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# *Publication activities and solution of research grant projects at the Faculty of Food and Biochemical Technology*

*This issue of the journal Food Sciences is again devoted to scientific papers written by authors from the Faculty of Food and Biochemical Technology Prague's Institute of Chemical Technology.*

*This form of presentation enables to the Faculty staff and chiefly of our younger members and postgraduate students to publish their new research results in a very short time after delivering for publication. I am sure this publishig activities of the Faculty members will be permanent. Almost all of research projects, which are solved on Faculty now, are by the part of various grants and projects. Publication of original papers is one of the main criterion for next evaluation of the successful project and opens the new possibilities for further competition in obtaining grants.*

*Workers of the Faculty received in 1994 in total 46 grants in the total financial amount 13.1 mil. CZK. From the Grant Agency of the Czech Republic it was 29 grants (6.3 mil. CZK), from the Agency of University Development of the Ministry of Education nine grants (1.1 mil. CZK), from the Ministry of Agriculture 1 grant (0.2 mil. CZK) and from international organisations seven grants (5.5 mil. CZK). Topics of these grants are focused on the sphere of healthy human nutrition and food quality assessment (improvement of plant food quality and their using - legumes, potatos, cereals, garlic, onion etc., identification and changes of natural toxins, antinutritional compounds, flavour and texture, development of modern analytical, biochemical and microbial technics in food evaluation and analysis), environmental aspects (biodegradation of phenol, polutants in gases, PCB) and applied biochemistry and biotechnology. Besides of these research topics on Faculty are also solved educational projects to improve and to optimize the university education of food specialists and technologists.*

*Our interest is also to present on the pages of the Food Sciences our common papers of foreign authors from European and world universities and research institutes, which are our partners in the solution of international research projects. It is even one of the way how to increase the international reputation of this Journal and to find its place among journals cited in Current Contents.*

*The journal Food Sciences is now one scientific journal in branch of food science and technology in our country, because publishing of The Scientific Papers of the Prague Institute of Chemical Technology, Serie E – Food was finished in 1993, after edition more than 60 issues in recent years.* This journal had also very good *scientific level, but its main disadvantage was irregularity in edition and very long time between receiving the manuscript and publication.*

*Prof. Pavel Kadlec, D.Sc.*
*Dean of the Faculty*

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# PREDICTION OF BAKING AND MILLING QUALITY CHARACTERISTICS OF CZECHOSLOVAK WHEAT

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Twenty two physico-chemical characteristics of 812 bread wheat samples were measured and analyzed by multivariate statistical methods to determine what characteristics are relevant for the corresponding milling and baking quality of wheat, flour and baking products. A representative set of about 400 wheat samples out of 812 objects was used as a calibration set. The multivariate analysis techniques employed were two different versions of partial least squares (PLS) regression. The statistical relationships between the most important milling and baking variables were calculated and the achieved results were discussed.

bread wheat quality; multivariate statistical methods; PLS regression; prediction of wheat characteristics; flour yield, loaf volume

The quality of bread wheat is primarily dependent upon its milling and baking quality. Milling quality is generally assessed by measuring the flour yield, flour ash content and yield according to Mohs. Baking quality is assessed by measuring the loaf volume. Other quality parameters (e.g. test weight, thousand kernel weight, grain ash content, grain protein content, gluten extensibility and farinographic data, etc.) are considered important as predictors of milling and baking quality.

End-use suitability of any given wheat sample is determined by the extent to which both genetic and environmental variability affects its physical and chemical properties. To date it has not been possible to determine milling and baking quality by prediction tests based on these properties alone. It will likely take some time before we can eliminate laboratory milling and baking and other end product simulation tests as the ultimate means of characterising wheat varieties and commercial lots for end-use suitability. Cereal scientists have made long strides in improving our understanding of the relation between the physical and chemical properties of wheat quality for milling and baking uses. Many attempts have been done in this field (Bogdanowicz, 1971; Seibel et al., 1979; Genzken, 1983; Holas, Kratochvíl, 1989; Preston et al., 1989; Sutton et al., 1990). However, many of these relationships are not straightforward and much further work remains to be done.

In this paper we discuss statistical relationships between quality parameters of wheat using partial least squares (PLS) regression. Special attention is focused upon milling and baking quality prediction tests.

## MATERIAL AND METHODS

Total number of 812 samples of different wheat varieties tested in the period 1980–1989 have been included into a computer processing. The numbers of samples tested in the respective years are listed in Table I.

I. List of wheat samples analyzed

| Year | No. of samples analyzed | Year | No. of samples analyzed |
|---|---|---|---|
| 1980 | 48 | 1985 | 63 |
| 1981 | 63 | 1986 | 170 |
| 1982 | 71 | 1987 | 102 |
| 1983 | 65 | 1888 | 91 |
| 1984 | 68 | 1989 | 71 |

The samples came from testing stations located in different growing regions of Czechoslovakia and covered the whole quality range of wheat varieties cultivated in the respective years. Apart from the standard varieties, new-bred cultivars are included in the above-mentioned sample set. The individual achieved variables (attributes) of these samples are listed in Table II. These 22 variables comprised two agronomic production variables, eight milling wheat characteristics, and 12 baking flour characteristics. For the purpose of correlation analysis, flour yield, flour ash content and flour yield according to Mohs were taken as dependent milling variables and the other five milling parameters as independent ones. Similarly, specific loaf volume and modified specific loaf volume were taken as dependent baking variables and the other ten baking parameters as independent ones. The agronomic variables, namely growing region and wheat quality index were assumed as independent variables.

II. Characteristics of wheat samples analyzed

| Variables (Attributes) | Mean | Std dev | Minimal | Maximal |
|---|---|---|---|---|
| **Agronomic** | | | | |
| Growing region | 1.89 | 0.81 | 1 (the best) | 4 (the worst) |
| Wheat quality index | 5.41 | 2.27 | 1 (the worst) | 9 (the best) |
| **Milling** | | | | |
| Test weight | 785.20 | 31.30 | 677 | 887 |
| Thousand kernel weight | 42.78 | 5.23 | 29.7 | 67.4 |
| Grain ash content | 1.73 | 0.13 | 1.25 | 2.13 |
| Grain protein content | 13.38 | 1.35 | 10.10 | 18.10 |
| Grain wet gluten content | 25.21 | 4.47 | 8.6 | 47.7 |
| Flour yield | 70.30 | 3.03 | 56.9 | 77.4 |
| Flour ash content | 0.50 | 0.05 | 0.40 | 0.68 |
| Flour yield according to Mohs | 6.53 | 6.22 | –9.99 | 36.90 |
| **Baking** | | | | |
| Gluten extensibility | 11.26 | 2.84 | 4 | 20 |
| Gluten swelling no. | 12.71 | 4.18 | 1 | 28 |
| Sedimentation value of flour | 39.27 | 12.16 | 10 | 71 |
| Falling no. | 316.43 | 87.20 | 61 | 604 |
| Farinographic data: | | | | |
| Water absorption | 60.42 | 4.44 | 50.1 | 74.5 |
| Dough development time | 3.22 | 1.46 | 1 | 9 |
| Dough stability | 4.06 | 2.09 | 1 | 14 |
| Decrease of consistency | 93.41 | 33.60 | 10 | 340 |
| Kopetz no. | 49.72 | 18.27 | 0 | 95 |
| Baking quality no. (according to Kalina) | 42.38 | 10.17 | 0 | 78 |
| Specific loaf volume | 351 | 50.35 | 243 | 560 |
| Modified specific loaf volume | 319.65 | 62.88 | 157 | 560 |

There were four different growing regions classified in four-level scale (Table II). The best growing region assumed as suitable for maize growing (value 1) was followed by the beet region (value 2), the potato region (value 3) and the mountain region (value 4) as the worst one. Wheat quality index was a value ranging from 9 to 1 (9 point system assigned to the given variety on the basis of a complex testing of milling and baking quality for three years). The wheat quality index value was 9 for the best variety and value 1 for the worst one. The sedimentation value of flour was determined using acetic acid solution. The specific loaf volume was expressed in ml per 100 g of baked loaves. This value was modified by subtracting of 0–40 ml for the loaf shape (height of loaf/diameter of loaf ratio), 0–50 ml for sensory evaluation and 50 ml for dough stickiness. By this way the modified specific loaf volume was obtained. All the other parameters were determined by common well-known methods.

### Statistical analysis

Statistical techniques employed were simple descriptive statistics, two-tailed correlation of variables and stepwise multiple linear regression which were performed on an IBM compatible computer using software purchased from SPSS/PS+, V3.1 (SPSS GmbH, München, Germany). Multivariate analyzes techniques involving two different versions of partial least squares (PLS) regression were done using UNSCRAMBER II software for multivariate data analysis (CAMO A/S, Trondheim, Norway).

## RESULTS AND DISCUSSION

The aim of the present research was to analyze the measured wheat variables to determine what variables are relevant for wheat quality and the corresponding milling and baking quality and to find a way to rationalize wheat grain and wheat flour quality control.

As it can be seen (Table III), the individual wheat attributes were strongly intercorrelated (multicolinear regressors). Therefore, the multivariate techniques based on PLS regression on variables have been used to explore the mathematical relationships between one or several dependent variables (regressands) and a block of independent variables (regressors).

**III. Correlation[a] of dependent variables with independent variables**

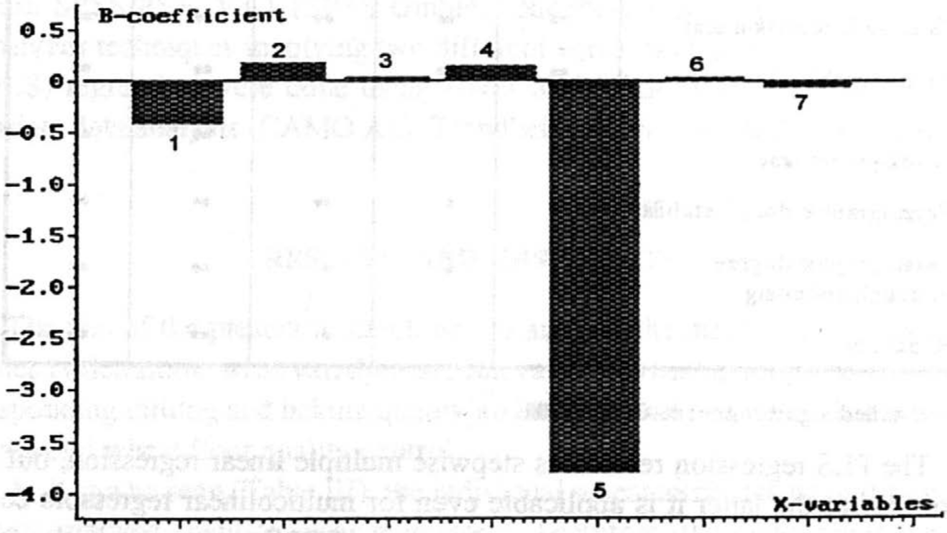
| Variable | Flour yield | Grain ash content | Mohs | Specific loaf volume | Modified specific loaf volume |
|---|---|---|---|---|---|
| Region | ** | | | | |
| Quality class | ** | ** | ** | ** | ** |
| Test weight | ** | | ** | | |
| Thousand grain weight | ** | | ** | * | * |
| Grain ash content | ** | ** | ** | * | |
| Grain protein content | | | | ** | ** |
| Grain wet gluten content | | | | ** | ** |
| Gluten extensibility | | | | | ** |
| Gluten swelling no. | | ** | ** | ** | ** |
| Baking quality no. (according to Kalina) | | ** | * | ** | ** |
| Falling no. | * | * | | ** | ** |
| Flour sedimentation test | ** | ** | ** | ** | ** |
| Farinographic absorption | ** | ** | | ** | ** |
| Farinographic dough development time | ** | | | ** | ** |
| Farinographic dough stability | ** | * | ** | ** | ** |
| Farinographic degree of dough softening | | * | * | ** | ** |
| Kopetz no. | ** | | * | ** | ** |

a = 2-tailed significance: *0.01, **0.001

The PLS regression resembles stepwise multiple linear regression, but in contrast to the latter it is applicable even for multicolinear regressors containing significant noise. Another advantage of PLS regression is the fact that all regresssors are included in the final solution and no one has to be discarded as in stepwise multiple linear regression. PLS is even more efficient than principal component regression (PCR), since it extracts from the achieved results represented by the regression matrix only those factors that are relevant to the prediction of the regressand(s). In addition, the technique can be used to predict values for the individual dependent variables, given a set of values for the corresponding independent variables (Martens et al., 1983).

Three of the above eight milling characteristics (i.e., flour yield, flour ash content and flour yield according to Mohs) and 2 of the 12 baking characteristics (i.e., specific loaf volume and modified specific loaf volume) were chosen as dependent milling and dependent baking variables, respectively. The growing region and wheat quality index were assumed as independent variables.

A representative set of 404 randomly selected wheat samples out of all 812 objects was used as a calibration (training) set. In order to reduce different variable effects, reduce the effect of noise and stress important variables having relatively small values, *a priori* weights equal to the inverse of the absolute noise standard deviation of the respective variables were employed. The outliers were assessed and removed from the calibration data set. When the model was established "unknown" dependent milling and baking variables of a test (validation) set of 30 entirely new randomly selected wheat



1 = growing region, 2 = wheat quality index, 3 = test weight, 4 = thousand kernel weight, 5 = grain ash content, 6 = gluten swelling no., 7 = grain protein content

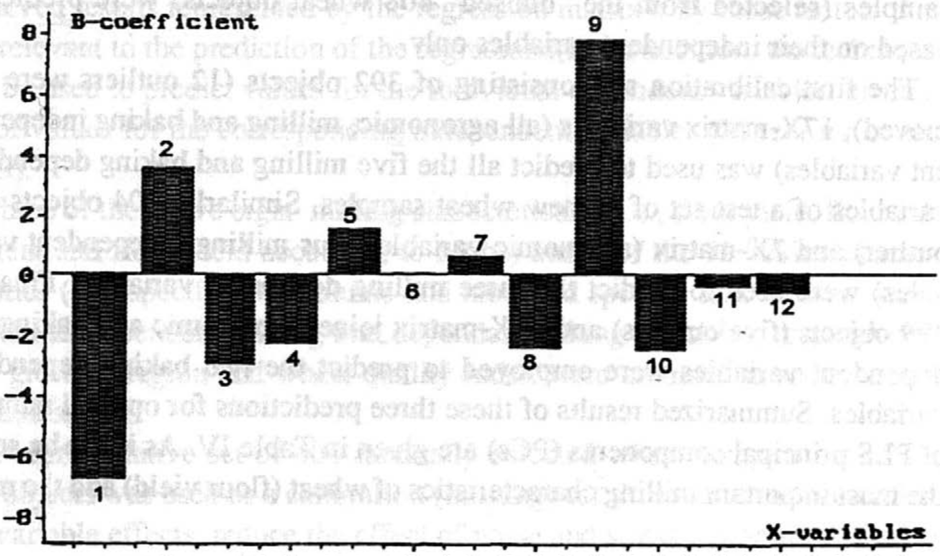
1. Estimated regression coefficients of agronomic and milling variables to flour yield

**samples (selected from the "unused" 408 wheat objects) were predicted based on their independent variables only.**

**The first calibration set consisting of 392 objects (12 outliers were removed), 17X-matrix variables (all agronomic, milling and baking independent variables) was used to predict all the five milling and baking dependent variables of a test set of 30 new wheat samples. Similarly, 404 objects (no outlier) and 7X-matrix (agronomic variables plus milling independent variables) were used to predict the three milling dependent variables. Finally, 399 objects (five outliers) and 12X-matrix joined agronomic and baking independent variables were employed to predict the two baking dependent variables. Summarized results of these three predictions for optimal number of PLS principal components (PCs) are given in Table IV. As it can be seen, the most important milling characteristics of wheat (flour yield) and the most**

**IV. Prediction of dependent milling and baking wheat characteristics**

| Dependent variable (Attribute) | Measured Value | Measured Uncertainty limit | Predicted Value | Predicted Uncertainty limit | Correctly classified Cases | Correctly classified [%] |
|---|---|---|---|---|---|---|
| **17X-variables-6 PCs** | | | | | | |
| Flour yield | 70.6 | 3.5 | 70.4 | 1.5 | 28/30 | 93.3 |
| Flour ash content | 0.52 | 0.02 | 0.51 | 0.02 | 21/30 | 70.0 |
| Mohs | 6.0 | 0.3 | 5.4 | 3.1 | 15/30 | 50.0 |
| Specific loaf volume | 344 | 17 | 352 | 24 | 21/30 | 70.0 |
| Modified specific loaf volume | 301 | 15 | 298 | 28 | 19/30 | 63.3 |
| **7X-variables-3 PCs** | | | | | | |
| Flour yield | 70.6 | 3.5 | 70.1 | 1.4 | 27{30 | 90.0 |
| Flour ash content | 0.52 | 0.02 | 0.51 | 0.02 | 20/30 | 66.7 |
| Mohs | 6.0 | 0.3 | 5.7 | 3.7 | 16/30 | 53.3 |
| **12X-variables-5 PCs** | | | | | | |
| Specific loaf volume | 344 | 17 | 335 | 26 | 22/30 | 73.3 |
| Modified specific loaf volume | 301 | 15 | 299 | 26 | 21/30 | 70.0 |



**1 = growing region, 2 = wheat quality index, 3 = gluten extensibility, 4 = gluten swelling no., 5 = baking quality no. according to Kalina, 6 = falling no., 7 = flour sedimentation, 8 = farinographic absorption, 9 = farinographic dough development time, 10 = farinographic dough stability, 11 = farinographic degree of dough softening, 12 = Kopetz no.**

**2. Estimated regression coefficients of agronomic and baking variables to dough volume**

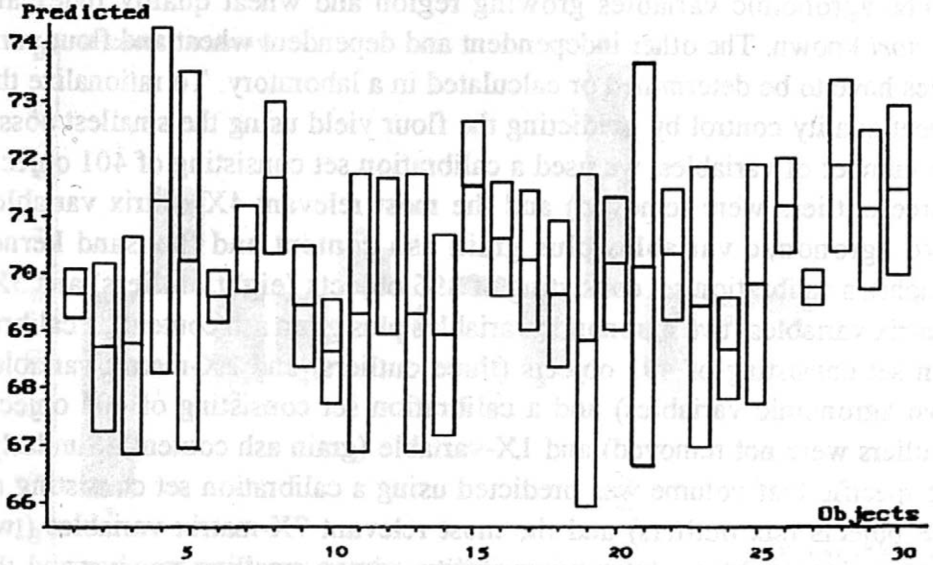
important baking characteristic of wheat (specific loaf volume) were successfully predicted. The predicted values are given with uncertainty limits which tell how much it is possible to trust the corresponding predictions. The measured values of uncertainty limits were ± 5 % of the measured values with the exception of flour ash content where the uncertainty limit was the measured value (in %, w/w) ± 0.02.

The estimated regression coefficients B of each dependent variable to each X-variable (independent variable) indicate the cumulative importance of the independent variables in the model. Thus grain ash content and growing region seem to be of high relevance, corresponding negatively to high flour yield (Fig. 1). The third relatively relevant variable (wheat quality index) corresponds positively to high flour yield. Farinographic dough development time and growing region seem to be of high relevance corresponding positively to high specific loaf volume (Fig. 2) while the variable wheat quality index corresponds negatively to this attribute.

The agronomic variables growing region and wheat quality index are *a priori* known. The other independent and dependent wheat and flour variables have to be determined or calculated in a laboratory. To rationalize the wheat quality control by predicting the flour yield using the smallest possible number of variables, we used a calibration set consisting of 401 objects (three outliers were removed) and the most relevant 4X-matrix variables (two agronomic variables plus grain ash content and thousand kernel weight), a calibration set consisting of 396 objects (eight outliers) and 3X-matrix variables (two agronomic variables plus grain ash content), a calibration set consisting of 401 objects (three outliers) and 2X-matrix variables (two agronomic variables) and a calibration set consisting of 404 objects (outliers were not removed) and 1X-variable (grain ash content). Similarly, the specific loaf volume was predicted using a calibration set consisting of 398 objects (six outliers) and the most relevant 7X-matrix variables (two agronomic variables, gluten extensibility, gluten swelling number and the farinographic data: water absorption, dough development time, dough stability), a calibration set consisting of 396 objects (eight outliers) and 5X-matrix variables (two agronomic variables and the same three farinographic data as above), a calibration set consisting of 398 objects (six outliers) and 3X-ma-

V. Prediction of flour yield and specific loaf volume

| Dependent variable (Attribute) | Independent variables no./ PCs no. | Measured | | Predicted | | Correctly classifieds | |
|---|---|---|---|---|---|---|---|
| | | Value | Uncertainty limit | Value | Uncertainty limit | Caes | % |
| Flour yield | 4/1 | 70.6 | 3.5 | 70.1 | 1.6 | 28/30 | 93.3 |
| | 3/1 | 70.6 | 3.5 | 70.0 | 1.6 | 27/30 | 90.0 |
| | 2/1 | 70.6 | 3.5 | 70.0 | 1.7 | 28/30 | 93.3 |
| | 1/1 | 70.6 | 3.5 | 70.2 | 0.1 | 25/30 | 83.3 |
| Specific loaf volume | 7/3 | 344 | 17 | 336 | 22 | 20/30 | 66.7 |
| | 5/2 | 344 | 17 | 335 | 19 | 20/30 | 66.7 |
| | 3/1 | 344 | 17 | 338 | 24 | 22/30 | 73.3 |
| | 2/1 | 344 | 17 | 338 | 18 | 17/30 | 56.6 |
| | 1/1 | 344 | 17 | 347 | 2 | 11/30 | 36.7 |



**3. Predicted flour yield using three independent variables**

trix variables (two agronomic variables and dough development time), a calibration set consisting of 398 objects (six outliers) and 2X-matrix variables (two agronomic variables) and a calibration set consisting of 404 objects (outliers were not removed) and 1X-matrix variable (dough development time).

