solution – in comparison with the heating of the same water layer – is in connection with the mechanism of microwave energy transfer (ionic motion must be accounted for during heating of salt solutions) and the different dielectric properties of the two materials during heating. The MW energy absorption significantly decreases in water with rising temperature (or the penetration depth grows, e.g. for 100 °C it is 57 mm). If NaCl is added, the energy absorption rises and further increases with the growing temperature (Ohlsson,

1990). The lower heating uniformity in materials containing salt was also observed by Dealler et al. (1992) and Prosetya, Datta (1991).

The rapid temperature growth during oil heating is – in spite of the very low value of MW energy absorption – explained by the low value of specific heat capacity and the lower density of oil in comparison with water (Ohlsson, 1983, 1990; Prosetya, Datta, 1991).

The relatively uniform temperatures both in radial and in axial direction can be explained by the values of penetration depth in oil that fluctuates near 180 mm for the given temperature range. Owing to the diameter of the container used for heating (185 mm), the contribution of conductivity to this effect can be excluded and an assumption can be made that the thermal field in the volume of oil during its heating can be assigned only to the distribution of the intensity of MW field in the individual sample locations. Linear growth of local temperatures in all oil layers during heating confirms this assumption.

CONCLUSION

The application of the fluoroptic thermometric system LUXTRON 755 resulted in obtaining concrete information on temperature distribution in liquid materials of different viscosities and chemical composition during their MW heating in a flat container of cylindrical shape.

The thermal field created during MW treatment in this type of materials changes with time and is affected by the shape and dimensions of the applied container and by the composition and physical properties and volume of the heated materials. The rate of heating and the temperature distribution are affected especially by the addition of NaCl and saccharides into water and by the content of fat. The location of the heated material in the MW equipment also has an influence on the course of heating.

The results obtained are very important also from the point of view of a closer elucidation of the mechanism of MW heating, for practical applications of the MW technology and furthermore for the development of products for MW processing. They prove that the interaction between microwaves and the food materials and products is a very complex process requiring further study for its complete understanding.

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Received May 30, 1994

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Rozložení a vývoj lokálních teplot v homogenních tekutých materiálech při mikrovlnném ohřevu

Mikrovlnný ohřev má řadu předností proti konvenčním metodám ohřevu, proces sám je však značně komplikovaný. Od konvenčních metod ohřevu jej odlišuje nejen

rychlost ohřevu, ale i rozložení teplot v ohřívaném materiálu. Z praxe je znám problém nerovnoměrného ohřevu, který v případě potravin představuje potenciální riziko poklesu kvality a hygienické bezpečnosti.

Řada faktorů ovlivňuje rozložení teplot v materiálu během mikrovlnného ohřevu: typ mikrovlnného pole, geometrie a rozměry ohřívaného materiálu, jeho složení, fyzikální vlastnosti a teplota.

Distribuce lokálních teplot ve čtyřech druzích modelových různě viskózních tekutých materiálů v průběhu ohřevu je předmětem tohoto článku. Jde o výsledky experimentální studie, při níž byl použit běžný typ mikrovlnné trouby pro domácnost (Moulinex, max. výkon 850 W, bez otočného talíře). Průběh mikrovlnného ohřevu byl sledován na matriálech: pitná voda (1 000, 500 a 1 500 ml), roztok NaCl (6 % hmotnostních, 1 000 ml), směs vody a sušené bramborové kaše (1 000 ml) a slunečnicový olej (1 000 ml). K moritorování teplot byl použit termometrický systém LUXTRON 755 se čtyřmi sondami typu MIW. Sondy byly postupně instalovány ve třech horizontálních rovinách. K ohřevu byla použita plochá skleněná nádoba kruhového tvaru o průměru 185 mm. Průběh teplot byl sledován do dosažení teplot kolem 80 °C (420 s při ohřevu vody, roztoku NaCl a bramborové kaše, 210 s při ohřevu oleje).

Průběh ohřevu a distribuce lokálních teplot se pro danou geometrii lišily podle chemického složení ohřívaného materiálu, jeho viskozity, dielektrických a dalších fyzikálních vlastností a objemu. Vliv na rozložení mělo i umístění nádoby s materiálem při ohřevu.

Pokles teploty v axiálním směru směrem od horní vrstvy materiálu k vrstvě u dna byl naměřen při všech zkouškách. Jeho velikost závisela na druhu materiálu, objemu i na vzdálenosti sondy od středu nádoby. Rozdíl průměrných teplot horní a spodní vrstvy oleje na konci ohřevu byl 8 °C, bramborové kaše a roztoku NaCl 45 a 50 °C, 500 ml vody 10 °C, 1500 ml vody 23 °C.