The achieved results are summarised in Table V. As it can be seen, three variables (growing region, wheat quality index and grain ash content) or even two agronomic variables alone or grain ash content alone can be used to predict flour yield as the amount of correctly classified cases was higher than 80 %. Similarly, specific loaf volume can be successfully predicted using only three variables (growing region, wheat quality index and dough development time), in which case the amount of correctly classified cases exceeds 70 %.

The relations between the independent variables and the dependent variable using the calculated regression coefficients (B–coefficients) can be expressed in the following form and calculated:

$$[\text{flour yield}] = 79.371 - 0.676\,[\text{growing region}] + 0.263\,[\text{wheat quality index}] - 5.33\,[\text{grain ash content}] \qquad [1]$$

or

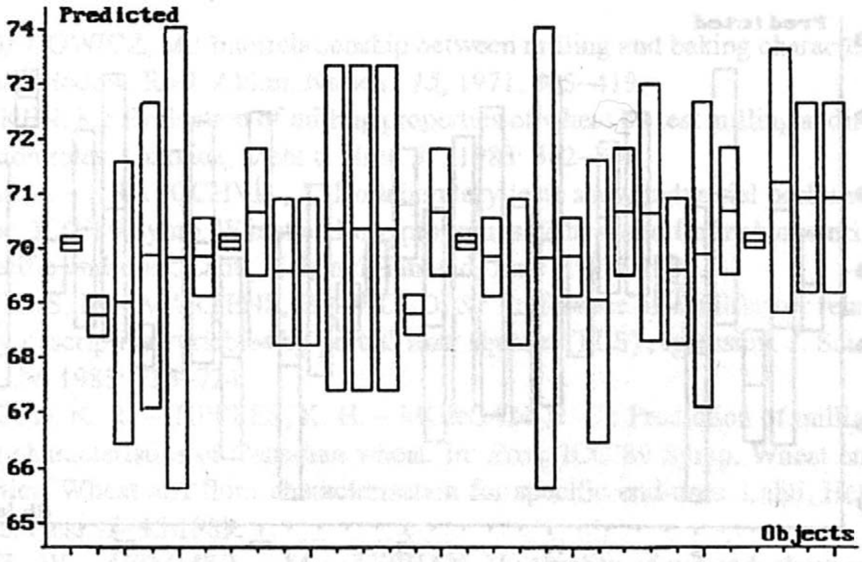
[flour yield] = 70.307 – 0.787 [growing region] +
+ 0.270 [wheat quality index] [2]

and

[specific loaf volume] = 295.676 – 2,449 [growing region] +
+ 6.446 [wheat quality index] + 6.551 [dough development time] [3]

From these equations it can be concluded: the higher the value of growing region the lower the flour yield (–0.676 % (eq. [1]) or –0.787 % (eq. [2]) per unit) and the lower the specific loaf volume (–2.449 ml per unit – eq. [3]). It means the better growing region the higher flour yield or the higher specific loaf volume. Analogically, the higher the value of wheat quality index the higher the flour yield (0.263 % (eq. [1]) or 0.27 % (eq. [2]) per unit) and the higher the specific loaf volume (6.446 ml per unit – eq. [3]). It means that the better the wheat variety the higher the specific loaf volume. As it can be seen from equation [3] every minute of prolongation of dough development time increases specific loaf volume by 6.551 ml.

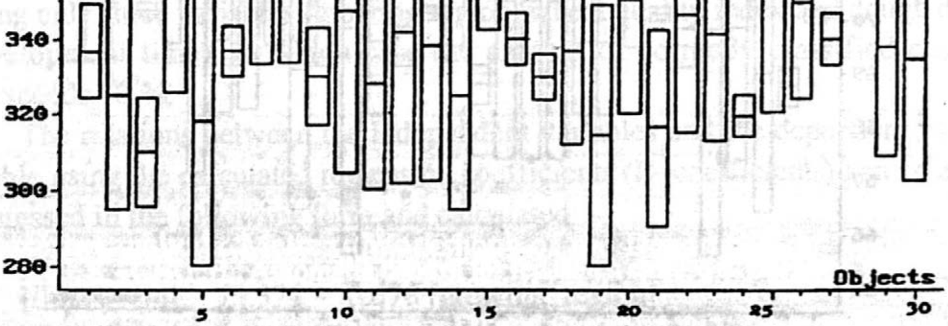
The reduced set of 565 samples of wheat varieties (analyzed from 1980 to 1987) was handled by the pair and multiple correlation analyzes (Holas, Kratochvíl, 1989). The flour yield and specific loaf volume were chosen as direct milling and baking parameters, respectively. It was found that test



4. Predicted flour yield using two independent variables

weight, grain ash content and also kernel hardness are the most important (significant) indirect milling indices. The flour sedimentation value and two farinographic data (dough stability and decrease of consistency) are the most important (significant) indirect baking indices. The authors have plotted the mean values of direct milling and baking parameters against growing region and wheat quality index. They stated on the basis of these plots that the influence of growing region and wheat quality index on milling quality is larger than it could be judged from the relatively low values of correlation coefficients (statistically significant). The baking quality (specific loaf volume) showed monotonous dependences on the above mentioned parameters in accordance with the high value of total pair correlation coefficients (statistically significant).

The predicted values of the above calculated dependent variables were plotted against their corresponding measured values. The calculated value of RMSEP (root mean square of prediction) gave an estimate of the prediction ability. If RMSEP was 2.961 and 3.238 for predicted *versus* measured flour yield using either three or two independent variables and 41.571 for predicted *versus* measured specific loaf volume using three independent variables, samples will have a prediction error of ± 3.0 %, 3.25 % and 41.6 %, respectively.



5. Predicted specific loaf volume using three independent variables

The plots in Figs 3–5 show the above three prediction results. The boxes show the deviations (uncertainty limits) of the prediction results. The predicted values are in the centre of the boxes.

Using the same calibration sets, the dependent milling and baking attributes (flour yield and specific loaf volume) were predicted for a larger test set consisting of 100 entirely new randomly selected wheat samples harvested in the period of 1980–1989. Using the above described three independent variables, the amount of correctly classified flour yields was 92 %. Using only two agronomic independent variables, the amount of correctly classified flour yields was 93 %. Using the above described five independent variables, the amount of correctly classified specific loaf volumes was 62 % and using the above described three independent variables, the amount of correctly classified specific loaf volumes was 65 %.

## Acknowledgement

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## Predikce pekařských a mlynářských ukazatelů potravinářské pšenice

V rozmezí let 1980 až 1989 bylo ve Výzkumném ústavu mlýnsko-pekárenském změřeno celkem 22 fyzikálně-chemických a chemických ukazatelů u 812 vzorků potravinářských pšenic a získané výsledky byly hodnoceny statistickými metodami vhodnými pro vícerozměrná data. Cílem tohoto výzkumu byla identifikace těch proměnných, které jsou relevantní jako pekařské a mlynářské ukazatele kvality potravinářské pšenice. K hodnocení výsledků byly pro kalibraci použity reprezentativní soubory asi 400 náhodně vybraných vzorků a za použití techniky PLS (principal least squares) byly vypočteny závislosti mezi nejvýznamnějšími pekařskými a mlynářskými ukazately vzorků, výtěžností mouky a měrným objemem pečiva. Na základě dat získaných s uvedeným kalibračním souborem byla úspěšně predikována výtěžnost mouky a měrný objem pečiva u dvou souborů náhodně vybraných vzorků (30 a 100 vzorků potravinářské pšenice).

potravinářská pšenice; pekařské ukazatele; mlynářské ukazatele; multivariační metody analýzy; PLS regrese; predikce ukazatelů pšenice; výtěžnost mouky; objem pečiva

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# CHANGES OF SUCROSE CRYSTAL SHAPE IN PRESENCE OF SOME IMPURITIES

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For measured and calculated data from model crystallization tests were carried out to evaluate the mutual effects of dextran, invert sugar and joint effect of both ones on the elongation of sucrose crystals, expressed as ratio of linear dimensions in direction of crystallographic axes C and B (c/b ratio). Further more there were evaluated the changes in sucrose crystal shape in presence of both observed impurities. These results are proved by evidence on the schemes of deformed crystals.

sucrose crystal; crystal shape and morphology; dextran; invert sugar

Paper follows on our previous study (Bubník, Kadlec, 1992) about influence of dextran and invert sugar on change in sucrose crystal shape and on detailed study of this problem reported in literature (Honig, 1963; Smythe, 1971; Mantovani et al., 1991; Vaccari et al., 1990, 1991). This matter is important from point of view of the quality factors of white sugar crystals and also owing to the influence to course of finishing technological operations in refinery, chiefly centrifugation, drying, classification, storage and packaging of crystals.

## MATERIAL AND METHODS

Material and methods of the crystallization tests were used and performed by the same procedure, which was described in our previous paper in this journal (Bubník, Kadlec, 1992). Experiments were carried out by the cooling crystallization technique with pure sucrose solutions and in presence of dextran, glucose, fructose and invert sugar. Concentration levels were for dextran with average molecular weight 87 000 g/mol from 0.2 to 50 g per 100 g of water, for invert sugar in range from 1 to 300 g per 100 g of water. Dimensions of crystals achieved from model crystallization tests were measured by microscope in direction of crystallographic axes C and B. During evaluation of crystals changes the development of individual crystal faces was also observed.

The results of our previous experiments (Bubník et al., 1992) confirmed the known effect of dextran on the sucrose crystal – i.e. elongation along C-axis. This relation was approximated by equation:

$$R_d = R_s + 0.101 \cdot c_d^{\,0.1} + 0.129 \cdot c_d^{\,0.7} \qquad [1]$$

where: $R_d$ – c/b ratio in the presence of dextran

$R_s$ – c/b ratio in pure sucrose solution

$c_d$ – dextran concentration [g per 100 g of water]

Analogically to dextran, also in case of invert sugar, the c/b ratio $R_i$ increases with the rising concentration, but the "retard effect" of concentration on the c/b ratio is yet more evident than by dextran.

This relation is approximated by equation:

$$R_i = R_s + 0.044 \cdot c_i^{\,0.350} \qquad [2]$$

where: $R_i$ – c/b ration in the presence of glucose, fructose or invert sugar

$c_i$ – glucose, fructose or invert sugar concentration [g per 100 g of water]

Taking into consideration the retard effect of concentration dextran and invert, we can assume the similar negative retard effect between these ones mutually. Results of this investigation were expressed by Bubník et al. (1992) by equations:

$$R = R_i + K_1 \cdot c_d^{\,0.1} + K_2 \cdot c_d^{\,0.7} \qquad [3]$$

where: $R$ – c/b ratio in the presence of all impurities (i.e. dextran and invert sugar)

$K_1, K_2$ – coefficients dependent on the invert sugar concentration $c_i$

$$K_1 = 0.101 + 0.108 \cdot c_i^{\,0.1} - 0.00165 \cdot c_i^{\,0.7} \qquad [4]$$

$$K_2 = 0.129 - 0.0144 \cdot c_i^{\,0.277} \qquad [5]$$

A competition between dextran and invert sugar was found, because the values computed by equations [3]–[5] differ from the theoretical ones. The discussion of this phenomenon was presented in the previous works (Bubník et al., 1991, 1992) and in the next chapter this effect is described quantitatively.

## RESULTS AND DISCUSSION

The effect of dextran on the c/b ratio $R_d$ can be described by the increase gradient $\mathrm{Grad}_d$, i.e. the first derivation of this ratio according to the concentration of dextran $c_d$ :

$$\mathrm{Grad}_d = \mathrm{d}\,(R_d)\,/\,\mathrm{d}\,(c_d) \qquad [6]$$

The values of the $\mathrm{Grad}_d$ are presented in the Table I. With the rising concentration of dextran the values of $\mathrm{Grad}_d$ are very strongly decreasing.

I. The values of the $\mathrm{Grad}_d$

| Dextran concentration [g per 100 g of water] | 0.2 | 1 | 5 | 20 | 50 |
|---|---|---|---|---|---|
| $\mathrm{Grad}_d$ . 1 000 | 198 | 100 | 56 | 36 | 27 |

The effect of low concentrations of dextran on c/b ratio is more evident in comparison with the high ones. Taking this fact into account we can assume that the increasing concentration of dextran retards the rate of crystal elongation.

Using the analogical values of the $\mathrm{Grad}_i$, i.e. in this case the first derivation of the c/b ratio $R_i$ according to the concentration of invert sugar $c_i$, it is possible to evaluate the following effects of invert sugar on the c/b ratio:

$$\mathrm{Grad}_i = \mathrm{d}\,(R_i)\,/\,\mathrm{d}\,(c_i) \qquad [7]$$

By comparison of values $\mathrm{Grad}_d$ with $\mathrm{Grad}_i$ at the same values of concentration of dextran and invert sugar, we can say that the values of $\mathrm{Grad}_i$ are approximately about 1 order lower than $\mathrm{Grad}_d$ and consequently the change in c/b ratio, which is raised by one concentration unity, is for invert sugar substantially less than for dextran.

II. The values of $Grad_i$

| Invert sugar concentration [g per 100 g of water] | 1 | 5 | 20 | 100 | 300 |
|---|---|---|---|---|---|
| $Grad_i$.1 000 | 16 | 5.6 | 2.3 | 0.8 | 0.4 |

But the retard effect of invert sugar on the rate of elongation is greater than by dextran. For example, by the increase in the dextran concentration from 1 to 20 g per 100 g of water, the value of $Grad_d$ for dextran decreases from 0.100 to 0.036 (i.e. 2.8 times), whereas for invert sugar at the same concentrations from 0.016 to 0.0023 (i.e. seven times).

The discussed mutual effect of dextran and invert sugar is possible again to describe by means of increase gradients $Grad_d$ and $Grad_i$, which characterize the rate of change of c/b ratio with concentration. The gradients were calculated as the first derivation of c/b ratio:

a. $Grad_d$ – according to the concentration of dextran at various fixed concentration of invert sugar (Table III),

b. $Grad_i$ – according to the concentration of invert sugar at various fixed concentrations of dextran (Table IV).

For all concentrations of invert sugar the dependence of $Grad_d$ on the dextran concentration has a similar tendency, i.e. the value of $Grad_d$ very progressively decreases with rising concentration of dextran. This retard effect is not constant, but it changes according to the concentration of invert sugar. By means of Table III we may determine these effects. The $Grad_d$ decreases

III. The values of $Grad_d$ at various fixed concentration of invert sugar

| Invert sugar concentration [g per 100 g of water] | $Grad_d$ . 1 000 for concentration of dextran [g per 100 g of water] | | | | | |
|---|---|---|---|---|---|---|
| | 0.2 | 1 | 5 | 10 | 25 | 50 |
| 0 | 198 | 100 | 56 | 45 | 36 | 27 |
| 5 | 221 | 95 | 49 | 38 | 30 | 22 |
| 40 | 205 | 84 | 42 | 33 | 26 | 19 |
| 100 | 188 | 76 | 37 | 29 | 22 | 16 |
| 300 | 152 | 60 | 29 | 22 | 17 | 13 |

with rising concentration of invert sugar in whole concentration range with an exception of dextran concentration 0.2 g per 100 g of water. For concentration of dextran above 5 g per 100 g of water the values of $\text{Grad}_d$ - by increase concentration invert from 0 to 300 g per 100 g of water – reduce to one half of their origin values.

Table IV was computed with an aim to describe the change in values of $\text{Grad}_i$ at various fixed concentrations of dextran.

**IV. The change of values $\text{Grad}_i$ at various fixed concentration of dextran**

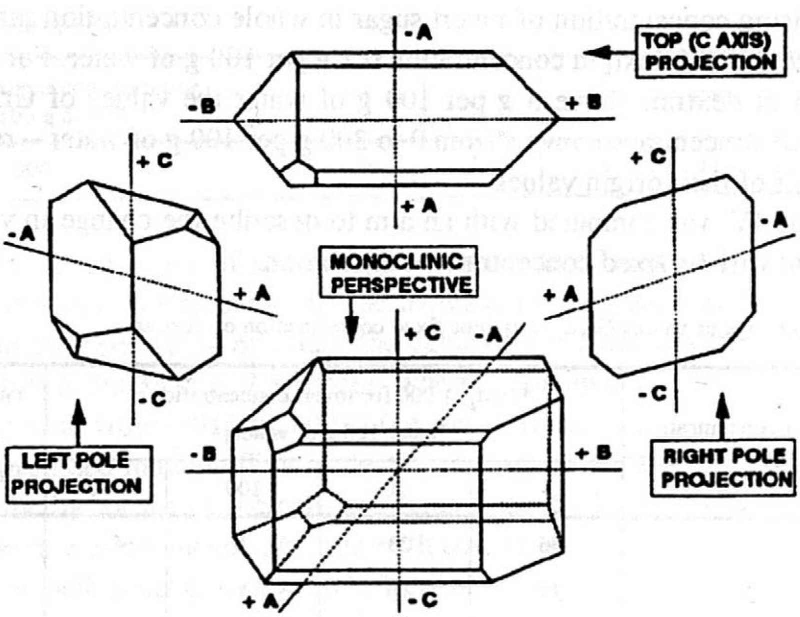
| Dextran concentration [g per 100 g of water] | $\text{Grad}_i$ . 1 000 for invert concentration [g per 100 g of water] | | | | Difference of $\text{Grad}_i$ |
|---|---|---|---|---|---|
| | 5 | 20 | 100 | 300 | Dif(5/300) |
| 0 | 56 | 23 | 8 | 4 | 52 |
| 5 | 37 | 10 | 2 | 0 | 37 |
| 15 | –5 | –6 | –4 | –3 | –2 |
| 30 | –55 | –25 | –10 | –5 | –50 |
| 50 | –110 | –45 | –17 | –8 | –102 |

Dif(5/300) is difference between Grad value for concentration 5 and 300 g per 100 g of water

As it is shown in Table IV, the evaluated effects are in this case more complicated. $\text{Grad}_i$ decreases with rising of invert sugar concentration only at small concentration of dextran (less than 15 g per 100 g of water). At approximately dextran concentration 15 g per 100 g of water the value of $\text{Grad}_i$ is nearly independent on the presence of invert sugar and over 15 g of dextran per 100 g of water the courses have the opposite tendency (i.e. $\text{Grad}_i$ increase with rising invert sugar concentration).

The discussed results in Table IV confirmed and quantitatively expressed the existence of very interested phenomenon – positive and negative mutual effects of the important impurities in cane sugar solutions on the sucrose crystal elongation.

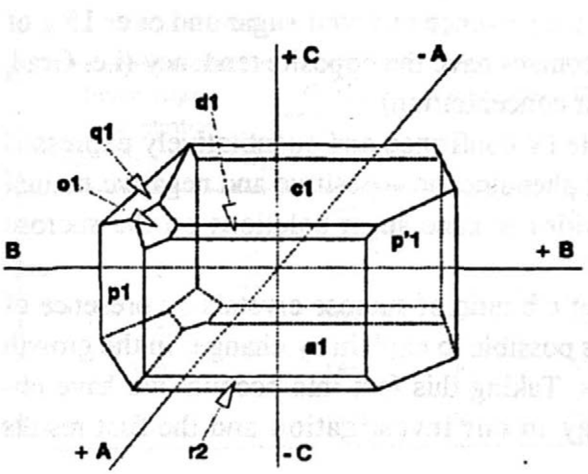
The reason of the variation of c/b ratio of sucrose crystals by presence of glucose, fructose and dextran is possible to explain by changes in the growth rate of individual crystal faces. Taking this fact into account we have observed the crystal morphology in our investigation and the first results



1.Typical sucrose crystal (crystal projection)

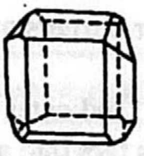
include photographs were presented in our previous papers (Bubník et al., 1991, 1992).

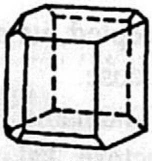
The main observed changes in crystal habits are described by the series of simple pictures – schemes. The typical sucrose crystals growing from pure solution are shown in the Figs. 1 and 2. The number in bracket following the number of scheme gives the concentration of impurities (g per 100 g of water) in order Glucose/Fructose/Dextran (from left to right) - for example

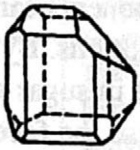


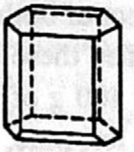
2. Typical sucrose crystal (crystal faces description)

(100/50/10) means: glucose concentration 100, fructose concentration 50 and dextran concentration 10 g per 100 g of water.


Sch.4(-|50|-)

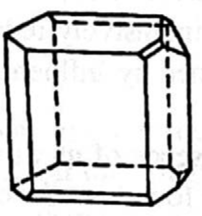

Sch.2(50|-|-)

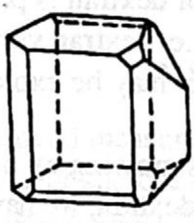

Sch.5(-|200|-)


Sch.3(200|-|-)

The main changes caused by the presence of glucose were observed on the left pole of crystals. With rising concentration of glucose the faces q were continuously reducing (Sch. 2) and by the highest measured concentration (200 g per 100 g of water) these faces of the most crystals disappeared completely (Sch. 3). Typical morphology for this case is a "square" flat projection (in direction of axis A). The phenomenon on the left pole is possible to explain by means of the negative effect of glucose on the growth rate of faces p. The more quickly growing faces q gradually disappear.

By increase in fructose concentration we may observe two main phenomenons – increase in faces q' on the right pole and presence of higher amount of twins. The increase in faces q' shows, that fructose reduces their growth rate and therefore the ratio between growth rates of faces p' and q' is growing. As a characteristic shape of sucrose crystal by projection in direction of axis A by presence of high concentration of fructose a square may be


Sch.6(50|50|-)


Sch.7(100|100|-)


Sch.8(-|-|1)


Sch.9 (-|-|15)


Sch.10a(-|-|50)


Sch.13


Sch.14

(150|150|50)


Sch.10b(-|-|50)


Sch.12(-|-|50)


Sch.11(-|-|50)

accepted, its all four corners are "cutting off" (sch. 4 and 5) as opposite to glucose.

The name "invert" indicates a mixture of the same amount of glucose and fructose. Taking this fact into account the combination or competition of the effect of both components can be supposed. The results of experiments confirmed these assumptions. By presence of high amount of invert (100-200 g per 100 g of water) in sugar solution the effect of glucose predominates on the left pole, because the faces q are disappearing. On the contrary the existence of big faces q' and often also o' on the right pole shows, that in this part of crystal the effect of fructose is predominant (Sch. 6 and 7). Result of these discussed effects is the crystal shape in the form near to the letter D (Sch. 7), what is in agreement with the previous works.

The influence of dextran on the habit of sucrose crystal has been studied by many authors with the aim to explain the reason which causes crystal elongation in the direction of axis C. The main examined phenomena can be divided into the following items:

crystal elongation along axis C,
decrease of faces a, r and c,
decrease and disappearance of faces q and o,
appearance of faces q' and o',
appearance of faces d.

These phenomena were summarized in the following Table V.

The reason of the well known effect of dextran is possible to explaine by very strong blocking of faces p and p', i.e. dextran very intensively reduces the growth rate of these ones. This effect may be explained by influence of dextran adsorption on the prism faces.

By observation of the sucrose crystals growing by presence of mixture of various amount of glucose, fructose and dextran, we have found out the common effect of all these components on the crystal habit. By comparison of the effects of all substances on the left and right poles, we can assume for the lower concentrations, that the behaviour of sucrose crystals is similar to individual effects of each of them. At the highest concentration of dextran (50 g per 100 g of water) and invert sugar (300 g per 100 g of water) the extremely simple shape appeared (Sch. 13 and 14). The crystal elongation along C-axis by high concentrations of all components is lower than the expected (comparison with simple addition effect of all impurities).

V. The phenomena of dextran

| Face | Changes in faces at dextran concentration range (g/100 g water) | | |
|---|---|---|---|
| | 1<br>(Sch. 8) | 10–25<br>(Sch. 9) | 30–50<br>(Sch. 10a, 10b, 11, 12) |
| a | The small difference between the width of faces a1 and a2 appears | The faces are changed to a strip, the widths of a1 and a2 are very different | The a1 and a2 have the form of very narrow strips, unfrequently a1 or a2 disappears |
| r<br>(c) | The faces r1 and r2 have approx. the equal area, (valid also for c1 and c2) | The faces r1 and r2 decrease, the rate of decrease is for both faces different | Further decreases of face r, sometimes r1 or r2 disappear |
| q<br>q'<br>o<br>o' | Common occurrence of face q, often face q'and unfrequently faces o and o' | Frequent occurrence of faces q'and o', face o' frequent with face d, the faces q and o disappear | The face q' occurs rarely, sometimes occurrence of face o' with face d |
| d | Unfrequently occurrence | Occurrence of face d is very often (with the face o'), often d1 or d2 only | Common occurrence of faces d, mostly d1 or d2 only |

## CONCLUSION

Evaluation of the proved measurement about influence of dextran and invert sugar on elongation of sucrose crystal gave these conclusions:

- the change of c/b ratio caused by invert sugar is substantially less than by dextran (for the same concentration of these impurities)
- the retard effect of invert sugar on the rate of elongation is bigger than by dex-tran
- mutual effects of invert sugar and dextran is positive and negative (in dependence of concentration on impurities)
- in presence of dextran are very strongly blocking of faces p and p' and crystals are extremely simple and prolonged needle-shaped.

## List of symbols

| | |
|---|---|
| A, B, C | crystallographic axes |
| c/b ratio | ratio of linear dimensions in direction of crystallographic axes C and B |
| $c_d$ | dextran concentration (g per 100 g of water) |
| $c_i$ | glucose, fructose or invert sugar concentration (g per 100 g of water) |
| Grad$_d$ | increase gradient of dextran |
| Grad$_i$ | increase gradient of invert sugar |
| $K_1, K_2$ | coefficients depended on the invert sugar concentration $c_i$ |
| $R$ | c/b ratio in the presence of impurities [1] |
| $R_d$ | c/b ratio in the presence of dextran [1] |
| $R_i$ | c/b ratio in the presence of glucose, fructose or invert sugar |
| $R_s$ | c/b ratio for pure solution [1] |

**Sucrose crystal faces:**

| Miller index | Symbol | Miller index | Symbol |
|---|---|---|---|
| {1 0 0} | $a_1$ | {1 1 0} | $p_1$' |
| {1 0 0} | $a_2$ | {1 1 0} | $p_2$' |
| {0 0 1} | $c_1$ | {0 1 1} | $q_1$ |
| {0 0 1} | $c_2$ | {0 1 1} | $q_2$ |
| {1 0 1} | $d_1$ | {1 1 1} | $o_1$ |
| {1 0 1} | $d_2$ | {1 1 1} | $o_2$ |
| {1 1 0} | $p_1$ | {1 0 1} | $r_1$ |
| {1 1 0} | $p_2$ | {1 0 1} | $r_2$ |

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## Změny tvaru krystalu sacharózy v přítomnosti některých necukrů

Práce je pokračováním předchozí studie (Bubník, Kadlec, 1992) o vlivu dextranu a invertního cukru na změnu tvaru krystalů sacharózy, což je důležité jak z hlediska kvalitativních ukazatelů krystalů bílého cukru, tak zejména z hlediska důsledků na průběh dokončovacích technologických operací v rafinerii, zejména při odstřeďování, sušení, třídění, skladování a balení krystalu.

U naměřených a početně zhodnocených výsledků modelových krystalizačních pokusů bylo provedeno vyhodnocení vzájemných efektů dextranu a invertního cukru na prodloužení krystalu sacharózy, které se vyjadřuje poměrem lineárních rozměrů ve směru krystalografických os C a B (c/b poměr).

Dále byly vyhodnoceny změny tvaru a jednotlivých ploch krystalu sacharózy ovlivněné přítomností uvedených necukrů. Tyto výsledky jsou dokumentovány na schematických obrázcích deformovaných krystalů.

krystal sacharózy; tvar a morfologie krystalu sacharózy; dextran; invert

Potrav. Vědy, *12*, 1994 (4) : 269-276

# USE OF NEAR-INFRARED SPECTROSCOPY IN THE CHOCOLATE ANALYSIS

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Preliminary studies were performed on dark and milk chocolate with different composition in order to establish how parameters like the fat and the moisture content can be determined with near-infrared reflectance spectroscopy (NIR). The resulting correlation for fat and moisture in dark and milk chocolate was represented with the calibration equations and correlation coefficients with standard errors. The NIR methode applied to dark chocolate provided single term prediction equations giving correlation coefficients of 0.966 for fat NMR and 0.895 for fat ČSN. The correlation coefficient for fat NMR in milk chocolate was 0.859 and the value for moisture ČSN in case of the group of dark and milk chocolate together was 0.738. The validation was carried out in the case of fat in dark chocolate.

near-infrared reflectance spectroscopy; dark; milk; chocolate; fat; moisture; analysis

Near-infrared reflectance analysis has become the attractive and useful technique in food qualitative and quantitative analysis during the last ten years. The most important features of non-polluting environment near-infrared analyzers are the speed of analysis, very low labor costs and simple sample preparation without its destruction. Scotter (1990) presented in the journal Food Control a short but exhaustive review providing brief and theoretical background and discussion of the techniques of near infrared spectroscopy, particularly mathematical.

NIR has been used for internal control of chocolate and cocoa products. Cadbury-Schweppes (Gardam, 1984) has been using NIR instrumentation for routine analysis and process monitoring of various chocolate products for many years. The evaluation of results of fat, sucrose and moisture demonstrated that the three products discussed in this contribution require little or no sample preparation. The precision of the instrumental NIR method was comparable in all cases to the classical laboratory method. **Permanyer** and **Perez** (1989) evaluated the potential application of the NIR spectroscopy in the quality control of powdered cocoa products. Results showed that moisture, fat, and sucrose could be analyzed in powdered cocoa products by near infrared reflectance spectroscopy because good correlation coefficients and low standard errors were achieved in prediction study.

**Kradjel** (1991) pointed out advantages of NIR analysis in confectionery industry, which enables rapid determination of fat, sugar and moisture in various stages of both chocolate and non-chocolate processing. **Davies** (1991) correlated sensory data on chocolate products and NIR spectra of raw cocoa beans. His results were very promising. The replacement of difficult and demanding sensory analysis by simple and reliable measurement of NIR absorption can be considered.

The objective of this study was to investigate the use of NIR spectroscopy as a rapid analytical method for determining moisture and fat in different types of chocolate which would facilitate its quality control.

## MATERIALS AND METHODS

Since the NIR technique is based on the correlation of spectroscopic response with constituent concentration, calibration of NIR instrument was carried out on a set of known samples in which moisture and fat varied. Seventy-five samples were collected from production.

The reference chemical methods were: for moisture the official method ČSN (1984a), and for fat the official method ČSN (1984b) and NMR method (**Doležal**, 1984).

The official method for moisture ČSN 56 0146 is drying at 105 °C for 3 hrs. The official method for fat ČSN 56 0146 is extraction with petroleum ether (b.p. 40–60 °C) and the NMR method for fat is based on nuclear magnetic resonance of protons in liquid phase.

For the NIR analysis, samples were grated and filled into the cell of the NIR instrument, Inframatic 8620, PerCon Ltd., equipped with 20 discrete narrowband filters :

| Filter [No.] | Wavelengths [nm] | Filter [No.] | Wavelengths [nm] |
|---|---|---|---|
| 1 | 2348 | 11 | 2050 |
| 2 | 2336 | 12 | 1982 |
| 3 | 2310 | 13 | 1940 |
| 4 | 2270 | 14 | 1818 |
| 5 | 2230 | 15 | 1778 |
| 6 | 2208 | 16 | – |
| 7 | 2190 | 17 | – |
| 8 | 2180 | 18 | 1722 |
| 9 | 2139 | 19 | 1680 |
| 10 | 2100 | 20 | 1445 |

The stepwise addition program verified wavelengths and the best pair, triple etc. of filters were selected by the multiple correlation. The multiple linear regression evaluated the regression coefficients of the calibration curve which correspond to the set of wavelengths chosen.

## RESULTS AND DISCUSSION

The compositional data for calibration and validation, namely fat ČSN, fat NMR and moisture ČSN are summarized in Tables I–IV.

The first task was a statistic comparison of the ČSN and NMR methods for fats (Table V) in dark and milk chocolate. The results of simple regression did not confirm the hypothesis that both methods were giving the values without any significant difference in the whole estimated range of fat content. It was the reason why the results of both methods for fats were used for the NIR calibration independently. The data treatment was processed after the interactive outlier rejection.

The results of processing the NIR spectra are summarized in Table VI. The resulting correlation for fat NMR and fat NIR in dark chocolate was represented by the calibration equation :

$$x_{NMR} = 36.6 + 36.3*H_{13} - 119.7*H_{14} - 377.9*H_{15} - 334.2*H_{20} \qquad [1]$$

$$H_i = \log (1/R_i) \qquad [2]$$

I. Content of fat ($x_{NMR}$ and $x_{SOX}$) and moisture in dark moulding chocolate

| Sample No. | $x_{NMR}$ [%] | $x_{SOX}$ [%] | Moisture | Sample No. | $x_{NMR}$ [%] | $x_{SOX}$ [%] | Moisture |
|---|---|---|---|---|---|---|---|
| **Calibration data** | | | | | | | |
| 1 | 33.16 | 33.64 | 1.355 | 11 | 33.11 | 33.10 | 1.374 |
| 2 | 31.55 | 32.70 | 1.213 | 12 | 31.99 | 32.20 | 1.290 |
| 3 | 32.19 | 33.13 | 1.282 | 13 | 32.65 | 33.13 | 1.253 |
| 4 | 33.95 | 33.38 | 1.149 | 14 | 32.91 | 31.60 | 1.264 |
| 5 | 33.08 | 33.34 | 1.079 | 15 | 32.58 | 31.86 | 1.327 |
| 6 | 33.68 | 34.17 | 1.069 | 16 | 32.39 | 33.14 | 1.326 |
| 7 | 31.74 | 33.42 | 1.172 | 17 | 32.43 | 32.54 | 1.397 |
| 8 | 32.73 | 33.09 | 1.253 | 18 | 32.22 | 32.64 | 1.245 |
| 9 | 32.74 | 32.97 | 1.198 | 19 | 32.17 | 33.29 | 1.228 |
| 10 | 33.04 | 33.70 | 1.267 | 20 | 32.54 | 33.26 | 1.320 |
| **Validation data** | | | | | | | |
| 21 | 32.33 | 33.48 | 1.164 | 24 | 30.66 | 31.77 | 0.845 |
| 22 | 33.87 | 34.82 | 0.876 | 25 | 36.37 | 37.10 | 1.309 |

The correlation coefficient of the calibration is CORR = 0.769 with the standard error SEE = 0.453, but the validation gave more convincing results with the correlation coefficient CORR = 0.966 and SEP = 0.434.

The resulting correlation for fat ČSN and fat NIR in dark chocolate was represented by the calibration equation :

$$x_{CSN} = 35.7 - 141.8*H_1 + 127*H_3 + 639.5*H_{10} - 911.1*H_{11} + 330*H_{14} \quad [3]$$

The correlation coefficient of the calibration is CORR = 0.872 with the standard error SEE = 0.371 and the validation confirmed this value (CORR = 0.895, SEP = 0.956).

As to a small group of milk covering and moulding chocolate and very low correlation for ČSN fat the calibration was carried out for the NMR fat only:

$$x_{NMR} = 32.2 + 1521.9*H_{10} - 2206.5*H_{11} + 819.5H_{14} - \\ - 602.8*H_{19} + 596*H_{20} \quad [4]$$

**II. Content of fat ($x_{NMR}$ and $x_{SOX}$) and moisture in dark covering chocolate**

| Sample No. | $x_{NMR}$ [%] | $x_{SOX}$ [%] | Moisture | Sample No. | $x_{NMR}$ [%] | $x_{SOX}$ [%] | Moisture |
|---|---|---|---|---|---|---|---|
| **Calibration data** | | | | | | | |
| 26 | 34.05 | 34.64 | 1.290 | 37 | 33.26 | 34.12 | 1.306 |
| 27 | 34.76 | 33.91 | 1.203 | 38 | 33.29 | 34.02 | 1.177 |
| 28 | 34.09 | 34.57 | 1.275 | 39 | 32.64 | 34.18 | 1.308 |
| 29 | 33.52 | 34.55 | 1.325 | 40 | 33.48 | 34.25 | 1.278 |
| 30 | 34.23 | 33.64 | 1.245 | 41 | 32.41 | 33.09 | 1.466 |
| 31 | 33.34 | 33.97 | 1.393 | 42 | 32.88 | 33.85 | 1.231 |
| 32 | 33.66 | 34.02 | 1.278 | 43 | 34.93 | 34.78 | 1.314 |
| 33 | 32.43 | 33.34 | 1.154 | 44 | 34.03 | 33.89 | 1.491 |
| 34 | 33.17 | 33.52 | 1.265 | 45 | 33.42 | 34.14 | 1.381 |
| 35 | 32.89 | 33.25 | 1.292 | 46 | 34.59 | 35.31 | 1.316 |
| 36 | 33.18 | 34.54 | 1.461 | 47 | 34.62 | 34.42 | 1.138 |
| **Validation data** | | | | | | | |
| 48 | 31.92 | 32.72 | 1.246 | 53 | 32.33 | 32.87 | 0.846 |
| 49 | 29.92 | 30.82 | 1.381 | 54 | 30.26 | 31.11 | 1.236 |
| 50 | 33.37 | 34.29 | 1.302 | 55 | 27.64 | 28.23 | 1.336 |
| 51 | 32.17 | 32.79 | 1.169 | 56 | 35.84 | 36.72 | 1.311 |
| 52 | 31.19 | 32.18 | 1.254 | | | | |

**III. Content of fat ($x_{NMR}$ and $x_{SOX}$) and moisture in milk moulding chocolate - Calibration data**

| Sample No. | $x_{NMR}$ [%] | $x_{SOX}$ [%] | Moisture |
|---|---|---|---|
| 1 | 30.47 | 31.45 | 1.398 |
| 2 | 30.86 | 32.23 | 1.569 |
| 3 | 32.23 | 32.27 | 1.451 |
| 4 | 32.39 | 33.41 | 1.478 |

The correlation coefficient of the calibration is CORR = 0.859 with the standard error SEE = 0.536.

Results for the moisture were not very promising and the calibration was done for the group of dark and milk chocolate together :

$$x_M = 1.51 + 1.9*H_4 - 0.87*H_5 - 124.9*H_7 + 117.5*H_8 + 4.9*H_{18} \quad [5]$$

The correlation coefficient of the calibration is CORR = 0.738 with the standard error SEE = 0.075. The validation was not carried out.

**IV. Content of fat ($x_{NMR}$ and $x_{SOX}$) and moisture in milk covering chocolate - Calibration data**

| Sample No. | $x_{NMR}$ [%] | $x_{SOX}$ [%] | Moisture | Sample No. | $x_{NMR}$ [%] | $x_{SOX}$ [%] | Moisture |
|---|---|---|---|---|---|---|---|
| 9 | 33.97 | 35.21 | 1.428 | 15 | 33.36 | 34.53 | 1.326 |
| 10 | 34.03 | 35.27 | 1.639 | 16 | 33.13 | 34.47 | 1.521 |
| 11 | 32.95 | 34.15 | 1.462 | 17 | 34.07 | 34.06 | 1.340 |
| 12 | 33.33 | 34.01 | 1.449 | 18 | 33.99 | 35.31 | 1.459 |
| 13 | 32.36 | 33.55 | 1.511 | 19 | 33.89 | 34.72 | 1.504 |
| 14 | 32.39 | 34.46 | 1.300 | | | | |

**V. Regression analysis on the CSN and NMR methods for fat in dark covering and moulding chocolate**

| Parametr | Estimate | Standard Error | T Value |
|---|---|---|---|
| Intercept | 0.32 | 1.94 | 0.16 |
| Slope | 0.97 | 0.06 | 16.73 |
| CORR = 0.917 | | | |

**VI. Simple regression analysis on the CSN and NMR methods for fat in milk covering and moulding chocolate**

| Parametr | Estimate | Standard Error | T Value |
|---|---|---|---|
| Intercept | 3.26 | 3.02 | 1.08 |
| Slope | 0.87 | 0.09 | 9.76 |
| CORR = 0.929 | | | |
| SEE = 0.404 | | | |

**Deleted points: 5, 17**

VII. Multiple regression for CSN fat, NMR fat and moisture in dark and milk chocolate

| Chocolate | Calibration | | Validation | | Selected filters |
|---|---|---|---|---|---|
| | CORR | SEE | CORR | SEP | |
| DARK | | | | | |
| fat NMR | 0.769 | 0.453 | 0.966 | 0.434 | 13, 14, 15, 20 |
| fat CSN | 0.872 | 0.371 | 0.895 | 0.956 | 1, 3, 10, 11, 14 |
| MILK | | | | | |
| fat NMR | 0.859 | 0.536 | – | – | 10, 11, 14, 19, 20 |
| DARK + MILK | | | | | |
| moisture | 0.738 | 0.075 | – | – | 4, 5, 7, 8, 18 |

The correlation results presented here indicate that near-infrared reflectance can be related to the composition analysis of dark and milk chocolate. It can be supposed that the accuracy of measurements can be increased if the liquid samples of chocolate are treated. In this case the influence of cocoa butter crystals is eliminated. We suppose next ineasuring with the cell for liquid samples with high viscosity and the estimated parameters will be enlarged on sugars.

**Acknowlegment**

The authors are grateful to Ing. Jiří **Cibulka** and Hana **Holubová** from Čokoládovny a.s. for excellent technical assistance and Dr. Dagmar **Šrámková**, Ing. Miloslava **Buriánková**, CSc. and Ing. Josef **Smíšek** from Čokoládovny a.s., who gave unstinting support to this investigations.

## List of symbols

| | |
|---|---|
| CORR | correlation coefficient |
| ČSN | abbreviation, index for the official methods |
| $H_i$ | wavelength or the filter number |
| $x$ | content of the constituent [%] |
| NMR | abbreviation, index for the nuclear magnetic resonance |
| NIR | abbreviation, index for the near-infrared reflectance spectroscopy |
| SEE | standard error of the calibration |
| SEP | standard error of the determination |
| T | value of *t*–test |

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ČSN 560146 – část 4. Stanovení obsahu tuku, 1984b.

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### Možnosti spektroskopie v blízké oblasti infračerveného záření v analýze čokolády

V této předběžné studii jsou uvedeny první experimentální zkušenosti se stanovením obsahu tuku a vlhkosti v čokoládových polevách tmavých a mléčných pomocí spektroskopie v blízké oblasti infračerveného záření v reflektančním uspořádání (NIR spectroscopy). Ke kalibraci NIR spektrofotometru Inframatic 8620 (PerCon Ltd.) byly použity v případě stanovení tuku hodnoty získané extrakční metodou ČSN a nukleární magnetickou rezonancí (NMR). Ke kalibraci při stanovení vody byly použity hodnoty získané sušením při 105 °C. Pomocí postupné korelace byly nalezeny vhodné kombinace filtrů a pomocí vícenásobné lineární regrese byly spočteny koeficienty regresních rovnic.

spektroskopie v blízké oblasti infračerveného záření; čokoláda tmavá; čokoláda mléčná; tuk; vlhkost; analýza

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# CHANGES IN BEHAVIOUR AND PHYSIOLOGY OF FED-BATCH *SACCHAROMYCES CEREVISIAE* CULTURE

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The impact of feeding strategies on physiology and behaviour of *Saccharomyces cerevisiae* cells in fed-batch culture was determined. For fed-batch cultivation of baker's yeasts molasses feeding controlled by the level of one parameter (ethanol, $CO_2$ or DOC) was applied. The growth characteristic and behaviour of the whole microbial system depend on feeding conditions. The feeding resulted in four main growth areas: aerobic growth, growth under aerobic fermentation conditions (Crabtree effect – the glycolysis over-flow metabolism resulting in ethanol production from the sugar that exceeds the respiration capacity of culture), growth on sugar and ethanol simultaneously in sugar limited culture (the sugar and ethanol are consumed at the same time without diauxic lag) and growth on produced ethanol. The most important is to find the optimum molasses feeding and the optimum specific growth rate to be in good agreement with the yeast physiology and activity. The best results were obtained in ethanol controlled cultivation.

fed-batch; yeasts; control; physilogy; feeding strategy

Fed-batch cultivations have many advantages such as easy operation, simple equipment and high cell concentrations depending on substrate concentration (Huang, Chu, 1981). In the fed-batch cultivation a small medium amount is added at the beginning and subsequently molasses medium is fed continuously in small doses during the fermentation process. Optimal feeding rate is especially important to avoid both substrate and product inhibitions and to increase productivity, yield and quality of produced microbial biomass. The influence of feeding strategy on cell metabolic pathways was described by several authors (Miskiewicz, 1985; Enfors, 1990). By successful substrate feeding a complete substrate utilization and promotion of productivity are expected (Huang, Chu, 1981). Since it is not usually possible to measure the substrate concentration directly and continuously, indirect parameters correlating with substrate utilization rate are often measured in order to control the feeding procedure. That is why some signals corresponding to changes of parameters in a fermentor, e.g. dissolved oxygen concentration, pH, vapour phase substrate concentration (e.g. ethanol) and $CO_2$ concentration in exhaust gas are chosen as controlled parameters (Huang, Chu, 1981).

## MATERIALS AND METHODS

### Microorganism

All cultivations were carried out with the yeast strain *Saccharomyces cerevisiae* var. *Hansen 03/2* from The Yeast Culture Collection of the Department of Fermentation Chemistry and Bioengineering, Institute of Chemical Technology, Prague. The culture was cultivated at 30 °C on a rotary shaker for 48 hours, centrifuged and used as an inoculum for fed-batch cultivation.

### Medium

The medium for inoculum contained per 1 liter: 40 g molasses, 1 g $(NH_4)_2SO_4$, 1 g $(NH_4)_2HPO_4$, 0.4 g $MgSO_4.7H_2O$ and 1 g dry yeast auto-lysate. Production molasses medium (molasses of solids concentration 28% w/v) contained per 1 liter 68 ml 26% $NH_4OH$, 17 ml 30% $H_3PO_4$ and 24 ml 25% $H_2SO_4$ and biotin additions.