Prakticky vyrovnané teploty v radiálním směru byly naměřeny při ohřevu oleje a rozdíly do 5 °C při ohřevu vody. Rozdíly 40 až 50 °C byly naměřeny mezi teplotami v radiálním směru při ohřevu bramborové kaše, teplota v tomto směru klesala od obvodu nádoby směrem k ose. Při ohřevu roztoku NaCl byly větší rozdíly mezi teplotami v radiálním směru naměřeny pouze v horní vrstvě (kolem 30 °C v 180. sekundě ohřevu, kolem 10 °C v 420. sekundě).

Při všech zkouškách bylo nejpomaleji ohřívané místo materiálu situováno do středu vrstvy u dna nádoby, nejrychleji ohřívané místo do horní vrstvy materiálu, v případě bramborové kaše a roztoku NaCl u obvodu nádoby.

mikrovlnný ohřev; distribuce teplot; rovnoměrnost ohřevu

Potrav. Vědy, *12*, 1994 (5) : 407-416

RETENCE VYBRANÝCH VITAMINŮ A MINERÁLNÍCH LÁTEK PŘI KULINÁRNÍ ÚPRAVĚ MASA

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Byla sledována retence thiaminu, riboflavinu, vitaminu B_6, niacinu, vitaminu E a minerálních látek při vaření, dušení, pečení a smažení hovězího, vepřového a kuřecího masa. Obsah riboflavinu, niacinu a minerálních látek se při kulinárních úpravách kromě vaření mění minimálně. Retence thiaminu se pohybuje od 20 do 85 %, retence vitaminu B_6 je v rozmezí 23 až 100 % původní hodnoty. Obsah vitaminu E klesá na 15–80 % původní hodnoty. Při vaření dochází k vyluhování 18 až 55 % hydrofilních vitaminů a 30–60 % minerálních látek do vývaru.

retence; vitamin E; thiamin; riboflavin; vitamin B_6; niacin; minerální látky; kulinární úprava masa

Maso je důležitou součástí stravy většiny populace v České republice. V průměru se spotřebuje přibližně 100 g masa a drobů (jedlý podíl) na osobu a den a 26 g drůbeže a drůbežích výrobků (jedlý podíl) na osobu a den (Ruprich, 1993). Maso je zdrojem základních živin a kromě toho též řady vitaminů, především skupiny B, a minerálních látek. Konzumu masa předchází vždy tepelná úprava. Tento proces může mít významný vliv na obsah výživových faktorů.

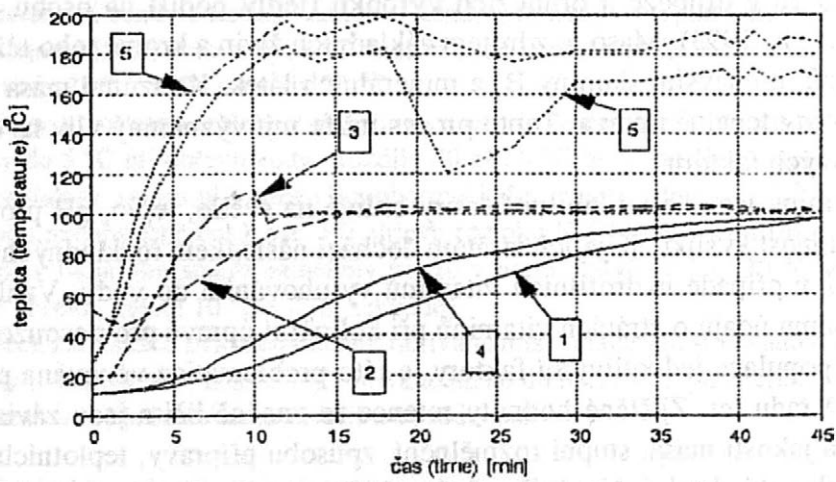
Vitaminy jsou velmi labilní faktory citlivé na světlo, teplo, pH prostředí a přítomnost kyslíku. K jejich ztrátám dochází následkem rozkladných reakcí a též v případě hydrofilních vitaminů vyluhováním do vody. Vzhledem k významu údajů o ztrátách vitaminů při kulinární úpravě pro posouzení saturace populace jednotlivými faktory je této problematice věnována pozornost již řadu let. Zjištěné hodnoty retence se značně liší a jsou závislé na druhu a jakosti masa, stupni rozmělnění, způsobu přípravy, teplotních podmínkách a skladování. Např. Bognár (1984) uvádí ztráty thiaminu při různých podmínkách tepelné přípravy masa v rozmezí 9 až 70 %. Pro vitamin B_6 jsou při kulinární úpravě masa uváděny ztráty 6,5 % (Olds et al., 1993) až 78 % (Uherová et al., 1993).

Příčinou ztrát minerálních látek během kulinární úpravy masa je především vyluhování. Kimura a Itokawa (1990) uvádějí, že vařené maso obsahuje v průměru 60 až 70 % původně přítomného množství minerálních látek, zatímco Seiler et al. (1977) zjistili ztráty až 50 %. Při pečení a grilování ztráty minerálních látek nepřesahují 10 % (Seiler et al., 1977).

V naší práci jsme sledovali retenci thiaminu, riboflavinu, vitaminu B_6, niacinu, vitaminu E a minerálních látek (Na, K, Ca, Mg, P, Fe, Cu, Zn) při kulinární úpravě hovězího, vepřového a kuřecího masa. Práce byla zaměřena na tradiční způsoby přípravy masa v našich domácnostech, tj. vaření, dušení, pečení a smažení. Tato volba odpovídá výsledkům průzkumu frekvence různých druhů kuchyňských úprav masa v ČR, provedeného CHPŘ Brno, SZÚ Praha (Ruprich, 1993).

MATERIÁL A METODY

Přehled použitých surovin a podmínek tepelných úprav masa je uveden v tab. I. K práci bylo použito běžné kuchyňské nádobí, pro pečení horkovzdušná trouba Tefal Maxi Ovn (příkon 1 250 W - Francie). Tepelný režim v průběhu kulinární úpravy byl sledován sadou kalibrovaných termočlánků Fe – konst. na přístroji Therm 3421. Příklad průběhu teplot při pečení kuřete je uveden na obr. 1. Sádlo bylo použito z důvodu minimálního přirozeného



1 = stehno – thigh; 2 = na plechu – baking tin; 3 = pod kůží – under skin; 4 = prsa – breast; 5 = vzduch – air

1. Průběh teplot při pečení kuřete – Temperature course during roasting of chicken

I. Přehled použitého materiálu a podmínek tepelných úprav masa – Review of used raw materials and culinary processing conditions

Druh a hmotnost[1]	Přísady[6]	Způsob úpravy[10]	Teplota prostředí[15] [°C]	Doba[16] [min]	Předběžná úprava[17]
Hovězí kýta[2] cca 500 g	NaCl, pitná voda[7]	vaření[11]	96–99	150	odblanění[18]
	sádlo[8], NaCl, pitná voda	dušení[12]	95–99	90	odblanění, kostky[19]
	sádlo, NaCl, pitná voda	pečení[13]	170–200	90	odblanění
Vepřová kýta[3] cca 500 g	sádlo, NaCl, pitná voda	dušení	95–99	90	odblanění, kostky
	sádlo, NaCl, pitná voda	pečení	170–200	60	odblanění
	NaCl, sádlo	smažení[14]	170–180	8	odblanění, plátky[20]
Kuřecí prsa mražená[4] cca 500 g	NaCl, máslo[9], pitná voda	dušení	95–99	45	plátky
	NaCl, máslo	smažení	170-180	8	plátky
Kuře kuchané s kůží[5] cca 650 g	NaCl, máslo, pitná voda	pečení	170–200	45	půlka[21]

[1]kind and weight; [2]beef; [3]pork; [4]frozen chicken breast; [5]chicken with skin; [6]ingredients; [7]water; [8]lard; [9]butter; [10]processing; [11]boiling; [12]stewing; [13]roasting; [14]frying; [15]medium temperature; [16]time; [17]preliminary treatment; [18]trimming; [19]cubes; [20]slices; [21]half

obsahu vitaminu E. Máslo bylo voleno pro dušení, pečení a smažení kuřete jako obecně nejběžněji používaný tuk při kulinární úpravě kuřete. Při vaření masa byl separátně analyzován též vývar, při smažení použitý tuk a při pečení a dušení byl vzorek získán homogenizací tepelně opracovaného masa a šťávy. Vzorky syrového i tepelně upraveného masa byly po homogenizaci na mixeru použity pro následující stanovení:

- thiamin: thiochromová metoda ČSN 56 0052
- riboflavin: lumiflavinová metoda ČSN 56 0054
- vitamin B_6: mikrobiologická metoda se *Saccharomyces uvarum* ATCC 9080, ČSN 56 0056
- niacin: mikrobiologická metoda s *Lactobacillus plantarum* ATCC 8014, ČSN 56 0051

- vitamin E (α-tokoferol): po alkalickém zmýdelnění, metoda HPLC s užitím stacionární fáze Separon SGX o zrnění 7 μm na koloně 250 x 4 mm, mobilní fáze hexan-2-propanol (99:1), průtok 1 ml/min, fluorescenční detekce λ_{ex} – 290 nm, λ_{em} – 330 nm
- minerální látky: mineralizace na suché cestě (520 °C, výluh HNO_3, c = = 1 mol/dm^3); plamenová AAS – Na, K, Ca, Mg, Fe, Cu, Zn; spektrofotometrie – P

VÝSLEDKY a DISKUSE

Výsledky analýz vzorků syrového masa jsou uvedeny v tab. II a III. Jsou průměrem analýz dvou nezávislých vzorků masa, přičemž každý vzorek byl analyzován nejméně třikrát. Ve většině faktorů vykazují výsledky dobrou shodu s tabulkovými hodnotami (Ciba-Geigy, 1981).