### Materials

Cultivations of yeasts were carried out in a standard laboratory fermentor (LF 2D, Development Workshops of the Czech Academy of Sciences) of the total volume 3 litres (working volume 1.5 litre). The bioreactor was temperature and pH controlled. Dissolved oxygen concentration (DOC) was measured by an oxygen electrode. Ethanol concentration was measured in vapour phase by analyzer Metrex (Workshops of the Department of Technical Physics and Electrotechnics, ICT Prague, CZ). $CO_2$ and $O_2$ were measured by Infralyt 5 (VEB Junkalor Dessau, D) and Permolyt 2 (VEB Junkalor Dessau, D), respectively. Medium was fed into the fermentor by a programmable dispensing pump (DP-200 NBS Co., New Jersey, U.S.A.) directly linked to computer CHP 3000. Cultivation time was always 10 hours.

### Analytical methods

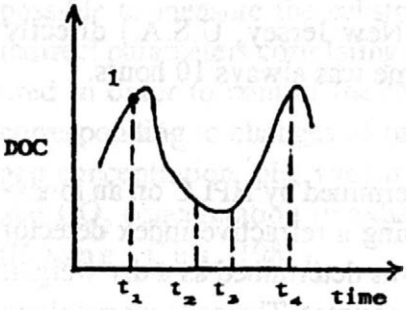
Ethanol and sugar concentrations were determined by HPLC on an ionexchanger Ostion LGKS 0800 $Ca^{2+}$ column using a refractive index detector (RIDK 101 LP, CZ).The cell concentration was determined as a dry weight. Cell budding was determined using a Bürker counter. The cells morphology was studied by microscope. The biomass elementary composition was determined in a Central Laboratory of Organic Analysis (Institute of Chemical Technology, Prague). The ATP, ADP and AMP levels were determined after perchloric acid extraction (Chapman et al., 1971) by HPLC on a Separon SGX C18 column using a UV detector at 254 nm (Kodíček et al., 1990). The glycogen content was determined by absorbance measurement at 660 nm after staining with an aqueous solution of iodine/potassium iodide (Quain, Tubb, 1983).

## RESULTS AND DISCUSSION

The aim of our work was to compare the behaviour and selected metabolic response of yeast *Saccharomyces cerevisiae* in fed-batch system depending on the various growth conditions and to propose the optimal feeding strategy. In all our experiments the only control variable was the feeding rate. One dose was always the same (0.1 ml) and the dose frequency was controlled according to corresponding measured signals. In our experiments the molasses medium was fed into the fermentor according to following parameters: dissolved oxygen concentration (DOC), ethanol and $CO_2$ concentrations.

### DOC controlled cultivation

Dissolved oxygen concentration (DOC) can be used as a parameter to control substrate feed rate. Oxygen and substrate are consumed simultaneously and the substrate feeding is connected with the DOC changes. Fig. 1 shows the full feeding cycle. Substrate is fed at point 1; ($t_1$–$t_2$) is a period of unlimited substrate uptake, ($t_2$–$t_3$) represents Crabtree effect (excess of substrate) and ($t_3$–$t_4$) period is substrate limitation phase – cell starvation (Miskiewicz, 1985).



**1. The full medium feeding cycle**

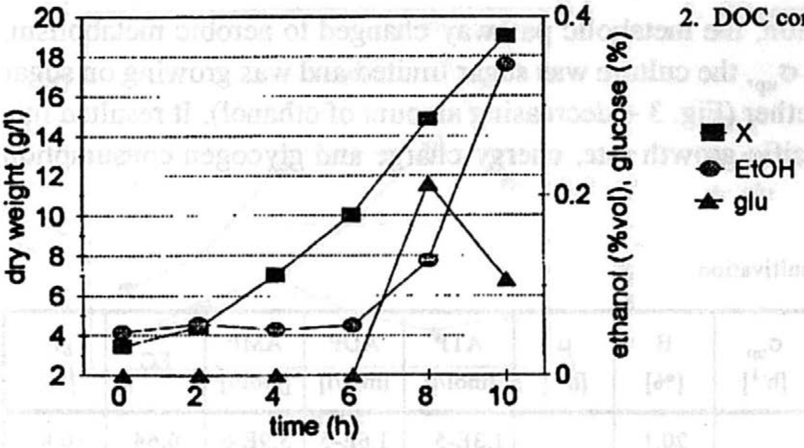
$t_1$–$t_2$ = unlimited sub strate uptake
$t_2$–$t_3$ = Crabtree effect
$t_3$–$t_4$ = cell starvation

In these experiments a new substrate dose was fed into the fermentor just after the last substrate dose was consumed (DOC started sharply to increase – at time $t_3$, Fig. 1) to eliminate the overall starvation period. During the first six hours the culture was grown aerobically – the specific substrate intake rate ($\sigma_{in}$) was equal to the specific substrate uptake rate ($\sigma_{up}$), no ethanol was produced and glycogen content was slowly increasing (Table I). Between the 2nd and the 4th hours the substrate dose was too low and the culture was substrate limited, it resulted in a decrease of specific growth rate ($\mu$), energy charge (EC) and glycogen consumption (Table I).

**I. DOC controlled cultivation**

| Time [h] | $\sigma_{in}$ [$h^{-1}$] | $\sigma_{up}$ [$h^{-1}$] | B [%] | $\mu$ [$h^{-1}$] | ATP [mol/l] | ADP [mol/l] | AMP [mol/l] | EC | gly [%] |
|---|---|---|---|---|---|---|---|---|---|
| 0 | | | 11.6 | | 1.3E-5 | 7.3E-6 | 1.4E-6 | 0.76 | 3.0 |
| 2 | 0.43 | 0.43 | 66.9 | 0.12 | 7.1E-6 | 6.2E-6 | n | n | 7.2 |
| 4 | 0.35 | 0.35 | 60.2 | 0.24 | 7.6E-7 | 6.3E-6 | 2.0E-6 | 0.43 | 4.5 |
| 6 | 0.35 | 0.35 | 55.8 | 0.18 | 8.5E-6 | 2.2E-6 | 1.4E-6 | 0.80 | 5.3 |
| 8 | 0.41 | 0.33 | 52.9 | 0.20 | 3.0E-6 | 1.5E-6 | 1.9E-7 | 0.80 | 6.1 |
| 10 | 0.51 | 0.47 | 52.4 | 0.13 | 1.2E-5 | 8.9E-6 | 2.3E-6 | 0.71 | 5.0 |

From the 6th hour of cultivation the molasses dosing frequency started to be higher, $\sigma_{in}$ increased slightly more than $\sigma_{up}$ which led to appearance of Crabtree effect demonstrated by higher ethanol production at the expense of sugar exceeding the respiration capacity of the culture (Fig. 2).



2. DOC controlled cultivation

During the whole fermentation time the culture energy charge and cell ATP levels were stable (EC = 0.8, ATP = $10^{-5}$–$10^{-6}$ mol/g) except the substrate limited period, when they were decreasing (EC = 0.4, ATP = $10^{-7}$ mol per g) (Table I). The ADP and AMP levels were constant ($10^{-4}$ mol/g), they decreased after the metabolic pathway change in the 6th hour ($10^{-7}$ mol/g) and then reached the original level again (Table I). The glycogen content of inoculum was low (3.1%) and varied during the whole fermentation time depending on amount of sugar fed to the culture. The average glycogen content was 5%.

### Carbon dioxide controlled cultivation

$CO_2$ concentration in outlet gas is always connected with fermentation or respiration of the cells. It was found out from our previous studies (Rychtera et al., 1993) that $CO_2$ concentration in fed-batch culture is a linear function of cultivation time. The molasses feeding was carried out to reach the increasing $CO_2$ concentration straight line to the 9th hour. Slope of this straight line was taken from height of $CO_2$ peaks dependence (Dušánek, 1993).

During the first eight hours the culture was growing under excess of sugar ($\sigma_{in}$ was higher than $\sigma_{up}$) and resulted in Crabtree effect and aerobic fermentation type of metabolism. The concentration of ethanol produced from the sugar exceeding the amount corresponding to respiratory capacity of cells was increasing to 0.38% vol. Under these conditions the specific growth rate was high (around 0.23 $h^{-1}$), energy charge of cells fluctuated from 0.56 to 0.83 (Table II) and glycogen content of cells increased to 8.6%. The last hour of cultivation, the metabolic pathway changed to aerobic metabolism. $\sigma_{in}$ was equal to $\sigma_{up}$, the culture was sugar limited and was growing on sugar and ethanol together (Fig. 3 – decreasing amount of ethanol). It resulted in a decrease of specific growth rate, energy charge and glycogen consumption (Table II).

II. $CO_2$ controlled cultivation

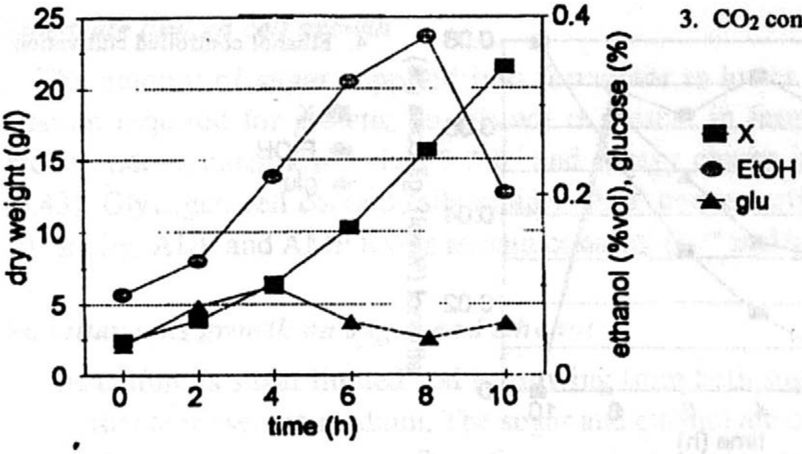
| Time [h] | $\sigma_{in}$ [$h^{-1}$] | $\sigma_{up}$ [$h^{-1}$] | B [%] | $\mu$ [$h^{-1}$] | ATP [mol/l] | ADP [mol/l] | AMP [mol/l] | EC | gly [%] |
|---|---|---|---|---|---|---|---|---|---|
| 0 | | | 20.1 | | 1.3E-5 | 1.6E-5 | 3.9E-6 | 0.64 | 0.8 |
| 2 | 0.53 | 0.40 | 78.1 | 0.23 | 1.2E-5 | n | 4.0E-7 | n | 6.1 |
| 4 | 0.55 | 0.45 | 76.4 | 0.26 | 1.5E-5 | 5.9E-6 | 1.1E-5 | 0.56 | 5.5 |
| 6 | 0.50 | 0.47 | 64.0 | 0.24 | 1.2E-5 | 5.0E-6 | 4.6E-7 | 0.83 | 8.6 |
| 8 | 0.46 | 0.44 | 53.8 | 0.21 | 6.0E-6 | 4.5E-6 | 1.0E-6 | 0.72 | 7.6 |
| 10 | 0.26 | 0.24 | 33.9 | 0.16 | 8.8E-7 | 3.3E-6 | 6.2E-7 | 0.53 | 5.9 |

The ATP cells content was almost constant for the first six hours ($10^{-5}$ mol per g) and then decreased (10-7 mol/g). The ADP cell content was slowly decreasing during the whole fermentation time ($10^{-5}$–$10^{-6}$ mol/g). The AMP cell content strongly fluctuated for the first six hours and subsequently the fluctuation weakened (Table II). The average glycogen content was 5.9%.

**Ethanol controlled cultivation**

Ethanol concentration is often used as a controlled variable (constant or changing profile), the value of which is maintained in the range 0.05–0.2% vol (Albrecht, 1989). In our experiment the molasses medium was fed into fermentor to keep the increasing straight line of ethanol concentration in vapour phase to 8th hour. During the last two hours of cultivation the straight line of ethanol decreased to reach zero.

During the first hours of cultivation (0.–4. h) the culture was growing under Crabtree effect conditions, when $\sigma_{in}$ was higher than $\sigma_{up}$ and ethanol was produced from excess of sugar. The specific growth rate and energy charge were higher (Table III) and ethanol concentration slowly increased to 0.07% vol (Fig. 4). The glycogen content of inoculum was high (11.6%) and differs



**3. $CO_2$ controlled cultivation**

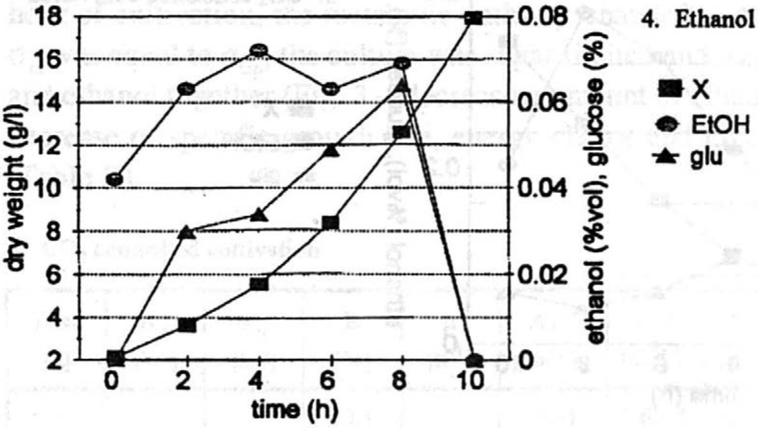
from values determined in previous two experiments. During the first two hours of experiment glycogen content increased even more (Table III).

During a short period around the half of experimental time (4.–8.h) the Crabtree effect ceased, no more ethanol was produced and the culture was growing only from supplied sugar (ethanol concentration was constant). It resulted in a slight decrease of specific growth rate and energy charge (Table III), decrease of glycogen content was however rapid (to 5%).

During the last two hours of fermentation the culture was sugar limited and was growing from sugar and ethanol simultaneously. This situation lead to further specific growth rate decrease but the glycogen content was constant (4.7%) – Table III.

**III. Ethanol controlled cultivation**

| Time [h] | $\sigma_{in}$ [$h^{-1}$] | $\sigma_{up}$ [$h^{-1}$] | B [%] | μ [$h^{-1}$] | ATP [mol/l] | ADP [mol/l] | AMP [mol/l] | EC | gly [%] |
|---|---|---|---|---|---|---|---|---|---|
| 0 | | | 17.7 | | 7.3E-4 | 3.4E-5 | 8.7E-6 | 0.97 | 11.6 |
| 2 | 0.45 | 0.40 | 71.7 | 0.26 | 5.5E-5 | 1.3E-5 | 1.1E-5 | 0.78 | 14.9 |
| 4 | 0.34 | 0.30 | 65.4 | 0.21 | 4.6E-5 | 8.3E-6 | 3.8E-6 | 0.86 | 13.2 |
| 6 | 0.27 | 0.24 | 40.8 | 0.21 | 6.2E-5 | 1.2E-5 | 9.4E-6 | 0.81 | 6.1 |
| 8 | 0.38 | 0.35 | 52.0 | 0.20 | 5.2E-5 | 1.5E-5 | 1.5E-5 | 0.72 | 4.7 |
| 10 | 0.18 | 0.18 | 14.2 | 0.18 | 3.1E-5 | n | 9.7E-6 | n | 4.8 |



**4. Ethanol controlled cultivation**

This experiment differs from two others because at the start there was quite a high glycogen content (11.6%), energy charge (0.97) and ATP level ($7.10^{-4}$ mol/g). The ATP content decreased during the first two hours, then being constant up to the 8th hour and during the last two hours of experiment was slowly decreasing again. The ADP content slowly decreased during the first four hours and then increased to the 8th hour. The AMP level fluctuated during the first 4 hours and subsequently was increasing up to the 8th hour and during the last two hours it was slowly decreasing (Table III).

## CONCLUSIONS

### Cell Physiology in Dependence on Growth Conditions

The molasses feeding effect results in four main culture growth areas: aerobic growth under balanced conditions ($\sigma_{in} = \sigma_{up}$), substrate limited cells growth, simultaneous growth on sugar and ethanol and growth under aerobic fermentation conditions (Crabtree effect).

### *Balanced aerobic growth*

Specific sugar intake rate is just equal to the specific sugar uptake rate, all the fed sugar is used for growth and maintenance process and no ethanol is produced. Specific growth rate of culture is lower than $0.2\ h^{-1}$, energy charge of cells is around 0.8 and glycogen cell content decreases slowly. ATP and ADP levels are constant in the range $10^{-5}$–$10^{-6}$ mol/g, AMP increases in the range $10^{-7}$–$10^{-6}$ mol/g.

### *Substrate limited cell growth*

The amount of sugar supplied into fermentor is lower than the sugar amount required for growth; no ethanol is present in fermentor. Specific growth rate is much lower than 0.2 $h^{-1}$ and energy charge is relatively low (0.43). Glycogen cell content falls rapidly. ATP concentration decreases to $10^{-7}$ mol/g, ADP and AMP levels remain constant ($10^{-6}$ mol/g).

### *Simultaneous growth on sugar and ethanol*

The culture is sugar limited and is growing from both sugar supplied and from ethanol present in medium. The sugar and ethanol are consumed simultaneously without diauxic lag. Specific growth rate is lower than 0.2 $h^{-1}$ and energy charge is about 0.5. Glycogen content is constant or slowly decreasing. ATP and AMP levels decrease slowly to $10^{-6}$ or $10^{-7}$ mol/g, respectively, ADP level is constant ($10^{-6}$ mol/g).

### *Growth under aerobic fermentation conditions (Crabtree effect)*

Specific sugar intake rate is higher than specific sugar uptake rate, ethanol is therefore produced from the sugar that exceeds the respiration capacity of the culture (i.e. over-flow mechanism). Specific growth rate is higher than 0.21 $h^{-1}$, energy charge is 0.7–0.8 and glycogen content increases. ATP level is constant ($10^{-5}$ mol/g), ADP level slowly decreases in the range from $10^{-5}$ to $10^{-6}$ mol/g and AMP level significantly fluctuates in the range from $10^{-5}$ to $10^{-7}$ mol/g.

The culture physiology and the metabolic responses depend on the feeding regime. The optimal specific growth rate was 0.2 $h^{-1}$. The value of energy charge of the growing cells was 0.6–0.8 and decreased to 0.43 when the culture was sugar limited. The ATP, ADP and AMP levels of the cells changed in the range from $10^{-5}$ to $10^{-7}$ mol/g depending on metabolic pathway. Glycogen content of cells depends on amount of fed sugar; it is produced under excess sugar conditions and consumed under sugar limited conditions.

## Comparison of Feeding Regimes

The feeding strategies are compared with regard to main parameters such as yield coefficient, average specific growth rate, consumption of molasses per unit compressed yeast and productivity (Table IV).

IV. Feeding regimes comparison

| Controlled parametr | $\mu_{aver}$ [$h^{-1}$] | $Y_{x/s}$ | $M_{50}/YB_{27}$ [ml/g] | $p_x$ [g.l/h] |
|---|---|---|---|---|
| DOC | 0.18 | 0.43 | 1.38 | 1.56 |
| $CO_2$ | 0.22 | 0.57 | 0.99 | 1.91 |
| EtOH | 0.21 | 0.77 | 0.74 | 1.58 |

The best results were obtained in ethanol controlled fermentation. In DOC controlled fermentation there were some problems with the estimation of new dose since DOC changes depend not only on fed sugar but on anti foam agent addition, too.

## List of symbols

| | |
|---|---|
| B | percentage of budding cells [%] |
| DOC | dissolved oxygen concentration [%] |
| EC | energy charge [–] |
| EtOH | ethanol concentration [% vol] |
| glu | glucose concentration [% w/v] |
| gly | glycogen concentration [% w/v] |
| $M_{50}/Y_2$ | consumption of standard molasses containing 50% sugar per unit of compressed yeasts of 27% w/v of dry weight [ml/g] |
| $p_x$ | dry biomass productivity [g.l/h] |
| X | dry weight [g/l] |
| $Y_{x/s}$ | yield coefficient [–] |
| $\mu$ | specific growth rate [$h^{-1}$] |
| $\mu_{aver}$ | average specific growth rate [$h^{-1}$] |
| $\sigma_{in}$ | specific sugar intake rate [$h^{-1}$] |
| $\sigma_{up}$ | specific sugar uptake rate [$h^{-1}$] |

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## Změny chování a fyziologie kvasinek *Saccharomyces cerevisiae* ve fed-batch kultuře

Byla sledována závislost fyziologického stavu kvasinek *Saccharomyces cerevisiae* na režimu přítokování substrátu ve fed-batch kultivaci. Při optimálním přítokování je požadována kompletní spotřeba substrátu, vysoká výtěžnost a produktivita. Protože koncentraci substrátu není obvykle možné měřit on-line, jsou k řízení přítokování melasového média použity parametry, které souvisejí se spotřebou substrátu a které lze snadno měřit (koncentrace rozpuštěného kyslíku, koncentrace $CO_2$ nebo koncentrace produkovaného etanolu). Bylo zjištěno, že růstové charakteristiky a chování celé kvasinkové kultury závisí na podmínkách přítokování. V závislosti na rychlosti přítokování substrátu lze rozlišit čtyři hlavní oblasti růstu kvasinek: vyrovnaný aerobní růst, růst za podmínek aerobní fermentace (Crabtree efekt – za aerobních podmínek je z té části cukru, která převyšuje respirační kapacitu kultury, produkován etanol), růst současně na dodaném cukru a vytvořeném ethanolu bez výskytu diauxického lagu a růst pouze na vyprodukovaném ethanolu. Nejdůležitějším úkolem je nalézt optimální rychlost přítokování melasového média a optimální velikost specifické růstové rychlosti tak, aby byla v souladu s fyziologií a aktivitou kvasinek. Nejlepších výsledků bylo dosaženo v kultivacích řízených podle koncentrace ehanolu.

fed-batch; droždí; řízení; fyziologie; přítokovací režim

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Potrav. Vědy, *12*, 1994 (4) : 289-296

# LEAD CONTENT IN WINES FROM THE CZECH REPUBLIC

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**The lead content of different types of Czech and Moravian wines was determined by differential pulse anodic stripping voltammetry without sample pretreatment. 54 different wines were analysed. Almost all samples of wine contained < 200 µg/l of lead. The mean lead concentrations were 41 and 49 µg/l for white and red wines, respectively. Lead level is generally higher in red wines, except wines from Znojmo-Mikulov area. The samples originating from this region also show the widest range of lead content. The lowest lead contents were found in wine samples from Bzenec-Strážnice (white – 11 µg/l, red – 27 µg/l). The lead concentrations in wines from the Czech central region are comparable with wines from Hustopeče-Hodonín (white – 25 µg/l, red – 62 µg/l).**

**lead; wine; differential pulse anodic stripping voltammetry**

Lead represents a very important trace element, which is highly toxic and could be accumulated in living organisms. For this reason lead together with cadmium and mercury having the similar properties, is often investigated the majority of food products. Some authors reported a positive correlation between wine consumption and lead level in blood (Shaper et al., 1982; Elinder et al., 1983; Elinder et al., 1988).

Recent investigations have shown that the lead levels in the table wines vary in various countries. In Italy, Germany and the United Kingdom levels of 50–100 µg/l were found (Jorhem et al., 1988). Average content of lead in wines sold in Sweden was found to be 73 µg/l (Jorhem et al., 1988). In Greek wines, the lead levels were higher ranging from 50 to 400 µg/l (Soulis et al., 1984) or from 50 to 560 µg/l (Lazos et al., 1989). Considerably higher lead concentrations (15–22 mg/l) were found in the case of several samples of Bulgarian wines (Ungurean et al., 1978). In wines from Macedonia and Herzegovina lead concentration ranging from 33 to 65 and from 26 to 68 µg/l were found for white and red wines, respectively (Maravic et al., 1990).

There are different methods to determine lead concentration in organic materials. In the most cases these methods are based on the mineralisation of samples so that the measurement is considerably time consuming.

One of the most useful method for determination of trace elements is flame or flameless atomic absorption spectrometry (AAS). Detection limit for lead is about of 0.1 mg/l for flame – and 1 µg/l for flameless AAS (Eschnauer, Neeb, 1988). Inductively coupled plasma atomic emission spectroscopy (ICP-AES) can detect less than 0.01 mg/l of lead (Interesse et al., 1984). These methods mostly require pretreatment of a sample. Direct determination of lead in wine is difficult due to contents of sugar and alcohol (Meranger et al., 1968). Voltammetric methods, namely differential pulse polarography (DPP) and voltammetry, enable very sensitive determination of some metals, e.g. Pb (Nürnberg, 1982). Unfortunately these methods require complete sample decomposition removing an organic matrix (Adeloju et al., 1985).

Cu, Pb and Zn in amounts of 0.03–0.08 mg/l were determined simultaneously by DPP in wine at pH of 3.1 (potassium hydrogen phtalate) after sample evaporation and decomposition by $HNO_3/H_2O_2$ treatment (Bruno et al., 1978). Higher sensitivities were achieved using stripping voltammetry, so that smaller sample volumes (1–10 ml) could be used (Mannino, 1982; Oehme, Lund, 1979; Popko et al., 1978). Small amounts of cadmium can also be determined in this way. These elements can also be determined in wine by differential pulse anodic stripping voltammetry (DPASV) after UV-irradiation of the acidified sample to which $H_2O_2$ has been added (Golimowski et al., 1979).

The maximum lead content in wine 0.3 mg/l was established in the Czech Republic by hygienic regulation (Hygienické předpisy, 1986). This value is very closed to those in some other countries (Table I) (Wai et al., 1979; Interesse et al., 1984; Lazos et al., 1989).

This paper is devoted to monitoring of lead content in wines from the Czech Republic. The wine-producing area in Bohemia extends along the valley of the Labe River (Elbe) and on the southern slopes of the Czech Central Mountains. The most important viticultural areas are Mělnik, Litoměřice,

**I. Hygienic limits for lead content in wine valid in some countries**

| Country | Pb [mg/l] |
|---|---|
| Czech Republic | 0.3 |
| Canada | 0.5 |
| Germany | 0.3 |
| Greece | 0.5 |
| Italy | 0.3 |

Karlštejn and Žernoseky, so called the Czech central region. Among the white sorts mainly Rhine Riesling, Traminer, Müller Thurgau and Chardonnay are cultivated here, among the blue sorts Vavřinecké, Portugieser and Pinot noir. Sparkling wines are also produced in Starý Plzenec. In Moravia, the Znojmo-Mikulov region is the largest viticultural area where particularly Rhine Riesling, Italian Riesling, Traminer, Veltliner and Müller Thurgau are produced, and of the blue sorts Vavřinecké, Portugieser and Frankovka (Limberger). The majority of the vineyards in the Hustopeče-Hodonín area are located on sloping grounds. In addition to the sorts just mentioned, Sylvaner and Neuburger are cultivated here. The vineyards in the Bzenec-Strážnice area are located in higher sloping localities, in a region with warm, dry climate and mild winters. The wines cultivated are the same as above (Farkaš, 1988).

54 samples of wines from these areas were analysed for lead content. Direct measurement by DPASV without sample pretreatment was applied.

## MATERIALS AND METHODS

### Chemicals and glassware

Standard solutions of lead and copper (*c* = 1 g/l in 0.3 mol/l $HNO_3$) for atomic absorption spectrometry were used for preparation of working standards. Hydrochloric acid (36 %) with a maximum lead content 1 µg/l (Analpur grade) was used for preparation of 1 mol/l HCl serving as supporting electrolyte. Except nitric acid analytical grade, used for soaking of glassware, all other chemicals were products of Analytika Praha of the highest purity. All glassware were decontaminated before use by three weeks soaking in dilute $HNO_3$ (1:10). All aqueous solutions were prepared using redistilled water.

### Sampling

In 1994, 54 bottles of different wines originating from several viticultural producers were analysed. Each sample of wine was described by its origin, sort and vintage. The samples included wines in bottles with the same type of cork closure.

### Procedure for lead and copper determination in wine

5 ml of wine and 5 ml of 1 mol/l HCl were transferred to a polarographic cell. After oxygen removing from the mixture by 5 min. bubbling with nitrogen lead was determined by DPASV using conditions described below. The quantification was made by standard addition technique. Potentials of lead and copper stripping peaks are −380±20 and −134±20 mV respectively.

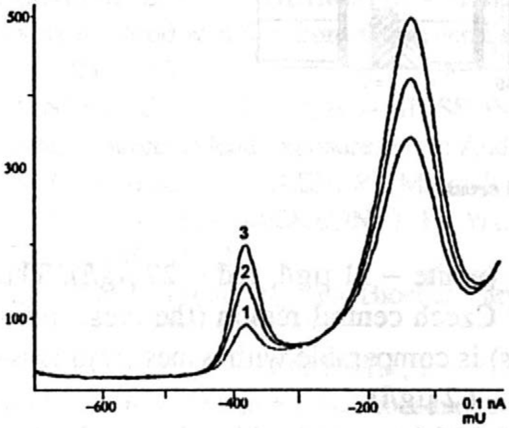
### Measuring instrument and conditions

Computer controlled polarographic analyzer ETP (Polaro-Sensors Ltd., Prague) was used. Parameters of determination were as follows:

- working electrode: HMDE
- reference electrode: Ag/AgCl/sat. KCl
- mode DPV stripping A
- drop expansion time 0.1 s
- pulse duration 100 ms
- pulse height 50 mV
- current sampling time 20 ms
- deposition potential −700 mV
- final potential +50 mV
- deposition time 120 to 600 s
- equilibration time 20 s
- current range 500 nA
- scan rate 5 or 20 mV/s

## RESULTS AND DISCUSSION

Stripping voltammetry is an often used method for lead measurement in food samples after decomposition. This highly sensitive method was utilized for lead and copper measurement in wine samples without any pretreatment. Suitable electrolyte is 0.5 mol/l HCl, in which stripping peaks of lead and copper are quite symmetrical and well resolved. For most samples (ranging in lead from 30 to 100 μg/l) 2 to 5 minutes electrolytic deposition is appropriate. Using common, normal measuring parameters (scan rate 20 mV/s, pulse height 50 mV) several samples showed another peak of interfering component in the proximity of lead peak. In these cases lead peak was distorted by partial overlapping and evaluation of its height was incorrect. Therefore slight modification of instrumental parameters (scan rate 5 mV/s, pulse height 10 mV, deposition time 10 minutes) was necessary to achieve a better shape of the stripping curve. An example of lead and copper determination in wine is given in Fig. 1.



**DPASV**
**$E_d$ = −700 mV**
**$t_d$ = 600 + 20 s**
**scan rate 5 mV/s**
**pulse height 10 mV**
**(1): 5 ml of sample + 5 ml of 1 mol.$l^{-1}$ HCl**
**(2): (1) + 100 ng Pb + 200 ng Cu V = 10.15 ml**
**(3): (1) + 200 ng Pb + 400 ng Cu V = 10.3 ml**

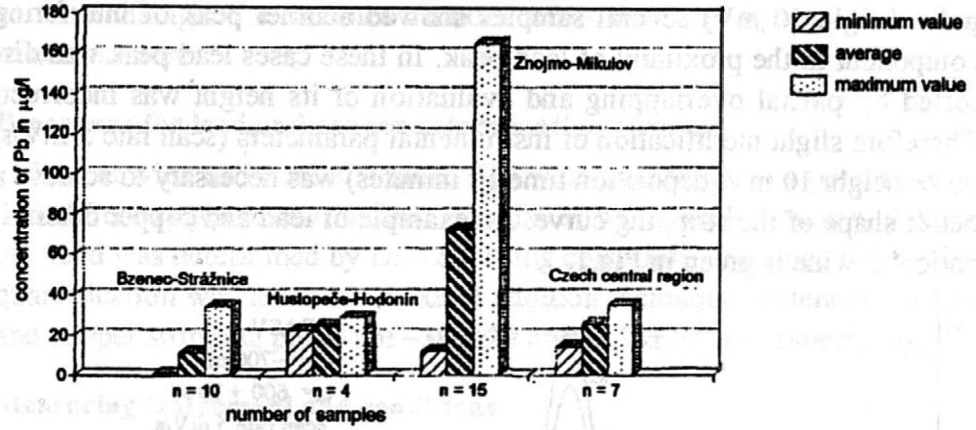
1. Simultaneous voltammetric determination of lead and copper in wine

Error of determination expressed as relative standard deviation is 10.6 % at 12.5 μg/l level.

Accuracy of the used method was proved by other independent methods (GF-AAS and DPASV after wet digestion of samples). These results will be presented in the next paper.

A total of 54 bottles of 54 different wines were analysed. Each sample of wine was analysed five times at least.

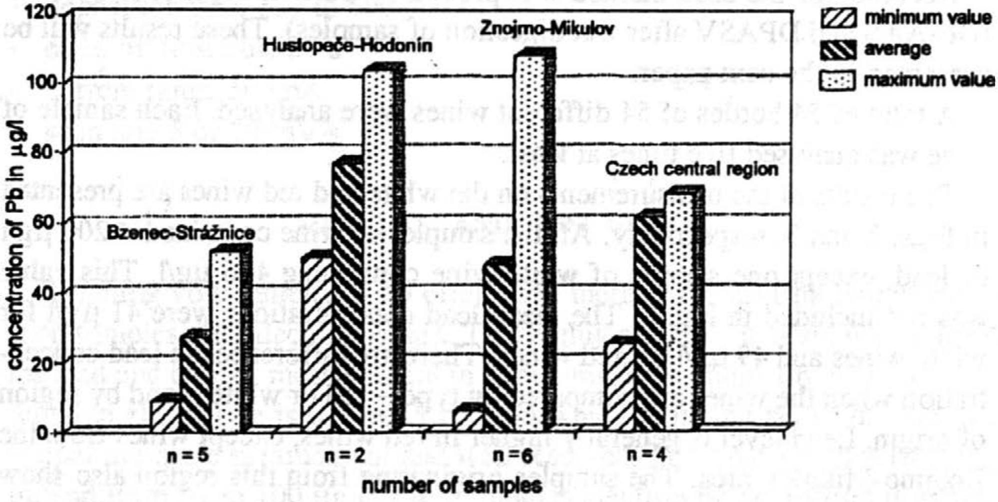
The results of the measurements on the white and red wines are presented in Figs. 2 and 3, respectively. All the samples of wine contained < 200 μg/l of lead, except one sample of white wine containing 466 μg/l. This value was not included in Fig. 2. The mean lead concentrations were 41 μg/l for white wines and 49 μg/l for red wines. There are differences in lead concentration when the wines are compared by type – red or white – and by region of origin. Lead level is generally higher in red wines, except wines from the Znojmo-Mikulov area. The samples originating from this region also show the widest range of lead content. The lowest lead contents were found in



3. Content of lead in white wines in individual areas

wine samples from Bzenec-Strážnice (white – 11 µg/l, red – 27 µg/l). The lead concentration in wines from the Czech central region (the most industrialized region from monitored areas) is comparable with wines from Hustopeče-Hodonín (white – 25 µg/l, red – 62 µg/l).

Assuming daily wine consumption 0.05 l (average lead level 44 µg/l), dietary intake of lead is 2.2 µg. This value represents only 0.5 % of tolerable daily intake of lead for adults proposed by a FAO/WHO expert group (WHO 1972).



3. Content of lead in red wines in individual areas

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## Obsah olova ve vínech vyráběných v České republice

Pro stanovení koncentrace olova ve vínech byla použita metoda diferenční pulsní anodické rozpouštěcí voltametrie na visící rtuťové kapkové elektrodě bez předchozí úpravy vzorku mineralizací. Voltametrická měření byla prováděna po zředění vzorku kyselinou chlorovodíkovou.

V 53 vzorcích analyzovaného vína byly nalezeny koncentrace olova menší než 200μg/l. Průměrná koncentrace olova v bílých vínech je 41 μg/l, v červených 49μg/l. Obsah olova je v červených vínech obecně vyšší než v bílých s výjimkou vín ze znojemsko-mikulovské oblasti. Tyto vzorky vykázaly největší variabilitu koncentrací olova. Nejnižší obsahy olova byly zjištěny ve vzorcích ze bzenecko-strážnické oblasti. Průměrné koncentrace ve vínech z české oblasti jsou srovnatelné s hodnotami nalezenými ve vínech z hustopečsko-hodonínské oblasti.

olovo; víno; diferenční pulsní anodická rozpouštěcí voltametrie

Potrav. Vědy, *12*, 1994 (4) : 297-308

# RAPE SEED PHOSPHOLIPASE D

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**Crude phospholipase D (PLD, E.C.3.1.4.4) has been isolated from seeds of winter rape seed (*Brassica napus* L.), variety Arabella, and its biochemical properties have been partly characterized. The rape seed PLD is mainly soluble, a minor quantity being identified in the microsomal fraction. Detergents (sodium deoxycholate, Tween 80, Triton X-100, sodium dodecylsulfate), reducing agents (dithiothreitol, mercaptoethanol), ethylenediaminotetraacetic acid and leupeptin do not improve extraction of PLD from mature rape seeds. Partial purification of the enzyme is obtained by ammonium sulfate precipitation (50% saturation) with 55–60% yields. The effect of sodium dodecylsulfate (SDS) and Triton X-100 on the enhancement of rape seed PLD activity has been shown evident in a limited range of concentrations. The stimulating effect of SDS on PLD activity is even much more pronounced at higher concentrations of Ca ions and it makes possible to reach threefold higher activity comparing to the original one.The optimum activity appears closely to pH 6 and at an incubation temperature of 40 °C. The enzyme is very stable at 35 °C while the temperature increase to 50 °C causes fast decline in its activity. Its isoelectric point has been found at pH 4.8. Isoelectric focusing has been demonstrated as one of promising options for further enzyme purification.**

**rape seeds; phospholipase D; localization; extraction; characterization**

**Phospholipase D (PLD, E.C.3.1.4.4) is known as an enzyme hydrolyzing the phosphodiester bond in phosphatidylcholine (lecithin) and related phospholipids. It is widespread in plants (Heller, 1978). PLD has been also reported to be involved in the hydrolysis of phospholipids of storage tissues and particularly in oil seeds, e.g. soybeans (Kouzeh Kanani et al., 1985)**

and peanuts (Heller et al., 1968). One of the products of the enzymic reaction is phosphatidic acid, the nonhydratable phospholipid remaining in the water degummed oil, which likely causes poor flavour of the final product and brings about technological problems during oil processing.

Plant PLD exists in two forms (Heller et al., 1968; Paliyath, Thompson, 1987); an insoluble one fixed to the subcellular structures and a soluble form, which does not sediment at high speed centrifugation (105 000 g).

Regarding the findings mentioned above, the authors expected the occurrence of PLD also in rape seeds, one of the main resources of edible plant oil production in the Czech Republic. The PLD activity levels in rape seed in various stages of the seed maturation and post-harvest period have been reported in our paper published earlier (Valentova et al., 1992). The highest PLD activity has been found in well matured seeds. The aim of this paper is to show the localization of this enzyme in plant cells and the best conditions for its extraction and activity determination. Biochemical characteristics of the partially purified rape seed PLD are described as well.

## MATERIAL AND METHODS

### Preparation of crude enzyme

Winter rape seed (*Brassica napus* L.), variety Arabella, was used in all experiments. A 10 g amount of mature dry seeds was milled three times for 20 sec (using coffee grinder), suspended in 50 ml of 0.05 M Tris-HCl buffer pH 7.4 and shaked at 4 °C for 45 min. The mixture was filtered through a cheese-cloth and centrifuged at 17 600 g at 4 °C for 30 min. The floating fat cake was removed. The supernatant (35 ml) referred to as the extract was saturated up to 50% with ammonium sulfate and shaked at 4 °C for 2 hours. The resulting precipitate was centrifuged at 17 600 g at 4 °C for 30 min, dissolved in 5 ml of the extraction buffer (see above) and desalted on PD-10 column (Pharmacia). This preparation was marked as crude enzyme. The protein content was determined by biuret method (Gornall et al., 1949).

### Assay of PLD

**Substrate emulsion**: 40 mg of L-α-phosphatidylcholine, type X-E from dried egg yolk (Sigma) was dissolved in 0.5 ml of ethanol and solvent evaporated under nitrogen. Then 0.33 ml of 1.83 M $CaCl_2$ solution and 0.67 ml of 0.4 M Tris-acetate buffer pH 6.0 containing 13 mM sodium dodecylsulfate (SDS) were added. The mixture was emulsified on vortex for 2 min.

**Incubation:** The reaction mixture containing 0.1 ml of 9% TRITON X-100, 0.1 ml of 0.2M MES buffer pH 6.0, 0.1 ml of phosphatidylcholine emulsion, 0.02 ml of 0.173M SDS and 0.2 ml of the rape seed extract was incubated at 30 °C for 10 min. Enzyme reaction was terminated by adding 0.2 ml of 50mM ethylenetetraacetic acid (EDTA) in 1M Tris-HCl buffer pH 8.0 and subsequent boiling for 5 min.

**Determination of choline:**The concentration of choline released by PLD action was determined using choline biosensor (Vrbová et al., 1993): 0.1 ml of the incubation mixture was added to the stirred reaction vessel (30 °C) with an inserted biosensor, containing 1.4 ml of 0.1M Tris-HCl buffer pH 8.0. The amount of choline was calculated from a calibration curve constructed from the responses measured for the standard solutions of choline chloride. The enzyme activity was expressed in international units (U) – 1 U was defined as 1 μmol of choline produced per 1 min.

### Isoelectric focusing (IEF)

IEF was carried out in 2 mm thick 6.5% polyacrylamide gels containing 5% ampholine (pH ranged from 3.5 to 10, LKB, Broma, Sweden). Crude enzyme (210 μl) was applied to the cathodic side of the gel on a 2.4 x 2 cm Whatman No 3 paper strips. Electrophoresis was performed without preforming the gradient. The voltage was progressively increased up to 600 V without outer exceeding the current of 20 mA. The separation was carried out at 4 °C for 5 h. Proteins were stained on gels by Coomassie Blue G-250 reagent (Neuhoff et al., 1988).

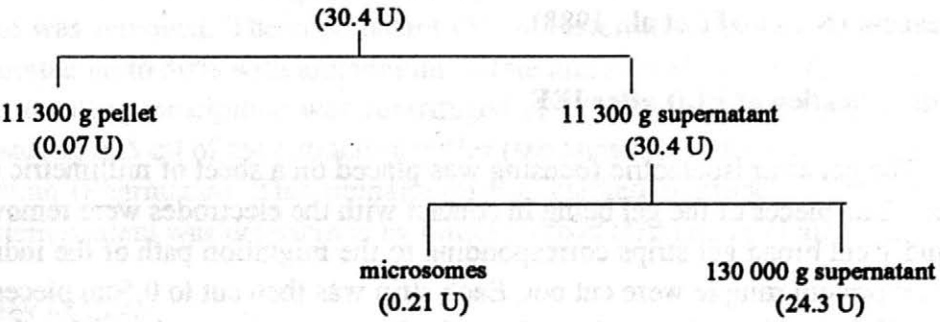
### Identification of PLD after IEF

The gel after isoelectric focusing was placed on a sheet of millimetric paper. The pieces of the gel being in contact with the electrodes were removed and 1 cm broad gel strips corresponding to the migration path of the individual protein sample were cut out. Each strip was then cut to 0.5cm pieces in the direction from the anode to the cathode, removed from the gel bond and proteins were eluated with the mixture of 0.2 ml 0.2M MES buffer pH 6.0, 0.2 ml of 9% TRITON X-100 and 0.04 ml of 0.173M SDS. The elution proceeded at room temperature for 30 min. The substrate emulsion (0.2 ml) was then added. 0.4 ml of 50mM EDTA in 1M Tris-HCl buffer pH 8.0 was added after 10 min incubation at 30 °C to stop the enzyme reaction. The content of choline was determined as described above.

## RESULTS AND DISCUSSION

### Subcellular distribution of PLD within the seed tissue

The procedure described by Heller et al. (1968) for the subcellular distribution of PLD in dry peanut seeds was slightly modified. Rape seeds (8 g) were homogenized as described at the experimental part and extracted with 40 ml of 0.05M Tris-HCl pH 7.4 containing 0.5M sucrose and 5mM dithiothreitol. After shaking at 4 °C for 30 min the extract was filtered through the cheese-cloth and centrifuged at 1 000 g for 10 min. The floating fat cake was removed and supernatant obtained was fractioned by differential centrifugation, first at 11 300 g for 20 min. The pellet was kept and the supernatant was then centrifuged at 130 000 g for 60 min in order to obtain microsomal fraction. All the operations were carried out at 4 °C. The pellets and supernatants were examined for PLD activity. The results described in Fig. 1 show that the major yield of PLD activity occurs in the supernatant of microsomal fraction, while only traces of PLD were located in microsomes.



1. Subcellular distribution of PLD in rape seeds. The total activity of each fraction is given in parenthesis

## Optimization of the extraction conditions

The first step in the isolation and purification of PLD involved the searching for the best conditions for the PLD extraction from the starting material. The effect of pH, detergents and stabilizing agents was therefore investigated.

For the determination of the pH effect on the activity of extract, Britton--Robinson buffer was used owing to the limited buffering capacity of Tris buffer which is most frequently used for the extraction of PLD from the plant tissues. The PLD activity of extracts prepared using both buffers of the same pH (7.4) differed only slightly. The activity in Tris-HCl buffer was higher only by 5%. Regarding the results given in the Table I, the use of Tris-HCl buffer pH 7.4 was preferred.