II. Obsah vitaminů v syrovém mase [mg/100 g] – Vitamin content in raw meat [mg/100 g]

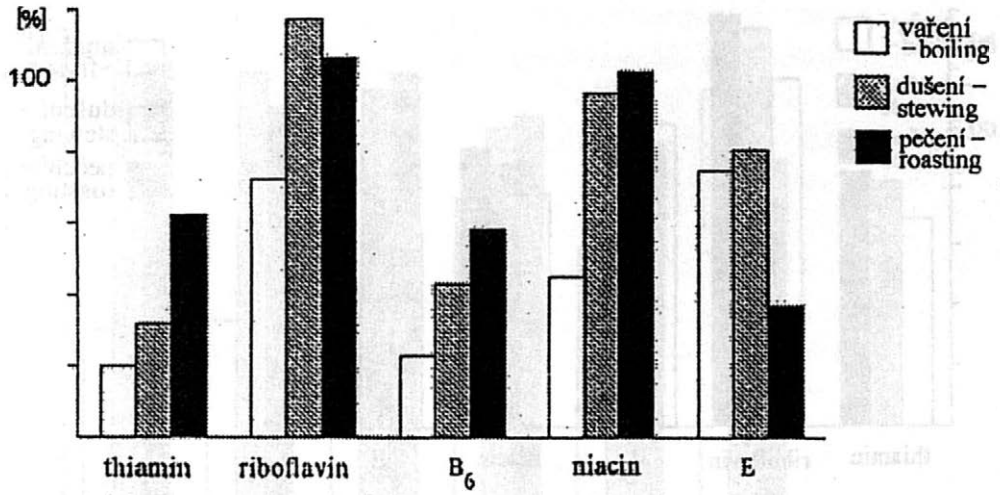
	Thiamin	Riboflavin	B_6	Niacin	E
Hovězí maso[1]	0,16	0,15	0,23	4,6	0,28
Vepřové maso[2]	0,84	0,18	0,26	5,6	0,11
Kuřecí prsa[3]	0,17	0,08	0,34	8,3	0,06
Kuře kuchané s kůží[4]	0,12	0,12	0,15	3,5	0,33

[1] beef ; [2] pork ; [3] chicken breast ; [4] chicken with skin

III. Obsah minerálních látek v syrovém mase [mg/100 g] – Mineral content in raw meat [mg/100 g]

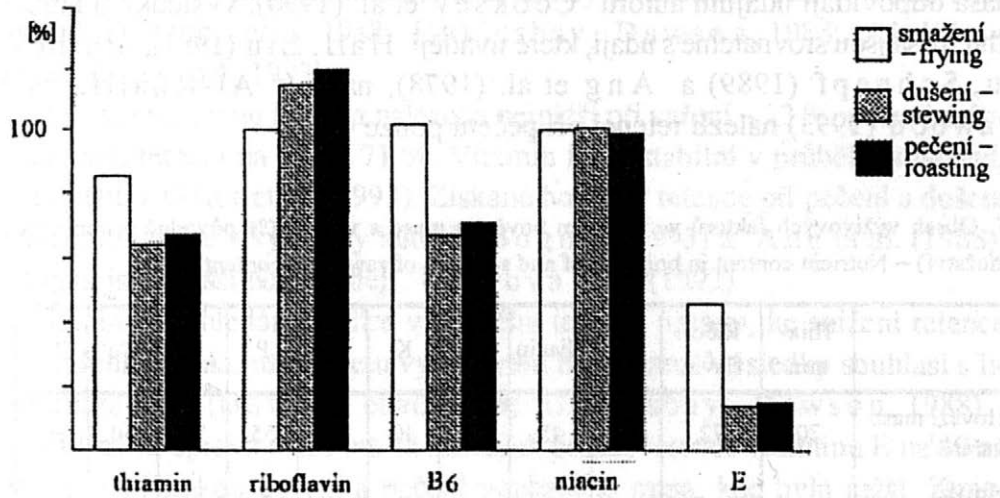
	Na	K	Ca	Mg	P	Fe	Cu	Zn
Hovězí maso[1]	63	325	6,6	20	176	1,8	0,09	4,6
Vepřové maso[2]	49	327	7	20	197	0,7	0,05	2
Kuřecí prsa[3]	41	297	4,6	26	220	0,5	0,09	0,5
Kuře kuchané s kůží[4]	72	255	7,1	20	217	0,7	0,08	1,1

[1] beef ; [2] pork ; [3] chicken breast ; [4] chicken with skin

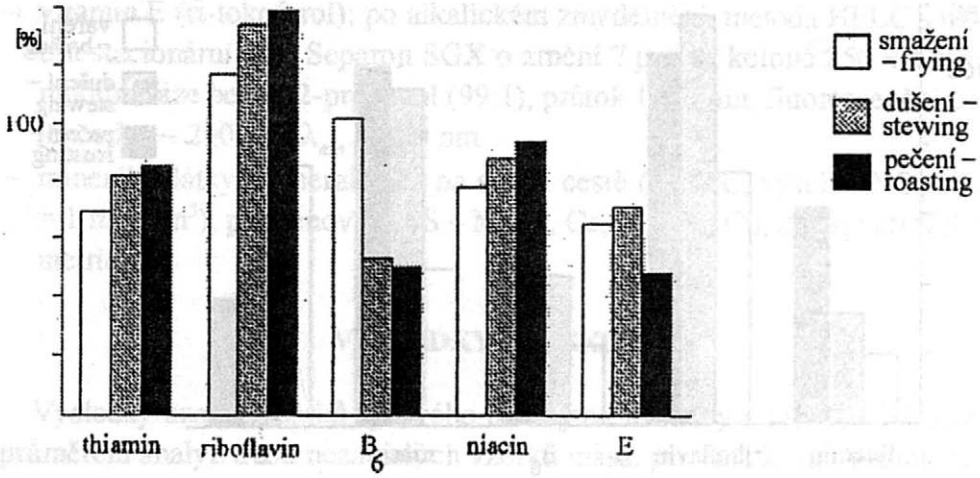


2. Retence vitaminů v hovězí kýtě po kulinárních úpravách [%] — Vitamin retention after culinary processing of beef [%]

Na obr. 2 až 4 jsou znázorněny hodnoty retence sledovaných vitaminů při různých způsobech kulinární úpravy hovězího, vepřového a kuřecího masa. Retence minerálních látek při tepelných úpravách hovězího masa jsou prezentovány na obr. 5. Uvedené hodnoty jsou vypočteny na základě hmotnostní bilance a vztaženy na obsah jednotlivých faktorů v původním syrovém mase (Unklesbay, Dawson, 1988).



3. Retence vitaminů ve vepřové kýtě po kulinárních úpravách [%] — Vitamin retention after culinary processing of pork [%]



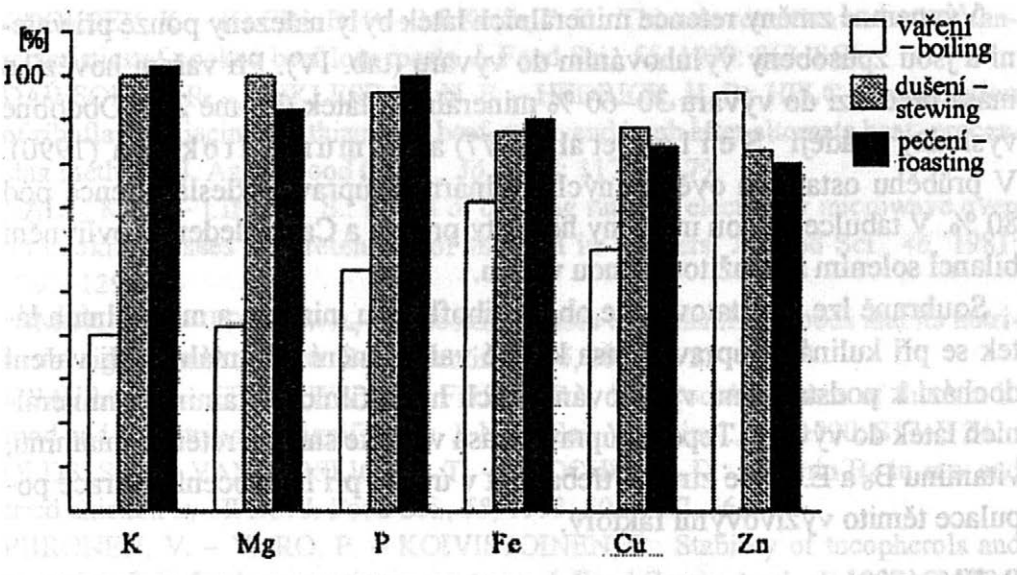
4. Retence vitaminů v kuřecím mase po kulinárních úpravách [%] — Vitamin retention after culinary processing of chicken meat [%]

Z výsledků je patrné, že při všech způsobech kulinární úpravy a u všech druhů mas dochází ke ztrátám thiaminu vzhledem k jeho značné termolabilitě. Retence se pohybuje v rozmezí 20 až 85 %. Nejšetrnější postup je smažení, nejnižší retence byla nalezena při vaření. Při této kulinární úpravě dochází k významnému vyluhování thiaminu do vývaru, jak je patrné z tab. IV. Stejnou závislost retence na způsobu kulinární úpravy nalezli K i m u r a et al. (1990). Námi zjištěné hodnoty retence při pečení hovězího masa odpovídají údajům autorů C o o k s e y et al. (1990), výsledky u kuřecího masa jsou srovnatelné s údaji, které uvádějí H a l l, L i n (1981), B a r b e a u, S c h n e p f (1989) a A n g et al. (1978), naopak A l - K h a l i f a a D a w o o d (1993) nalezli retenci při pečení pouze 45 %.