I. Effect of extraction buffer pH on the PLD activity of the extract

| pH | 5.4 | 6.0 | 6.4 | 7.0 | 7.4 | 8.0 | 9.0 |
|---|---|---|---|---|---|---|---|
| Activity [U/ml] | 0.26 | 0.63 | 1.40 | 1.90 | 2.10 | 2.10 | 2.18 |

Any positive effect on the total PLD activity of the extract caused by added detergents to the extraction medium was not observed (Table II). Tween 80, Triton X-100 and SDS (in concentrations higher than 5 g/l) affected the activity decline while sodium deoxycholate (SDC) remained without any effect.

Similar results were achieved by the use of reducing agents (dithiothreitol and mercaptoethanol). Proteinase inhibitor leupeptin did not affect the PLD activity indicating that no proteolysis of the enzyme occurred. In contrast, the presence of EDTA decreased the extracted PLD activity by 12% indicating the role of C$^{2+}$a for the rape seed PLD activity.

## Partial purification of the rape seed PLD

The ammonium sulfate precipitation of the extract, examined in the range from 20 to 90% of saturation (w/v), showed the highest PLD yield at 50% saturation. Up to 30% saturation only small amount of active protein is pre-

II. Effect of detergents in extraction buffer on the PLD activity of the extract

| Detergent | Concentration [g/l] | Activity [U/ml] |
|---|---|---|
| SDC | 5 | 2.20 |
| | 10 | 2.20 |
| | 20 | 2.20 |
| Tween 80 | 5 | 1.74 |
| | 10 | 1.79 |
| | 20 | 1.74 |
| Triton X-100 | 5 | 1.97 |
| | 10 | 1.97 |
| | 20 | 1.56 |
| SDS | 5 | 2.20 |
| | 10 | 2.10 |
| | 20 | 0.87 |
| Control | | 2.20 |

cipited. Two-step precipitation was also investigated and the characteristics of both procedures applied are given in Table III.

III. Partial purification of PLD by ammonium sulfate precipitation

| Fraction | Total activity [U] | Total protein [mg] | Specific activity [U/mg] | Purification [-fold] | Recovery [%] |
|---|---|---|---|---|---|
| Crude extract | 113.4 | 1 309.7 | 0.086 | – | 100 |
| 50% precipitate | 69.1 | 860.8 | 0.080 | 1 | 61 |
| Desalting PD-10 | 64.6 | 547.1 | 0.118 | 1.4 | 56.9 |
| Two-step precipitation 30–50% | 66.1 | 525.5 | 0.126 | 1.5 | 58.3 |
| Desalting PD-10 | 57.4 | 324.9 | 0.177 | 2 | 50.6 |

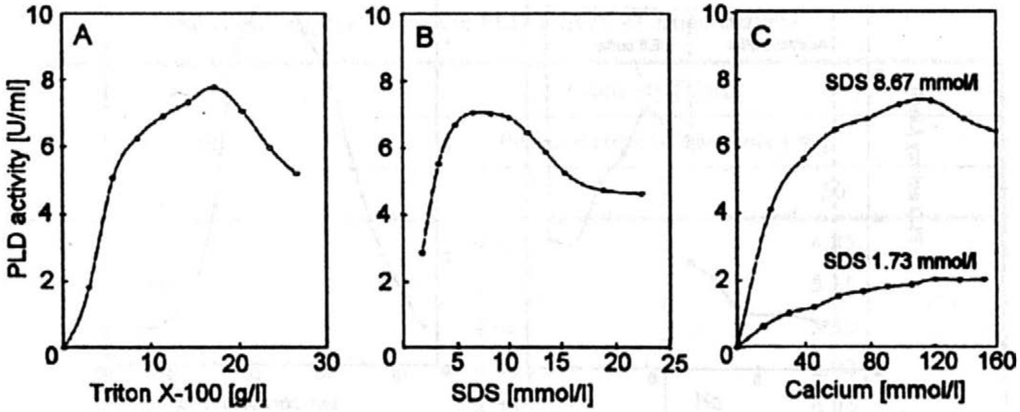
## Properties of the crude enzyme

The effect of activators Triton X-100, SDS and $Ca^{2+}$ on PLD activity have been investigated.

The optimal concentrations have been achieved at 18 g/l for Triton X-100 and 8.7 mmol/l for SDS (Fig 2A, B). The molar ratio of phosphatidylcholine to SDS 6.3 : 8.7 mM in the incubation mixture is not in agreement with the value 2:1 found for peanut PLD (Tzur, Shapiro, 1972). The activity level is most probably influenced by the change in the substrate form from vesicles to mixed micelles which are formed in the presence of detergents (Reynolds et al., 1991) and not by the activation of the enzyme molecule itself.

Phospholipase D from rape seed, as most of plant PLD, is not able to hydrolyze phosphatidylcholine unless calcium ions are added to the reaction mixture (Fig. 2C). The optimum $Ca^{2+}$ concentration (120 mM) is higher than the values published for other plant PLD. For example 40 – 60 mM concentration of $Ca^{2+}$ is required by PLD from peanuts (Heller et al., 1968 ). The substantial increase in the PLD activity has been achieved in the simultaneous presence of SDS and Ca ions as a result of the combined effect of both activators. The optimum influence of Ca ions has been observed at very high concentrations (about 120 mmol/l) regardless of SDS addition. Comparison of SDS given at 1.73 mmol/l or at its optimal concentration (8.7 mmol/l) shows an enhancement of the PLD activity about three times, most probably, due to the better accessibility of the substrate.

Diethylether has been described to increase the activity of nearly all plant PLD except that one from soy beans and beans. The activity of rape seed



2. The effect of Triton X-100 (A), SDS (B) and $Ca^{2+}$ (C) concentration on the PLD activity. Concentration of other components in the incubation mixture are described in experimental part

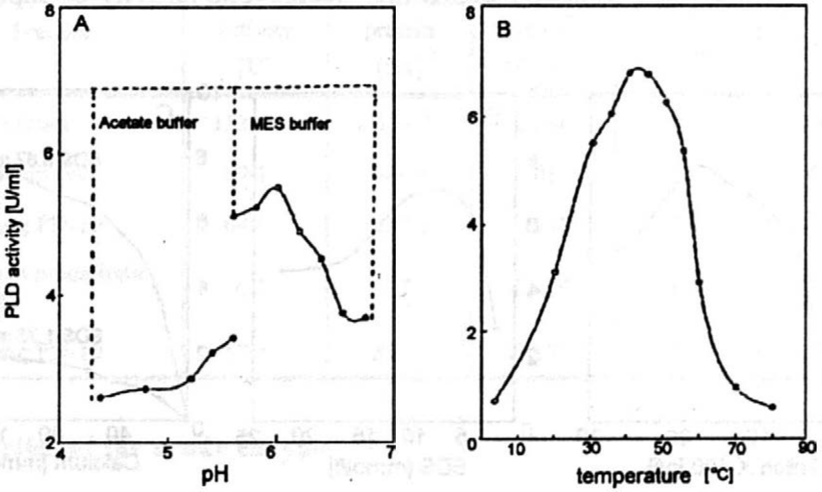
**IV. Effect of diethylether (DEE) on the PLD activity of the crude enzyme**

| Activators | | |
|---|---|---|
| Concentration of DEE [mol/l] | Concentration of SDS [mmol/l] | Activity [U/ml] |
| – | 8.67 | 3.96 |
| 0.67 | 8.67 | 3.47 |
| 0.67 | 1.73 | 3.99 |
| 0.67 | – | 1.16 |
| – | – | ND |

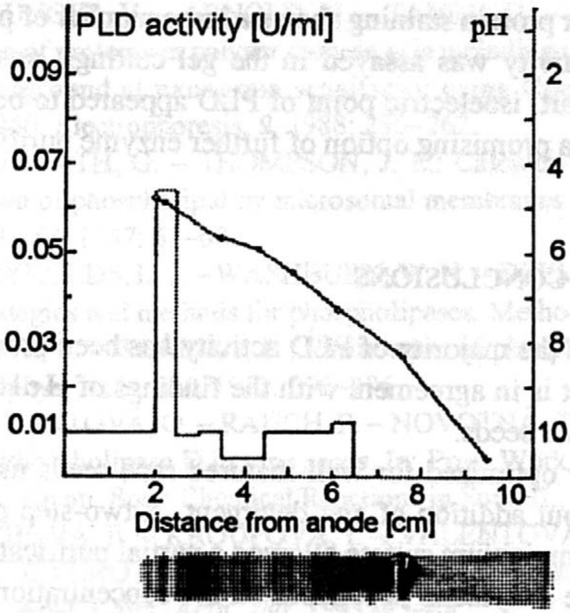
ND = not detected

PLD was not affected in the presence of diethylether alone (Table IV). In combination of diethylether with SDS in concentration 1.73 mmol/l the activation effect reached the same level as with the SDS in optimal concentration.

Other biochemical characteristics of the enzyme were determined. The pH optimum 6.0 (Fig. 3A) corresponds to those ones of the other plant phospholipases D. The investigation was carried out in two buffering systems: 0.2M acetate buffer for the pH range 4.4–5.6 and 0.2M MES for pH range 5.6–6.8. MES buffer enhanced the PLD activity more than acetate buffer.



3. pH (A) and temperature (B) dependence of the PLD activity of crude enzyme. The experimental conditions are described in the text



**4. Determination of pI using IEF. Conditions of the electrophoresis and subsequent PLD activity localization on the gel are described in experimental part**

The value of pH-optimum for rape seed PLD does not depend on the concentration of the activator SDS (results not shown).

The temperature optimum for the rape seed PLD (Fig. 3B) has been found between 40°C and 45 °C. The enzyme is quite stable at 35 °C (Table V). The PLD activity remained at that temperature on the same level for more than six hours while at 50 °C increased a little bit within the first 10 min and then dropped to zero within three hours. Crude enzyme stored in 0.05M Tris-HCl pH 7.4 at –20 °C still showed 82 % of the original activity after four weeks.

V. Effect of preincubation temperature on the PLD activity of crude enzyme

| Time [min] | Activity [U/ml] | |
|---|---|---|
| | Preincubation temperature [°C] | |
| | 35 | 50 |
| 0 | 4.85 | 4.85 |
| 10 | 4.75 | 5.15 |
| 30 | 4.68 | 3.50 |
| 90 | 4.90 | 2.00 |
| 180 | 4.70 | 0.80 |
| 300 | 4.70 | ND |

ND = not detected

Electrophoretic pattern after protein staining shows a large number of protein bands but when PLD activity was assayed in the gel cuttings, as described in the experimental part, isoelectric point of PLD appeared to be at 4.8 (Fig. 4). This results give a promising option of further enzyme purification.

## CONCLUSIONS

In well matured rape seeds, the majority of PLD activity has been proved in soluble fraction. This result is in agreement with the findings of Heller et al. (1968) for matured peanut seeds.

Extraction conditions were optimized for well matured rape seeds using Tris-HCl buffer pH 7.4 without addition of any detergent. A two-step precipitation up to 30 and 50% ammonium sulfate allowed a partial purification of PLD. The activity of crude enzyme is dependent on the concentration of Ca ions, presence of the detergents SDS and Triton X-100. Findings of the proper concentrations of these activators together with the optimal values of pH and temperature assess the conditions of PLD activity assay. Results of the present study allow us to continue with purification and further characterization of PLD.

**Acknowledgement**

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## Fosfolipasa D z řepky

Fosfolipasa D (PLD, E.C.3.1.4.4) je enzym hydrolyzující fosfodiesterové vazby fosfolipidů, zejména fosfatidylcholinu (lecithin). Výskyt, přečištění a charakterizace tohoto enzymu byla popsána u celé řady rostlinných materiálů (Heller, 1978). Kromě funkce PLD v počáteční fázi degradace membrán během stárnutí buněk hraje zřejmě také důležitou roli při štěpení fosfolipidů v zásobních tkáních rostlin, zejména v semenech olejnin (Kouzeh Kanani et al., 1985; Heller et al., 1968). Jedním z produktů reakce katalyzovavané PLD je kyselina fosfatidová, jejíž přítomnost ovlivňuje senzorické vlastnosti finálních produktů a působí určité technologické problémy při zpracování olejů.

Ve vyzrálých semenech řepky (*Brassica napus* L., odrůda Arabella) byla nalezena PLD aktivita převážně v rozpustné formě, pouze malá část byla lokalizována v mikrosomální frakci. Enzym byl částečně purifikován a charakterizován.

**Vliv detergentů (SDS, Tween-80, Triton X-100 a SDC), redukujících činidel (dithiothreitol a merkaptoethanol), EDTA a proteasového inhibitoru leupeptinu na množství vyextrahovaného enzymu nebyl ani v jednom případě prokazatelný.**

**Aktivita enzymového preparátu částečně přečištěného dvoustupňovým srážením síranem amonným je závislá na koncentraci $Ca^{2+}$, SDS a Tritonu X-100. Nalezené optimální hodnoty koncentrací těchto aktivátorů spolu s pH a teplotním optimem byly také použity pro optimalizaci podmínek stanovení enzymové aktivity. Tento preparát je poměrně velmi stabilní při 35 °C, při 50 °C jeho aktivita rychle klesá. Při skladování preparátu při –20 °C poklesla jeho aktivita po čtyřech týdnech zhruba o 20 %.**

**Po izoelektrické fokusaci surového extraktu z řepky byla PLD aktivita nalezena v jediném proužku odpovídajícím pH 4,8.**

**řepka; fosfolipasa D; lokalizace; extrakce; charakterizace**

Potrav. Vědy, *12*, 1994 (4) : 309-318

# MICROSTRUCTURE OF HYDROGENATION Ni-CATALYST

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**The chosen microstructure characteristics were studied in six chosen types of commercial catalysts (Nysel DM-3, Nysosel 222, Harcat DM-2, Nysofact 101, Nysel SP-7 – producer Harshaw Chemie B. V., De-Meern, The Netherlands) and in the STZ catalyst (producer SETUZA, a. s., Ústí-upon-Elbe): specific surface area, medium size of crystallites and distribution of pore sizes. Medium crystallite size keeps within 2–7 nm in catalytic systems on the Ni/NiO basis on the carrier. The values of specific surface area (BET) in the range 7–18 m$^2$/g are rather lower. From the point of view of catalyst pore size distribution there are complicated polydispersed texture systems in all cases. The pore sizes is greater than 6 nm in all cases.**

**crystallite; distribution of pore sizes; fats; hydrogenation; Ni-catalyst; oils; specific surface area; microstructure of catalyst**

Catalyst microstructure (texture) is one of the basic characteristics of catalysts which as sorbents are interpenetrated by a labyrinth of pores creating the inner catalysts surface. It is a collective term which includes a characteristic set as e.g. pore radius, pore volume, surface etc. (Muchljonov et al., 1985, p. 69).

Heterogeneous Ni-catalysts on a carrier represent so far the most spread type of hydrogenation catalyst used for the partial catalytic hydrogenation of vegetable oils and fats with the aim to obtain plastic fat for the production of margarines and shortenings. It is usually a catalytic Ni/NiO system on the $SiO_2$ carrier first suggested by Normann (1903). This original concept of a heterogeneous Ni-catalyst on the carrier was basically preserved and is systematically being developed by top producers of catalysts through the carrier development and modification, and through the modification of preparation of an active catalytic Ni/NiO system on the carrier. Sufficient activity, selectivity, stability of these properties in the course of the reaction, and related to these, resistance to catalytic poisons and catalyst particle size in the range 3–50 μm (Beckmann, 1983) and particle filterability belong to the main demands laid on the hydrogenation catalyst. Especially $S_I$ selectivity (linoleic), specific isomerisation and triglyceride selectivity (Coenen, 1976) are usually considered from the various selectivity aspects. The decisive criterion is created by the triglyceride blend properties after the hydrogenation characterized usually by the physical parameters – namely SFC profile and consistency properties.

Triglyceride hydrogenation, as every heterogeneously catalysed reaction, can be directed by the reaction rate or by the mass transport in dependence on the reaction parameter influence (Lefebvre, Dizier, 1984). The reaction itself takes place inside the catalyst pore and its microstructure is applied in this case. The important role is played by the size of catalyst specific surface area, the size of active catalytic system surface area which is usually roughly half in Ni-catalysts on the silica carriers (Rodrigo, Mendioroz, 1992), the size of elementary Ni/NiO crystallites (Dafler, 1977) and predominantly by the arrangement of catalyst inner surface area as far as the diameter and pore length are concerned. Coenen (1976, 1981) distinguishes three types of pores according to their sizes:

- those with a diameter less than 2–2.5 nm which with respect to the size of triglyceride molecule – 1.5 nm show the zero effective diffusion coefficient.
- those with a diameter of 10 nm or more. The inner diffusion in pores is negligible in this case, and the bulk diffusion is applied. Good correlation between the catalyst activity and the size of the active catalytic surface area is valid for these surfaces (Linsen, 1971).
- those with a diameter within the interval of the stated extreme values. The triglyceride inner diffusion is applied in this case.

The stated intervals of the pore medium size (the distribution of pores according to size is applied in real systems) have the essential influence on linoleic selectivity and predominantly on triglyceride selectivity (Coenen, 1976, 1981). According to this aspect the reaction takes place in wide pores selectively where the concentration gradients for individual fatty acids are minimum. The striking concentration gradients of reactants originate in narrower pores – there is an increase in the stearic acid concentration and finally in the tristearoylglycerol concentration. Random saturation of unsaturated acyls bonded in triglycerides can be assumed in wide pores, and, in this way, also great triglyceride selectivity.

The elementary crystallite size in heterogeneous catalysts is usually an important parameter (Dafler, 1977). Ni/NiO crystallites are applied in the considered catalytic systems and so it is necesseary to consider the mutual Ni and NiO ratio which is changed at the catalyst reduction. The medium value 4 nm is considered to be a medium size in common catalysts (Beckmann, 1983). The elementary crystallite size in precipitated catalysts is dependent on the way of precipitation, the type of used nickelous salt, a precipitating agent, the interaction between the carrier surface and originated precursor (Martin et al., 1981). Further, especially temperature conditions of calcination (Kundu et al., 1980) and reduction are important. When the temperature range of 400–500 °C is exceeded, sintering starts to take place - i.e. enlargement of elementary crystallites and consequently the carrier surface cover with an active metal is reduced (Richardson, Crump, 1979; Kuo et al.,1980).

## MATERIAL AND METHODS

The following catalysts were chosen for the study:

| Type | Producer | Usage |
|---|---|---|
| Nysel DM-3 | Harshaw Chemie B. V., DeMeern | hydrogenation of oils and fats |
| Nysosel 222 | The Netherlands | highly active and selective catalyst for oils and fats |
| Harcat DM-2 | | hydrogenation of fatty acids and oils |
| Nysofact 101 | | hydrogenation of fatty acids |
| Nysel SP-7 | | special catalyst with increased isomeric properties on the nickel sulfide basis |
| Cat. STZ | SETUZA, a. s., Ústí-upon-Elbe (CR) | universal catalyst for hydrogrogenation of oils, fats and fatty acids |

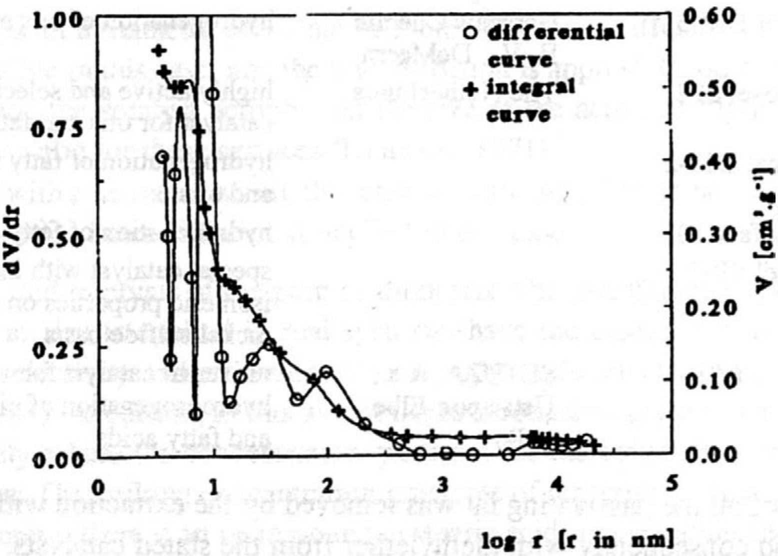
First of all the passivating fat was removed by the extraction with chloroform and consequently with diethylether from the stated catalysts. The solvent residues were removed under the presssure of 0.5 kPa.

The specific surface area of the catalyst was determined in a chromatographic way with the use of nitrogen as an adsorbate. The calculation was carried out according to the equation BET (Muchljonov et al., 1985, pp. 314-316). The experiments were carried out at the department of catalysts LTI, Petersburg. The distribution of pores size was determined at the same department by the method of mercury porosimetry (Muchljonov et al., 1985, pp. 319-323).

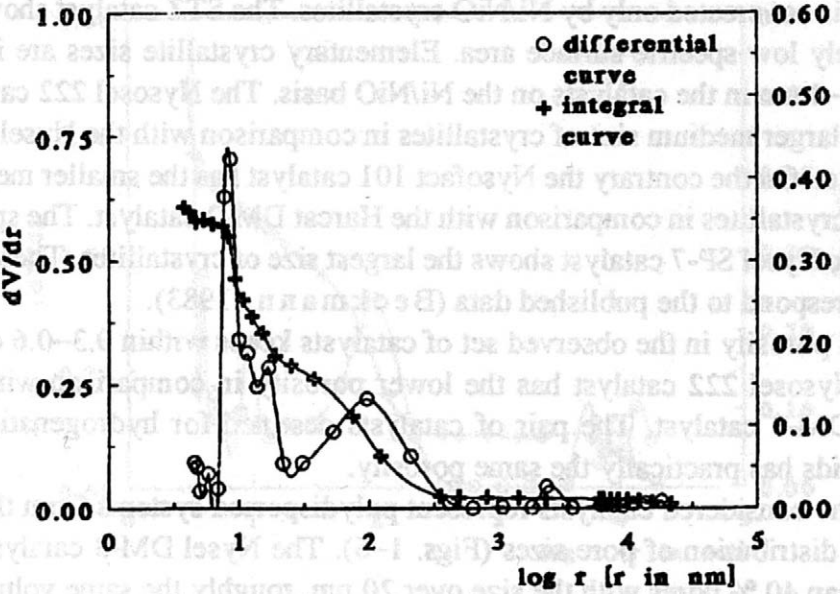
The size of elementary crystallites was determined by the x-ray diffraction method according to Scherrer (Dafler, 1977) on the apparatus Rikagaku Denki of a Geigerflex type with the use of Cu lamp with the wave length of 0.154178 nm with a graphic record of the diffraction intensities.