IV. Obsah výživových faktorů ve vařeném hovězím mase a vývaru (% původně přítomného množství) – Nutrient content in boiled beef and soup (% of raw meat content)

	Thiamin	Riboflavin	B_6	Niacin	E	K	Mg	P	Fe	Cu	Zn
Hovězí maso vařené[1]	20	72	23	45	74	40	42	55	71	60	96
Hovězí vývar[2]	47	18	37	55	0	60	58	45	29	40	4

[1]boiled beef ; [2]soup



5. Retence minerálních látek v hovězí kýtě po kulinárních úpravách [%] — Mineral retention after culinary processing of beef [%]

Riboflavin je ve srovnání s thiaminem odolnější vůči působení tepla. Ke ztrátám riboflavinu dochází pouze při dlouhodobém varu (tab. IV). Retence v mase představuje 72 %, do vývaru přechází 18 % původně přítomného množství. Při ostatních kulinárních úpravách se retence pohybovala od 100 do 139 %. Důvodem tohoto nárůstu může být uvolnění vázaných forem riboflavinu vlivem tepelného zásahu. Hodnoty retence nad 100 % uvádí řada autorů (Dawson et al., 1988; Unklesbay, Dawson, 1988; Al-Khalifa, Dawood, 1993).

Retence vitaminu B_6 byla nalezena nejnižší při vaření – 23 %, pečení a dušení sníží retenci na 43 až 71 %. Vitamin B_6 je stabilní v průběhu smažení, jak zjistili i Olds et al. (1993). Získané hodnoty retence při pečení a dušení jsou srovnatelné s výsledky autorů Bognár (1993) a Ang et al. (1988), naopak jsou vyšší než uvádějí Uherová et al. (1993).

Niacin byl shledán stabilní v průběhu tepelné úpravy, ke snížení retence při vaření dochází následkem vyluhování do vývaru. Výsledky souhlasí s literárními údaji (Dawson et al., 1988; Unklesbay, Dawson, 1988).

Kulinární úprava masa má za následek pokles retence vitaminu E na 40 až 80 %, s výjimkou dušení a pečení vepřového masa, kde byla nižší. Ztráty vitaminu E 33 až 44 % při úpravě hovězího masa uvádí Bennink a Ono (1982) a pouze 5% ztráty zjistili Piironen et al. (1987).

Významné změny retence minerálních látek byly nalezeny pouze při vaření a jsou způsobeny vyluhováním do vývaru (tab. IV). Při vaření hovězího masa přechází do vývaru 30–60 % minerálních látek (kromě Zn). Obdobné výsledky uvádějí Seiler et al. (1977) a Kimura, Itokawa (1990). V průběhu ostatních ověřovaných kulinárních úprav neklesla retence pod 80 %. V tabulce nejsou uvedeny hodnoty pro Na a Ca vzhledem k ovlivnění bilancí solením a použitou pitnou vodou.

Souhrnně lze konstatovat, že obsah riboflavinu, niacinu a minerálních látek se při kulinární úpravě masa kromě vaření mění minimálně. Při vaření dochází k podstatnému vyluhování všech hydrofilních vitaminů a minerálních látek do vývaru. Tepelná úprava masa vede ke snížení retence thiaminu, vitaminu B_6 a E. Výše ztrát je třeba brát v úvahu při hodnocení saturace populace těmito výživovými faktory.

Poděkování

Autoři děkují ing. K. Hokemu za laskavé proměření průběhu teplot při kulinárních úpravách.

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Došlo 1. 7. 1994

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Vitamin and mineral retention in meat in various cooking methods

Meat is a source of vitamins of particularly group B and of minerals. Meat consumption is always preceded by culinary processing, which may exert a significant effect on the content of these nutrients.

Retention of thiamine, riboflavin, vitamin B_6, niacin, vitamin E and minerals (Na, K, Ca, Mg, P, Fe, Cu, Zn) was investigated during culinary processing of beef,

pork and chicken meat. The investigation was focused on traditional methods of meat processing in the Czech households, i.e. on boiling, stewing, roasting and frying. Table I shows a review of used raw materials and culinary processing conditions. Tables II and III indicate the results of analyses of raw meat samples. The values are in good coincidence with tabular data in most indicators (C i b a - - G e i g y , 1981). Figs. 2–4 represent the values of vitamin retention during various culinary processing of beef, pork and chicken meat. Fig. 5 presents mineral retention during culinary processing of beef. The indicated values have been calculated on the basis of weight balance and related to the content of the particular nutrients in raw meat (U n k l e s b a y , D a w s o n , 1988) – true nutrient retention.

Thiamine loss was observed in all methods of culinary processing and in all kinds of meat. Retention ranges from 20 to 85%. Frying is the most favorable as for the thiamine loss, while cooking revealed the lowest retention. Besides cooking, riboflavin retention ranged from 100 to 139% in the other applied culinary processing methods. Vitamin B_6 content is constant during frying while cooking showed the lowest retention – 23%. Roasting and stewing reduce retention to 43–71%. Niacin content was constant during culinary processing. In all cases, vitamin E content decreased to 15–80%.