## RESULTS AND DISCUSSION

Two older types of Ni-hydrogenation catalysts of the Harshaw Chemie firm destined for the hydrogenation of oils and fats (Nysel DM-3) and of fatty acids (Harcat DM-2) and two new generation catalysts of analogous destination (Nysosel 222 and Nysofact 101) were chosen for the comparison of microstructure characteristics. These new catalysts are declared to have strikingly improved use properties. The Nysosel 222 catalyst attains strikingly higher activity and selectivity in comparison with the preceding Nysel



1. Distribution of pores in the calatyst Nysel DM–3



2. Distribution of pores in the calatyst Nysosel 222

DM-3 catalyst at its reduced dosing. The comparison of use properties of the Nysofact 101 and Harcat DM-2 catalysts is analogous. It is obvious from the data in the Table I that the newer types of catalysts have got a lower specific surface area. Their absolute values are at the level of 10 $m^3/g$ which is by one order less in comparison with the published data (Rodrigo et al., 1992). The special Nysel SP-7 catalyst produced on the basis of Ni-subsulphide has the largest specific surface area. It is the only catalyst where the catalytic

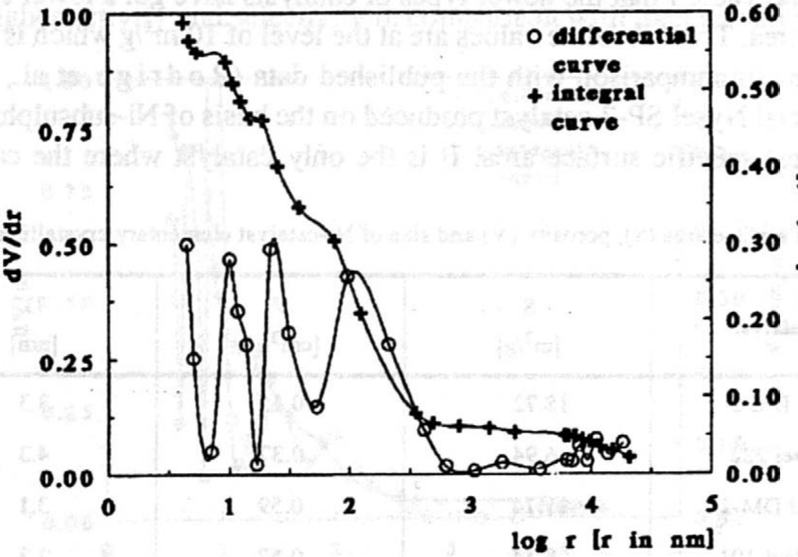
I. Specific surface area (S), porosity (V) and size of Ni-catalyst elementary crystallites (D)

| Catalyst | S [$m^2/g$] | V [$cm^3/g$] | D [nm] |
|---|---|---|---|
| Nysel DM-3 | 18.72 | 0.55 | 3.3 |
| Nysosel 222 | 6.94 | 0.37 | 4.3 |
| Harcat DM-2 | 11.74 | 0.59 | 3.1 |
| Nysofact 101 | 9.34 | 0.52 | 2.3 |
| Nysel SP-7 | 14.19 | 0.37 | 7.2 |
| Kat. STZ | 0.38 | 0.33 | 3.1 |

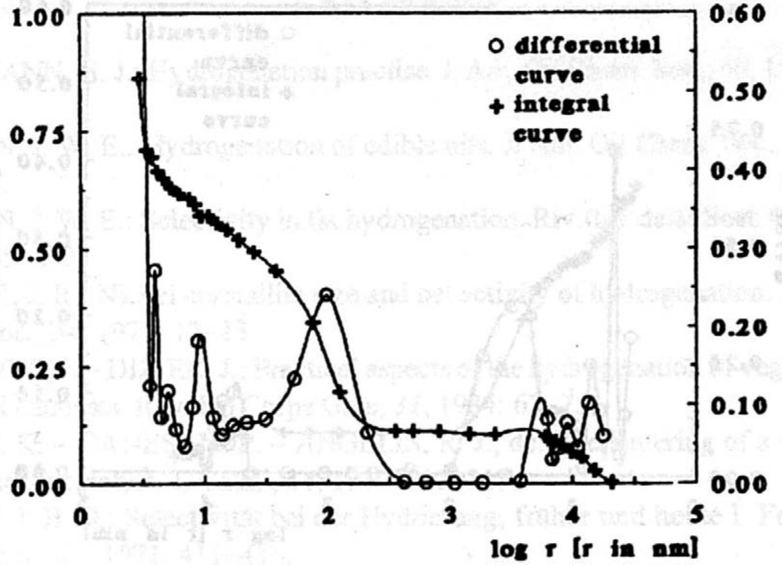
system is not created only by Ni/NiO crystallites. The STZ catalyst shows an extremely low specific surface area. Elementary crystallite sizes are in the range 2–4 nm in the catalysts on the Ni/NiO basis. The Nysosel 222 catalyst has the larger medium size of crystallites in comparison with the Nysel DM-3 catalyst. On the contrary the Nysofact 101 catalyst has the smaller medium size of crystallites in comparison with the Harcat DM-2 catalyst. The special isomeric Nysel SP-7 catalyst shows the largest size of crystallites. These values correspond to the published data (Beckmann, 1983).

Total porosity in the observed set of catalysts keeps within 0.3–0.6 cm$^3$/g The Nysosel 222 catalyst has the lower porosity in comparison with the Nysel DM-3 catalyst. The pair of catalysts destined for hydrogenation of fatty acids has practically the same porosity.

All the considered catalysts represent polydispersed systems from the aspect of distribution of pore sizes (Figs. 1–6). The Nysel DM-3 catalyst has more than 40 % pores with the size over 20 nm, roughly the same volume is represented by the pores with the size between 6–20 nm. Only a small part of the total volume is created by the pores with the diameter lower than 6 nm.



3. Distribution of pores in the calatyst Harcat DM–2



**4. Distribution of pores in the calatyst Nysofact 101**

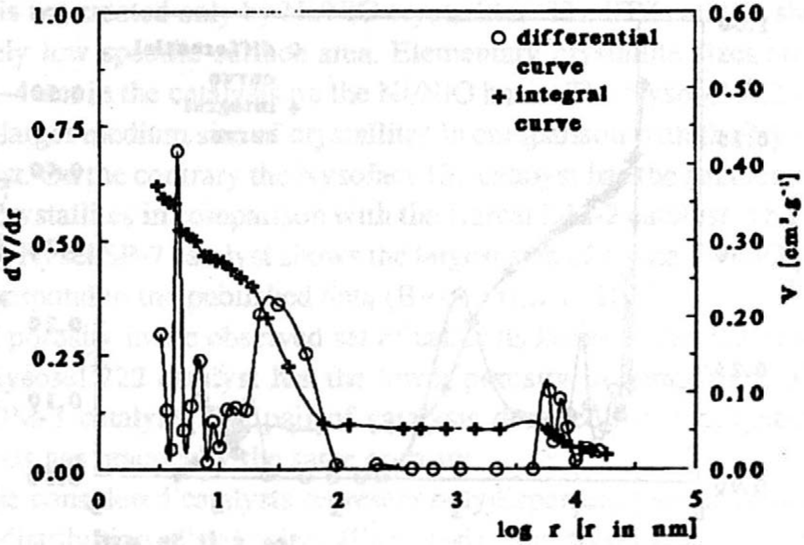
At the Nysosel 222 catalyst the distribution of pore size is shifted in the direction to higher values (Fig. 2), pores with the diameter larger than 20 nm prevail. Satisfactory triglyceride selectivity can be assumed in both the catalysts.

The two catalysts destined for hydrogenation of fatty acids (Figs. 3 and 4) represent more complicated polydispersed systems but in both cases the pore size exceeds the value of 6 nm. Neither in this case should the inner diffusion represent a limiting factor.

Pores with the size of 30–200 nm (ca. 50 % of pore volume) strikingly prevail in the special Nysel SP-7 catalyst (Fig. 5). The smallest pores achieved values over 8 nm.

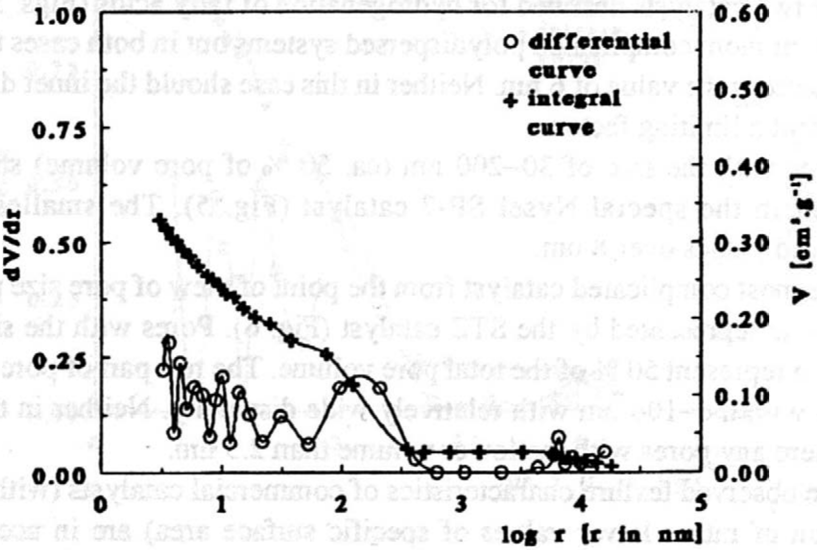
The most complicated catalyst from the point of view of pore size polydispersity is represented by the STZ catalyst (Fig. 6). Pores with the size over 100 nm represent 50 % of the total pore volume. The rest part of pore volume keeps within 6–100 nm with relatively wide dispersity. Neither in this case are there any pores with the lower volume than 2.5 nm.

The observed texture characteristics of commercial catalysts (with the exception of rather lower values of specific surface area) are in accordance with the published data for this catalyst type as far as medium crystallite size



5. Distribution of pores in the calatyst Nysel SP–7

(Beckmann, 1983) and pore sizes are concerned. From the point of view of pore size distribution there is not monodispersed (bidispersed) microstructure at any type of catalyst.



6. Distribution of pores in the calatyst STZ

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### Mikrostuktura hydrogenačních Ni–katalyzátorů

**U šesti vybraných typů komerčních katalyzátorů (Nysel DM–3, Nysosel 222, Harcat DM–2, Nysofact 101, Nysel SP–7 – výrobky fy Harshaw Chemie B.V., DeMeern, Holandsko a u katalyzátoru STZ – výrobce SETUZA, a. s., Ústí n. L.) byly sledovány vybrané mikrostrukturní charakteristiky: specifický povrch, střední velikost krystalitů a distribuce velikosti pórů. U katalytických systémů na bázi Ni/NiO na nosiči se střední velikost krystalitů pohybuje v rozmezí 2–7 nm. Poněkud nižší**

jsou hodnoty specifického povrchu (BET) v rozmezí 7–18 $m^3$/g. Z hlediska distribuce velikosti pórů katalyzátoru se ve všech případech jedná o složité polydisperzní texturní systémy. Velikost pórů je všech případech větší než 6 nm.

krystality; distribuce velikosti pórů; tuky; hydrogeance; Ni–katalyzátor; oleje; specifický povrch; mikrostruktura katalyzátoru

Potrav. Vědy, *12*, 1994 (4) : 319-324

# ELECTROCHEMICAL REDUCING CHANGES IN FOOD

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In the presence of oxygen the spontaneous oxidative changes dominate in foods. Reduction changes which accompany fermentation processes are characterised mainly by electron exchange in systems consisting of foodstuff itself as well as the microorganisms growing there. Electrochemical reductions in food are caused by chemical changes related to corrosion of metal packings when the packing material provides electron donors. There are three basic types of those changes: i) reduction of oxygen to water, ii) reduction of hydrogen ions to hydrogen and iii) reduction of food constituents (e.g. quinoid pigments). In consequence of those reactions the redox potential of food system decreases. It was demonstrated for two types of canned fruit. The experiments on electrochemical reduction and oxidation of several types of fruit and vegetable juices using inert electrodes in apparatus with separated anode and cathode space are further described. Sensory evaluation of electrochemically treated samples, namely vegetable juices, showed disagreeable changes in flavour compared with untreated control which is probably caused by reduction of food constituents containing sulphur.

reduction; redox-potential; canned food; cans

In agreement with basic thermodynamic relations the spontaneous oxidation changes with atmospheric oxygen as acceptor of electrons dominate during processing and storage of food. It is interesting that food constituents susceptible to oxidation constitute also the most important nutritive components, e.g. ascorbic acid, unsaturated fatty acids, carrotenoids, etc. It is due to an important role of redox reactions in human organism during digestion.

The reducing changes of food components during food processing and/or storage are rarer. There are two principal types of those changes, i.e. biochemical reductions which are typical for many fermentation processes used in food technology (e.g. ethanol and/or lactic acid fermentation, etc.) and electrochemical reductions, the most frequent case of which is connected with corrosion of metal food packings (e.g. cans).

Biochemical reductions in consequence of microorganisms action consist of systems of redox reactions with a corresponding charge transfer within food and/or microorganism system without external donor of electrons. Nutritional substrates are dehydrogenated by microorganisms and all available acceptors of hydrogen in system are reduced. This situation results in a very low value of redox potential which is typical for such foods, e.g. wine.

During electrochemical changes the metal packing provides electrons for food components reduction. There are three main groups of these changes, i.e. i) reduction of hydrogen ions to hydrogen, ii) reduction of oxygen to water and iii) reduction of other food constituents, e.g. quinoid pigments, etc.

The first type of electrochemical reduction is typical for acid canned foods in absence of oxygen. The course of those changes can be followed by the volumetric determination of formed hydrogen and/or by the measurement of redox potential by the platinum electrode, which works as the gas hydrogen electrode. Table I shows the difference of redox conditions in stewed fruit packed in cans and jars. For example, the low value of redox potential in canned plums (rH ≤ 1) indicates the inside pressure of hydrogen exceeding 0.1 MPa. In extreme case cans can swell in consequence of development of higher hydrogen amount during intense corrosion.

More complex reaction systems can occur in tinned steel cans without lacquer in presence of ascorbic acid and residual oxygen. This case is rather common in many fruit and vegetables cans. It is difficult to predict the rate of oxygen consumption both as the depolarisator for metal (mainly tin) corrosion and as the electron donor for ascorbic acid oxidation. By other words it is not easy to predict both the extend of the stabilisation of vitamin C consisting in oxygen reduction during tin dissolving and in return the effect of ascorbic acid autooxidation on metal corrosion, i.e. oxygen depolarisation. The following course of mentioned oxidation changes can be supposed:

**I. Redox potentials of stewed fruit in cans and jars after fourteen months storage**

| Fruit | Type of packing | pH | Redox potential | |
|---|---|---|---|---|
| | | | [mV] | rH |
| Plums | lacquered cans | 2.8 | –147 | 0.4 |
| | jars | 2.9 | 428 | 20.9 |
| Pears | non lacquered cans | 2.9 | –130 | 1.4 |
| | jars | 2.9 | 488 | 22.6 |

$$Sn + 1/2\,O_2 + 2\,H^+ = Sn^{2+} + H_2O$$

for tin corrosion with oxygen depolarisation and

$$AA + 1/2\,O_2 = DAA + H_2O$$

for ascorbic acid oxidation. Moreover, the kinetics of the system is further complicated by heterogeneity of the tin dissolving process and by the effect of increased concentration of metal ions on the rate of ascorbic acid oxidation.

Although any more precise prediction of the courses of the mentioned reaction system is rather difficult it is obvious from our previous experiments that ascorbic acid is much more stable in canned food (in cans with as well as without lacquer) compared with the same products packed in jars (namely in those with closure not enabling "thermal exhaust" of content, e.g. twist--off caps).

The situation is even more complicated in systems with more electrodes, e.g. in presence of lead solder, when the rate of lead dissolving decreases with lower oxygen concentration and so the amount of oxygen, or the rate of its consumption by constituents of food, is also important (Warwick, Müller, 1980).

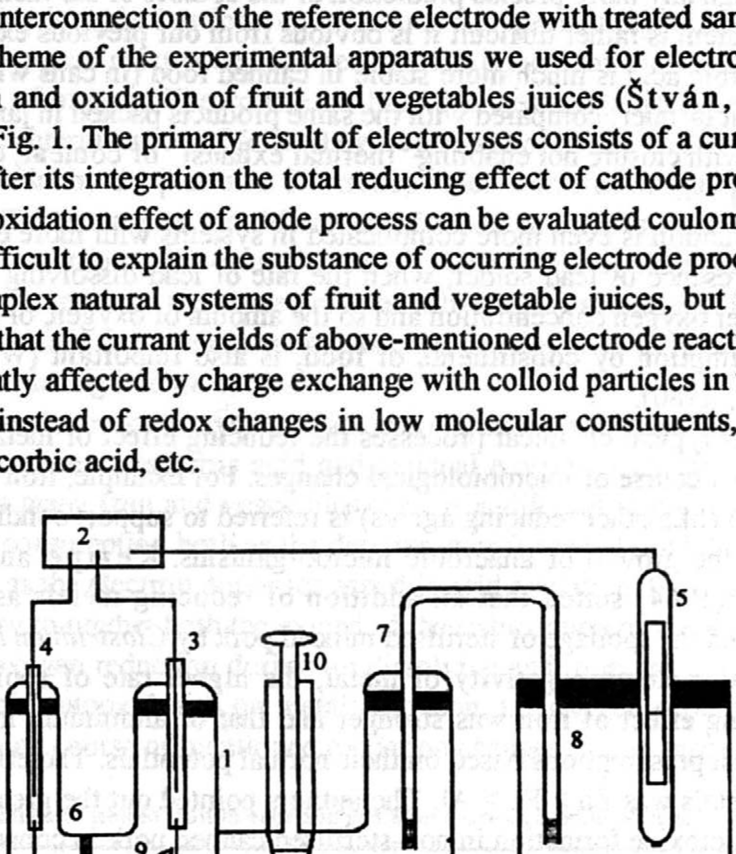
Besides typical chemical processes the reducing effect of metals can affect even a course of microbiological changes. For example, iron (Reed et al., 1951) (like other reducing agents) is referred to support conditions suitable for the growth of anaerobic microorganisms. Kempe and Graikovski (1964) stated that an addition of reducing metals as powders accelerated the spoilage of sterilised minced pork by *Clostridium botulinum*. The greater electronegativity of metal, the higher rate of spoilage. The stimulating effect of iron was stronger and that of aluminium lower compared with presumptions based on their normal potentials. The efficiency of tested metals was Zn > Fe > Al. The authors pointed out the greater danger of a botulotoxine formation in non-sterilised canned pork in consequence of presence of reducing metals.

As the probability of occurrence of mentioned changes in practice is negligible, the reducing effect of metals in consequence of reasonable corrosion of cans can be considered positive especially with regard to the reduction of oxygen and so to the protection of oxilabile constituents of food. On the other hand, the reduction of oxygen to water by tin, as the typical example of depolarisation reactions in acid medium, can cause an undesirable increase in metal ion concentration in canned products, i.e. in that case 7 mg Sn for 1 mg of reduced oxygen.

Nevertheless, the electrochemical processes occurring during corrosion of cans provide an interesting example for the realization of an electrochemical treatment of food without dissolving of used electrodes. Achievement of “clean“ reduction under definite experimental conditions is not easy even in the case of electrolyses using inert electrodes. There are problems namely with a separation of anode and cathode spaces of the electrolytic vessel and with the interconnection of the reference electrode with treated samples.

The scheme of the experimental apparatus we used for electrochemical reduction and oxidation of fruit and vegetables juices (**Štván**, 1977) is given in Fig. 1. The primary result of electrolyses consists of a current-time curve. After its integration the total reducing effect of cathode process and the total oxidation effect of anode process can be evaluated coulometrically.

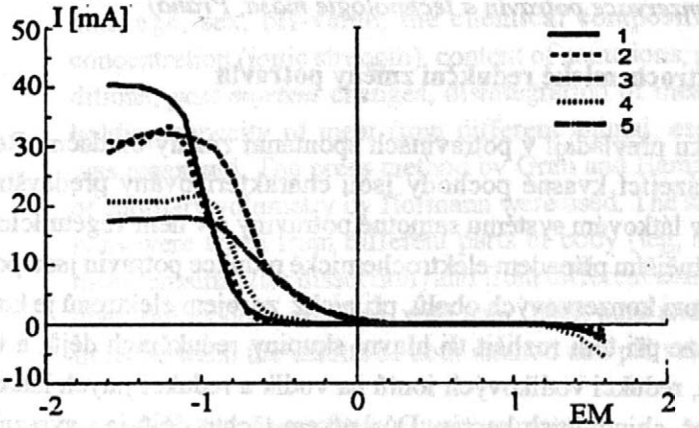
It is difficult to explain the substance of occurring electrode processes for such complex natural systems of fruit and vegetable juices, but it is very probable that the currant yields of above-mentioned electrode reactions were significantly affected by charge exchange with colloid particles in the tested systems, instead of redox changes in low molecular constituents, e.g. pigments, ascorbic acid, etc.



**1 - electolytical vessel; 2- potentiostat; 3- Pt measuring electrode; 4 - Pt keep alive electrode; 5 - reference electrode; 6- sintered glass; 7 - bridge of unsatureted KCl solution; 8 - vessel with saturated KCl solution; 9 - Luigi capillary; 10 - closing cock**

**1. Shema of the apparatus for electrochemical oxidation and/or reduction of fruit and vegetable juice**

**Polarisation curves, i.e. currant-potential dependences obtained under stationary currant conditions of electrolyse (approximately after 30 minutes), were plotted for each tested juice. Fig. 2 gives the polarisation curves for five fruit and/or vegetable juices (1 – red beet, 2 – celery, 3 – carrot, 4 – apple and 5 – grapefruit). The remarkable long part separating anode and cathode portions of polarisation curves at the zero currant which is characteristic for irreversible processes was typical for all tested samples. In potentiometry under zero currant this irreversibility causes a low reproducibility of potential.**



**2. Polarisation curves**

**Sensory analyses showed a worse flavour of electrochemically treated samples, mainly vegetable juices, compared with untreated samples. Their disagreable flavour was probably caused by a reduced form of sulphur substances.**

**The possibility of application of electrochemical treatment in food processing is still unclear. So far there is little information on its influence on the quality of different foodstuffs. Theoretically it could be useful for example in wine processing where low values of redox-potential in product are required. Perhaps, it could be one way of elimination of sulphur dioxide in wine production.**

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## Elektrochemické redukční změny potravin

Za přítomnosti kyslíku převládají v potravinách spontánní změny oxidační. Redukční procesy doprovázející kvasné pochody jsou charakterizovány především elektronovými přesuny v látkovém systému samotné potraviny a v něm vegetujících mikroorganismů. Nejběžnějším případem elektrochemické redukce potravin jsou pochody doprovázející korozi konzervových obalů, při nichž zdrojem elektronů je kov uvolňující se z obalů. Lze při tom rozlišit tři hlavní skupiny redukčních dějů, a to redukci kyslíku na vodu, redukci vodíkových iontů na vodík a redukci jiných látkových složek náplně, např. chinoidních barviv. Důsledkem těchto dějů je i výrazný pokles redox potenciálu, jak je demonstrováno na dvou druzích kompotů. Dále jsou popsány experimenty s elektrochemickou redukcí a oxidací několika druhů ovocných a zeleninových šťáv na inertních elektrodách v elektrolytické nádobce s odděleným anodickým a katodickým prostorem. Senzorické hodnocení elektrochemicky ošetřených vzorků ukázalo nepříznivý vliv na redukované vzorky zejména zeleninových šťáv, což je připisováno především redukci sirných sloučenin.

redukce; redox potenciál; konzervové plechovky; konzervované potraviny

Potrav. Vědy, *12*, 1994 (4) : 325-335

# THE WATER HOLDING CAPACITY OF VENISON

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Very important technological property of meat is water holding capacity (WHC). This characteristic of meat is usually defined as the ratio between bound water (hydration and immobilised water) and total content of water in meat. WHC depends on several factors: species of animal, age, sex, pH-value, the chemical composition of meat, salt concentration (ionic strength), content of some ions, pre slaughter conditions, *post-mortem* changes, disintegration of meat, etc. The water holding capacity of meat from different animal, especially of game, was measured. The press method by Grau and Hamm and the method of capillary volumetry by Hofmann were used. The samples to the analysis were taken from different parts of body (leg, shoulder, loin and meat remains after dissection) and from different animals (red deer, roe deer, fallow deer, mufflon, wild boar, beef, pork and sheep). The relations between the results of both method and pH value are presented.

water holding; free water content; venison

The properties of game meat have recently taken much interest because of a large offer of this meat. Besides the animals shot in the wild, the use of farmed venison seems to be of great importance. A very important technological property of meat, considered in meat production, is its capacity to bound and hold both own and added water. Good water holding capacity (WHC) is necessary for the meat product structure, holding of meat juice and thus of sensory properties (taste, flavour, consistency).

Water holding capacity (WHC) is usually defined as the ratio of bound water (hydration and immobilised water) to total content of water in meat. The value of WHC is influenced by many factors and the methods used to determine the WHC give very different values. Thus the comparison of published data is very difficult. Usually the amount of released water is taken into consideration under the experimental conditions, such as pressure, centrifugation and heating, the drip losses are other possibility of the WHC determination. Thus the WHC is the amount of water bound in meat under method conditions.

Water holding capacity depends on several factors: pH-value, chemical composition of meat, salt concentration (ionic strength), content of some ions, preslaughter conditions, *post-mortem* changes, disintegration of meat, etc. It is possible to influence many of these factors by technological treatment and to achieve good WHC in this way.

The changes of pH-value in meat occur during the *post-mortem* changes and during some technological processes. The pH-value of meat and meat products is usually in the range 4.2 and 7.0 (mostly between 5.5 and 6.0). Minimum WHC is at an isoelectrical point of meat (pH = 5.3) (Hamm, 1972).

The content of fat in sausage mixture also influences the WHC. The small fat particles keep the proteins in structure matrix in free state. More water is maintained after heat treatment in the sausage structure with fat than without fat. The degree of disintegration of meat influences the amount of release of proteins and the immobilisation of water (Honikel, 1982).

WHC depends upon different preslaughter conditions, such as animal species, sex, maturity, seasons and composition of feed. Type of growth are also of great importance. These differences are related to the different fat and protein contents, muscle structure and the process *post-mortem* changes. It is reported that the WHC increases in the order poultry, beef, pork, sheep. The best WHC is in the beef of cows, followed by the beef of heifer and bull, the lowest WHC is *in ox* (Gilka et al., 1981).

Water holding capacity falls *post mortem* due to the pH decrease and the firm structure is formed (*rigor mortis*). WHC increases during ripening. The decrease of pH-value is in several cases different (PSE or DFD meat). WHC is either lower (PSE) or higher (DFD) than in the normal meat.

While a large amount of data on WHC under different conditions exists for beef and pork, the information about WHC of venison is very poor. It is usually reported that the venison has the better WHC than beef or pork.

The meat of buffalo (*Bubalus bubalis*) had the better WHC than beef (Šukjurov et al., 1985).

Smaller drip losses were found in the meat of fallow deer in comparison with beef and pork (Freudenreich, Fisher, 1989). The average value of WHC [press method by Grau and Hamm (Hofmann, Hamm, 1982)] in *musculus semimembranosus* one hour *post mortem* is 82.56%, after 24 hours it is 78.70% and after 48 hours 78.90%; this is a much higher value than in beef and mutton (Matzke et al., 1986).

No differences of WHC were found between farmed animals and those in the wild. The stress caused the higher pH-value and the drip losses were thus much lower in stressed animals (0.55–1.05%) than in normal animals (0.85 to 1.95%) ( Schwark et al., 1990). The meat of very excited animals had the character of DFD (Matzke et al., 1986).

The same character can also occur in relation to the quality and amount of feed and the year season. There was 20% of DFD meat of the reindeer in October, 30–60% in November and up to 80% in December in some areas. In January the portion of DFD meat was 70% (a half of the cases had pH-value 6.4). In February the DFD meat occurred in 100% nearly, and in March all carcasses were DFD (Petäjä, 1983).

The drip losses in the meat of red deer (loin muscle) packed under vacuum (or in the modified atmosphere) and stored for 6–18 weeks at the temperature 1–3 °C were 39% (Seman et al., 1989).

The meat of the red deer and roe deer shot in the wild in Bavaria is reported to be very exudative in 4.5% cases (Riemer, Reuter, 1979).

A relation was found between the pH-value and WHC in the meat of wild boar in the wild, the WHC increased with the increasing pH. The WHC-coefficient (measured by Braunschweiger device*) ranged between 0.35 and 0.68. The high values 0.67-0.68 were found in two kinds of animals that were not killed immediately and were found later. The high pH-value was observed. The exsudative muscles were found in 6% cases (Ring, Scherling, 1990).

The meat of elk was compared with beef. The level of bound water (measured by Grau and Hamm method) (Hofmann, Hamm, 1982) was higher in elk meat than in beef. The exception was elk meat from rib. In this case

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* Braunschweiger device uses two glass plates for the pressing of meat sample on chromatographic paper. The ratio of both areas (meat and exudate) is measured and expressed as a quotient. (Classical press method expresses this ratio in percentage.) (Ring, Scherling, 1990, Baur, Reiff, 1976).

the WHC was better in beef. If the water addition was 20%, the WHC was better in beef (Volkov, Fedorenko, 1975).

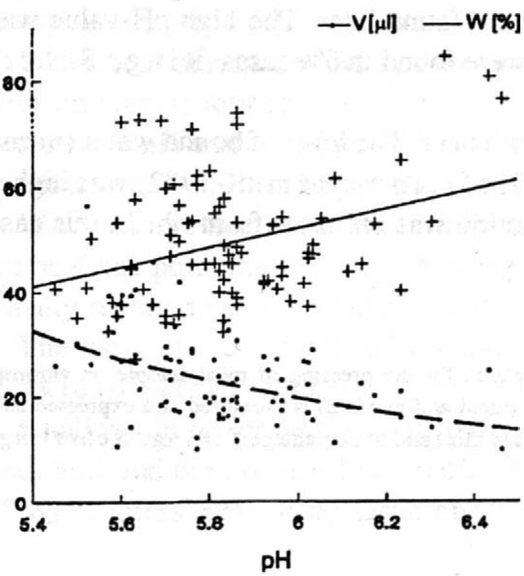
## MATERIAL AND METHODS

The water holding capacity of meat from different animal species, especially of the game, was measured. The classical Grau and Hamm press method in the last modification by Hofmann and Hamm (1982) was used (W). The method of capillary volumetry (V) according to Hofmann (1979) was used, too. The pH-values of all samples were measured by the pH-metre ACIDIMETR 333 with the combine electrode.

The samples for the analysis were taken from different parts of carcasses and from different animals ($n_w$, $n_v$ are numbers of samples taken into analysis of WHC, free water content, resp.). The meat remains after dissection were studied separately. The samples were homogenised twice through the table grinder (the slot diameters of the plates 2 a 4 mm).

## RESULTS AND DISCUSSION

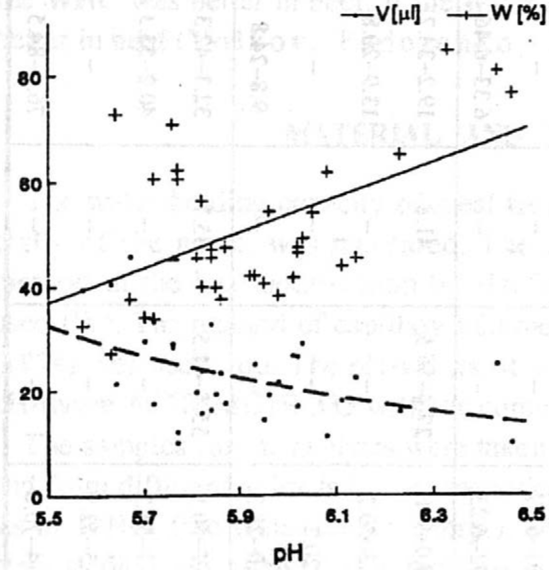
The water holding capacity of the different animals and different carcass parts was compared. The aim of these investigations was to compare different animals and to a find a common relation between pH and WHC and relation between pH and the free water content.



**1. The dependence of WHC (W) and free water content (V) on pH-value (all measured samples)**
**$W = -58.0 + 18.4\ \text{pH}$, $r = 0.299$, $n_w = 86$**
**$V = -82.8 + 619/\text{pH}$, $r = 0.398$, $n_v = 80$**

I. The pH-values, WHC (W) and free water content (V) for different animals and carcass parts

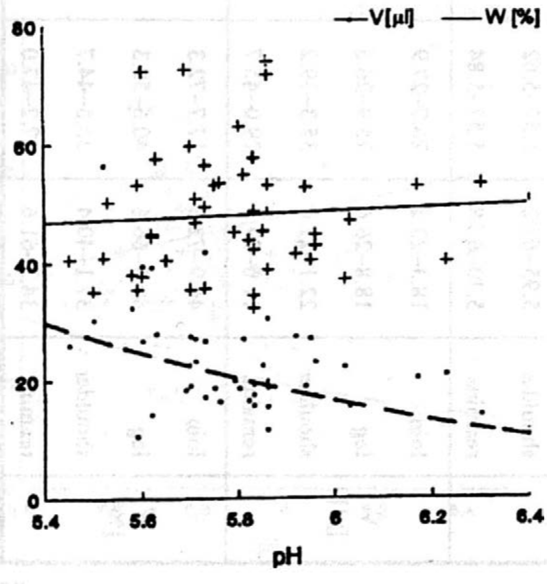
| | | Red deer | Roe deer | Fallow deer | Mufflon | Wild boar | Beef | Pork | Sheep |
|---|---|---|---|---|---|---|---|---|---|
| pH | loin | 5.69–5.80 | 5.60–5.63 | 5.76–5.86 | 5.81–5.83 | 5.58–5.71 | 5.52–5.92 | 5.73 | 5.83–5.86 |
| | leg | 5.65–5.94 | 5.45–5.53 | 5.75–5.86 | 5.82–5.86 | 5.50–5.73 | – | – | 6.03–6.30 |
| | shoulder | 5.95–6.02 | 5.59–5.62 | 5.93–5.86 | 5.96 | 5.83–6.17 | – | – | – |
| | reamins | 5.70–6.08 | 5.57–5.84 | 5.64–5.87 | 5.81–6.14 | 5.72–6.23 | – | – | 6.33–6.46 |
| V [µl] | loin | 18.3–23.2 | 26.7–27.9 | 11.4–16.3 | 17.4–26.9 | 27.0–9.4 | 27.4–56.5 | 41.8 | 19.2–34.2 |
| | leg | 18.8–26.6 | 25.9–26.3 | 15.3–18.6 | 16.4–19.7 | 10.5–30.2 | – | – | 13.9–20.8 |
| | shoulder | 22.1–27.0 | 35.3–39.2 | 15.6–18.6 | 17.2–23.0 | 20.2 | – | – | – |
| | remains | 11.0–29.5 | 29.0–45.7 | 10.1–21.3 | 16.7–28.8 | 12.0–28.3 | – | – | 9.8–24.8 |
| W [%] | loin | 46.9–72.6 | 57.7–72.3 | 53.6–73.9 | 55.0–57.8 | 37.7–50.9 | 35.5–41.5 | 35.7 | 32.3–42.3 |
| | leg | 40.5–56.6 | 40.6–50.3 | 53.2–71.7 | 43.9–48.5 | 35.1–53.3 | – | – | 40.2–53.3 |
| | shoulder | 37.1–40.4 | 35.5–44.7 | 48.8–53.2 | 42.9–44.7 | 34.5–52.9 | – | – | – |
| | reamins | 34.2–61.6 | 27.2–47.0 | 47.4–72.7 | 39.7–49.0 | 40.4–70.8 | – | – | 76.5–84.5 |



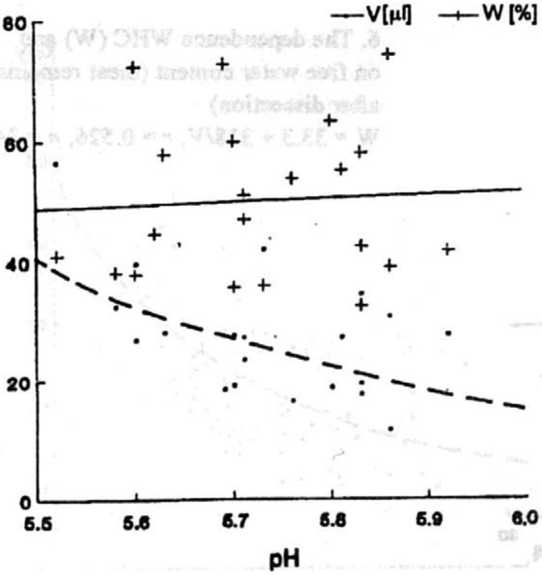
**2. The dependence of WHC (W) and free water content on pH-value (meat remains after dissection)**
**$W = -146 + 33.2\ \mathrm{pH}$, $r = 0.493$, $n_w = 37$**
**$V = -82.2 + 623/\mathrm{pH}$, $r = 0.399$, $n_v = 34$**

The measured values are summarised in Table I. The pH-values of the investigated samples ranged between 5.5 and 6.2. Only in the case of the sheep the pH-value was a little increased (6.23–6.6); the WHC increased, too (76.5–84.5%).

For all samples the relation between pH and WHC is shown in Fig. 1; the water holding capacity slowly increases with the increasing distance from



**3. The dependence of WHC (W) and free water content on pH-value (leg, shoulder and *musculus longissimus dorsi)***
**$W = 30.7 + 3.00\ \mathrm{pH}$, $r = 0.052$, $n_w = 49$**
**$V = -105 + 742/\mathrm{pH}$, $r = 0.455$, $n_v = 46$**



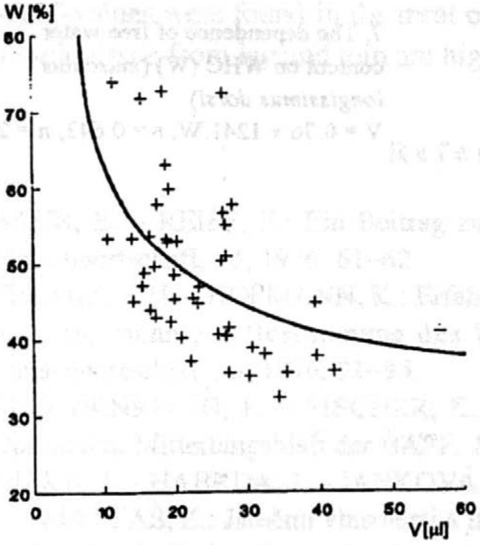
**4. The dependence of WHC (W) and free water content on pH-value (*musculus longissimus dorsi*)**

**$W = 20.7 + 5.10\ pH$, $r = 0.043$, $n_w = 21$**

**$V = -266 + 1680/pH$, $r = 0.537$, $n_v = 20$**

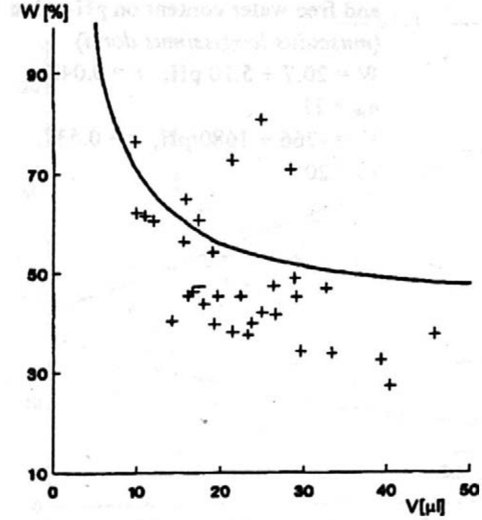
thr isoelectrical point. The free water content (volumetric method) on the contrary decreases. The variance of these values is very large because the WHC depends on many other factors, such as animal species, composition of meat, etc.

The same relation is sharper in the case of meat remains after dissection (the parts with the higher level of fat and stromatic proteins) as Fig. 2 shows. The variance of the values is large in the case of muscle samples (shoulder,



**5. The dependence WHC (W) and on free water content (leg, shoulder and *musculus longissimus dorsi*)**

**$W = 33.0 + 329/V$, $r = 0.509$, $n = 46$**

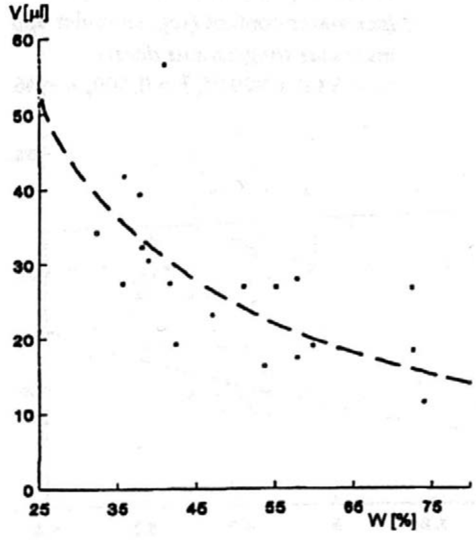


**6. The dependence WHC (W) and on free water content (meat remains after dissection)**
**$W = 33.3 + 318/V$, $r = 0.526$, $n = 34$**

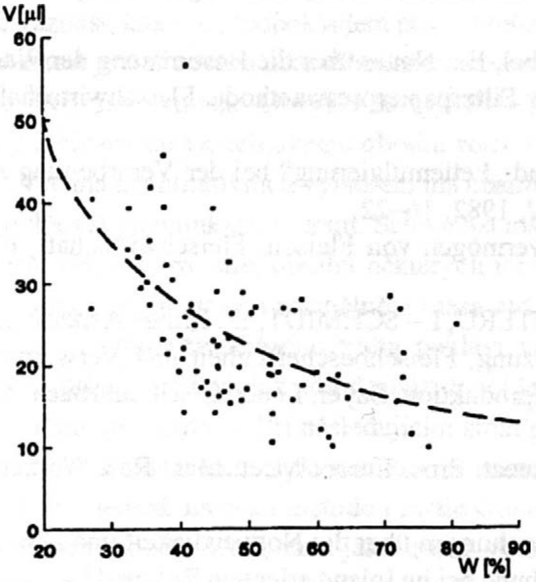
leg and loin – Fig. 3). In comparison with preceding cases the variance of data on (*musculus longissimus dorsi*) was smaller (Fig. 4).

The influence of pH-value upon the free water content is higher than that upon WHC (Table I). Besides, the suitability of meat of different animals for the meat production was also influenced by the other factors.

The dependence of WHC (by press method) on free water content (by volumetry) is hyperbolic. The variance of the values is great, the correlation is not so significant (Figs. 5 and 6). In the case of *musculus longissimus dorsi*



**7. The dependence of free water content on WHC (W) (*musculus longissimus dorsi*)**
**$V = 0.76 + 1241/W$, $r = 0.643$, $n = 20$**



**8. The dependence WHC (W) and on free water content (all measured samples)**
**W = 2,86 + 970/V, $r$ = 0.530, $n$ = 80**

the variance of the values is smaller (Fig. 7), the dependence of WHC on free water content is influenced by the other animal specific factors. Honikel (1987) also reported that the correlation between both values was not simple. These parameters characterise two different properties of meat.

The water holding capacity of the most samples ranges between 30 and 50%. The differences can be found between the animals and carcass parts. The highest WHC-values were found in the meat of fallow deer. The values of WHC of the muscle tissue from leg and loin are higher than those in the shoulder.

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## Vaznost masa lovné zvěře

Vzhledem k rozšiřování nabídky masa lovné zvěře i v souvislosti s chovem zvířat farmovým způsobem je v poslední době soustředěna pozornost na vlastnosti tohoto masa. Jednou z významných vlastností masa, důležitou při výrobě masných výrobků, je vaznost, která je předpokladem pro vytvoření kvalitní struktury masných výrobků. Vaznost je definována jako schopnost masa udržet vlastní, případně i přidanou vodu za daných podmínek. Obvykle se vyjadřuje jako podíl vody vázané (tj. hydratační a imobilizované) k celkovému obsahu vody v mase.

Většina kvantitativních vyjádření má charakter uzančních metod a je třeba je vždy doplňovat podmínkami měření. Schopnost masa vázat vodu závisí na četných faktorech: pH, iontové síle, obsahu některých iontů, intravitálních vlivech, průběhu posmrtných změn, stupni rozmělnění masa atd. Odlišná vaznost bývá nalézána mezi zvířaty různého živočišného druhu, pohlaví, věku; význam má i způsob chovu zvířat. V průběhu posmrtných změn dochází k poklesu pH až k izoelektrickému bodu, kde je minimum vaznosti. Při následujícím zrání pak pH i vaznost opět rostou.

Při vlastním měření byla hodnocena vaznost homogenizovaného masa, zejména zvěřiny, jednak lisovací metodou podle Graua a Hamma, jednak metodou kapilární volumetrie podle Hofmanna jako obsah volné vody. U všech vzorků byla měřena hodnota pH.

Hodnota pH se pohybovala mezi 5,5 až 6,2. Pouze v případě skopového masa bylo pH mírně zvýšené (6,23 až 6,46), čemuž odpovídal i výrazný nárůst vaznosti (76,5 až 84,5%).

Srovnáním vaznosti a obsahu volné vody se ukazuje náznak nepřímé (hyperbolické) závislosti, ale vzhledem k rozptylu hodnot je taková závislost neprůkazná. V té souvislosti je třeba zohlednit, že obě veličiny vyjadřují odlišné vlastnosti. Vaznost se u většiny vzorků pohybuje v rozmezí 30 až 50 %. Rozdíly lze nalézt jak mezi jednotlivými živočišnými druhy, tak i mezi různými svalovými partiemi. Zvěřina má vyšší vaznost než hovězí nebo vepřové maso, přičemž nejvyšší vaznost byla nalezena u masa daňka. Svaloviny kýty a hřbetu mají poněkud vyšší hodnoty vaznosti než svalovina plece.

Závislosti vaznosti (lineární vztahy) a obsahu volné vody (hyperbolické vztahy) na pH jsou zatíženy značným rozptylem naměřených hodnot, což souvisí s řadou intravitálních vlivů.

vaznost; obsah volné vody; zvěřina

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