Significant changes in mineral contents were observed in cooking only and they are mostly due to extraction into soup (Table IV). 30 to 60% of the minerals are extracted into soup by cooking. Similarly, hydrophilic vitamins are extracted into soup at an amount of 18 to 55%.

The final values of retention are used as a basis for evaluation of actual uptake of these nutrients.

cooking methods; meat; vitamin E; thiamine; riboflavin; vitamin B_6; niacin; minerals; retention

Z VĚDECKÉHO ŽIVOTA

XXV. Sympozium o nových směrech výroby a hodnocení potravin

Ve dnech 18. až 20. května 1994 se ve Skalském dvoře konalo XXV. Sympozium o nových směrech výroby a hodnocení potravin. Jeho hlavním organizátorem byla Odborná skupina pro potravinářskou a agrikulturní chemii České společnosti chemické. Zúčastnilo se ho 112 odborníků z oblasti potravinářské, zemědělské a zdravotnické, a to jak z výzkumu, tak i z praxe.

Na sympoziu byly prezentovány nové poznatky z oblasti nutričně významných složek potravin, aditiv i kontaminantů, týkající se analytických metod a jejich užití při hodnocení potravin a sledování změn, ke kterým dochází při zpracování a skladování potravin. Celkem bylo uvedeno 34 referátů a 26 posterů.

Z oblasti modernizace a zlepšení analytických postupů se autoři referátů věnovali užití bio- a chemiluminiscenční metody v analýze potravin, použití mikrovlnných systémů pro přípravu vzorků, možnostem užití multivariačních metod k predikci, využití magnetických separací v potravinářské mikrobiologii. Dále bylo referováno o zjednodušeném postupu stanovení těkavých kyselin, hustoty a alkoholu ve víně, o separaci izomerních terpenoidů pomocí HPLC a o stanovení nikotinu a dehtu v cigaretách.

Početná skupina příspěvků se týkala využití senzorické analýzy při hodnocení potravin a korelace výsledků senzorické analýzy s fyzikálně-chemickými metodami. Souhrnně byly diskutovány výhody a nevýhody užití pořadové zkoušky a hodnocení pomocí absolutních ukazatelů při senzorické analýze, dále vliv jednotlivých znaků pro celkovou senzorickou hodnotu ovoce a interakce hořké a sladké chuti. Byly uvedeny výsledky senzorické analýzy při hodnocení piv a vlivu oxidace monoterpenů na senzorický profil výrobků. Referáty se týkaly senzorické jakosti masa, možnosti jejího ovlivnění a relací k fyzikálně-chemickým parametrům. Přítomní se seznámili s výsledky korelací mezi senzorickým a přístrojovým hodnocením tvrdosti a korelací mezi senzorickým a GLC hodnocením silic.

Pět příspěvků se zaměřilo na problematiku vitaminů. Byly prezentovány výsledky stanovení vitaminu A a E v olejích a margarínech, vitaminu E v obilovinách, hydrofilních vitaminů v cereálních výrobcích. Přednesené práce sledovaly dále pokles obsahu vitaminu C ve skladovaných jahodách, vliv kulinární úpravy na obsah vitaminů a minerálních látek v mase a ovlivnění stability vitaminu E při smažení bramborových lupínků.

Pozornost byla věnována zdrojům a stanovení vlákniny a možnosti přípravy sójových jogurtů.

Výsledky výzkumu v oblasti změn tukové složky potravin uváděly referáty týkající se interakcí oxidovaných olejů s mléčnými bílkovinami, vlivu saprofytické mikroflóry na stabilitu tukové složky, vlivu užitého oleje na oxidační změny v majonézách. Do této oblasti směřovaly referáty o antioxidační aktivitě bílkovinných hydrolyzátů a Maillardových produktů a o zdrojích a antioxidačních účincích (−)-epikatechinu. Pro studium oxidačních změn byla uváděna metoda izotermální mikrokalorimetrie.

Přehledný referát o vývoji oboru zpracování masa po roce 1990 uvedl blok referátů týkajících se výroby a jakosti masa a masných výrobků. Další příspěvky byly zaměřeny na zlepšení údržnosti masných výrobků, na aplikaci tekutého udicího preparátu ve fermentovaných masných výrobcích, na srovnání jeleního a hovězího masa z hlediska solení a na retenci jodu v masných produktech získaných s přídavkem jodidované soli.

K problematice hygienické jakosti potravin se vztahovaly referáty pojednávající o některých polyfenolických látkách v semenech luskovin a obilovin, dále o obsahu glukosinolátů v hořčičných semenech a brukvovitých zeleninách, o obsahu glykoalkaloidů v bramborách a toxických kovů v minerálních vodách. Autoři referátů se také věnovali stanovení zbytkového kysličníku siřičitého, migraci obalových materiálů do potravin, možnosti náhrady konzervačních činidel přírodními fytoncidy, aktivitě fosfolipázy během zrání rajčat, identifikaci neionogenních tenzidů v kontaminovaných potravinách. Bylo upozorněno na význam rozložení teplot při mikrovlnném ohřevu. Zajímavé byly i první výsledky projektu týkajícího se sledování expozice člověka některým chemickým látkám z poživatin.

Diskuse se zaměřila rovněž na nutriční, hygienickou a technologickou jakost produktů ekologického zemědělství.

Zpracování rostlinných produktů se věnovaly referáty o problémech termosterilace a řízení jejího procesu, o mléčně fermentovaných zeleninových šťávách. Předneseny byly referáty sledující aminokyselinové složení cereálních výrobků a změny mikrostruktury kukuřičného zrna při extruzi.

Souhrny přednesených referátů a posterů jsou k dispozici ve VÚPP, Radiová 7, 102 31 Praha 10.

Ing. Marie Holasová

Potrav. Vědy, *12*, 1994 (5) : 419-420

INFORMACE

Výzkumný ústav potravinářský Praha se představuje

Stává se již dobrou tradicí věnovat některá z čísel časopisu Potravinářské vědy jedné instituci – tentokrát vám předkládáme výběr prací Výzkumného ústavu potravinářského Praha.

I přes určitá omezení a problémy, se kterými se potýká v současné době naše výzkumná sféra, představuje Výzkumný ústav potravinářský Praha komplexní pracoviště, které řeší úkoly jak průřezového, tak aplikovaného výzkumu v oblasti chemie, biochemie, technologie potravin, výživy, ale i potravinářského inženýrství a ochrany zásob před škůdci. Kromě pracovišť v sídle ústavu v Praze-Hostivaři má ústav vývojovou základnu potravinářské techniky i v Hrušovanech nad Jevišovkou. Toto pracoviště je zaměřeno na vývoj, konstrukci a výrobu potravinářské, balicí a manipulační techniky podle požadavků zákazníků.

V současné době ústav řeší celkem 16 resortních výzkumných projektů, z nichž dva mají předpokládaný konec řešení v letošním roce, zbylých 14 v roce 1995. Náklady na tyto projekty jsou hrazeny z příspěvku Ministerstva zemědělství ČR.

Do budoucna by se dalším podstatným zdrojem finančních prostředků na výzkum měla stát i Grantová agentura ČR. Již koncem minulého roku získal ústav jak vlastní grant, tak spoluúčast na řešení grantů jiných pracovišť – VŠCHT, ČVUT a VŠZ v Praze.

Ústav dále průběžně zajišťuje některé specifické – tzv. trvalé nebo expertní činnosti, např. udržování a doplňování sbírek roztočů a hmyzu, mikroorganismů, databanky fyzikálních vlastností potravin, dílčí monitoring cizorodých látek, expertní a poradenskou činnost.

Významné místo v aktivitách ústavu zaujímá i zahraniční spolupráce, které v poslední době věnujeme stále větší pozornost. Spoluráce se zahraničními partnery probíhají po několika liniích. Česká republika je zastoupena prostřednictvím našeho výzkumného ústavu v několika komisích, např. v Mezinárodní organizaci pro cereální vědy a technologie ICC a v Mezinárodní organizaci pro biologický a integrovaný boj se škůdci. Řada odborných pracovníků ústavu má přímý kontakt s předními vědeckými pracovišti a univerzitami, např. s Univerzitou v Piacenze (Itálie), Státním zdravotním ústavem pro hubení škůdců v Berlíně (SRN) a Univerzitou v Albertě (Kanada).

Důležitá je rovněž spolupráce při řešení výzkumných problémů. Zde připomenu spolupráci se Silsoe Research Institute v Bedfordu (Velká Británie) při řešení matematických modelů chlazení kapalných potravin, spoluúčast na řešení programu PECO - Matematické modelování vlastností potravin během výroby a skladování a spolupráci s NIZO (Holandsko) na projektu zemí ES zaměřeném na problémy zemí střední a východní Evropy.

Po přechodu Výzkumného ústavu potravinářského Praha na příspěvkovou organizaci je pouze část jeho rozpočtu, konkrétně příspěvek na řešení resortních úkolů a trvalých činností, hrazena jeho zřizovatelem (Ministerstvem zemědělství). V roce 1993 činil tento podíl asi dvě třetiny rozpočtu ústavu. Zbylý díl je nutné zajistit jinými aktivitami. S tím souvisí stoupající aktivita a podpora prací a služeb za úhradu na objednávku z podnikatelské sféry. Služby poskytují v oboru své působnosti prakticky všechny útvary ústavu.

Ing. Jiří Celba, CSc.
ředitel Výzkumného ústavu potravinářského Praha

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UPOZORNĚNÍ PRO ODBĚRATELE

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