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# Starch granule size distribution in caryopses of selected malting varieties of spring barley

In ten varieties of spring barley (*Hordeum vulgare* L.) of Czech, Slovak, and German origin, starch granule size distribution in a caryopsis endosperm was followed for a three-year period by means of the Low Angle Laser Light Scattering Method (LLALS). Distinct intervarietal differences were detected in starch granule size distribution. A share of the varieties in starch granule size distribution variability was 70 %. Effects of growing localities (12 %) and years (6 %) did not play a significant role. According to a ratio of large starch granules the varieties could be split into three groups. The varieties Akcent, Forum, and Atribut had the highest ratio of large starch granules, the varieties Krona, Tolar, Amulet, Olbram, and Novum had the lowest. Increase in small starch granule content was accompanied with reduction of values of relative extract at 45° C, wort viscosity, germination index,  $\beta$ -glucans in barley, in malt and wort and prolongation of saccharification time and increase of values of homogeneity and malt modification by the Carlsberg method. However, among the followed technological parameters and starch granule size distribution, there was only a low statistical dependence.

(Descriptors: Barley, starch granules, size distribution, LALLS)

These are some of the reasons for which continuous attention is devoted to this starch aspect study.

## 1 Introduction

Starch in a form of large (type A) and small (type B) starch granules is an essential component of caryopses' endosperm (May, Buttrose 1959). Generally, the small granules represent about 90 % of the total sum of present granules but only about 10 % of the total starch weight (Bathgate, Palmer 1972). The small starch granules tend to fast embedding into a protein matrix and inside cell walls (Palmer 1972). And the small starch granules are those ones that can cause technological problems. During malting and mashing only about half of the small starch granules is degraded, it means that approximately 5 % of the total starch content is not degraded into fermentable sugars for the consequent conversion to alcohol. The remaining small granules can form starch haze and block filtration beds in lauter tanks (Tillet, Bryce 1993).

In connection with study of an effect of the structure and composition of a barley caryopsis, further research direction was outlined (McGregor 1991) into the following areas:

- Identification of factors influencing and controlling small and large starch granule ratio in a barley endosperm so that cultivars containing a minimal quantity of small granules but with a maximal total starch content could be developed.
- Identification of factors influencing the relation between content and classification of small starch granules and mealiness and malting potential.

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Tables see Appendix

## 2 Material and methods

### 2.1 Varieties

Starch granule size distribution was followed in ten following malting varieties of spring barley (Psota, Jurečka 2000, Psota et al. 2001, Psota, Jurečka 2001): Akcent, Amulet, Atribut (Selgen, CZ), Novum, Kompakt (Hordeum, SK), Olbram, Forum (Monsanto CR, CZ), Tolar (Plant Select, CZ), Krona (Semundo Saatzucht, D), Scarlett (Saatzucht J. Breun, D).

Seed samples were obtained from testing stations of the Central Institute for Supervising and Testing in Agriculture of the Czech Republic. In each harvest year the above mentioned assortment of varieties was grown at four testing stations. It means that they were equally influenced by the growth conditions. The varieties were followed for a three-year period (1997–1999) and thus 120 samples were processed. The samples were graded and sieving fractions above 2.5  $\mu$ m were used for further observations.

### 2.2 Micromalting

A micromalting test procedure follows the traditional method used in the Malting Institute of the RIBM in Brno and the trial malting method accepted on the 102<sup>nd</sup> session of the EBC committee for barley and malt in Perugia on May 31 2000. Temperature of steeping and germination was 14.5° C, time of steeping and germination was 144 hours. Temperature at the beginning of kilning was 50° C and at the end 80° C, time of kilning was 23 hours.

### 2.3 Analysis of caryopses and malt

Parameters followed in caryopses, malt and wort were analysed according to EBC (1998) and MEBAK (1997) methods. Germination homogeneity was calculated pursuant to authors Riis, Bang-Olsen (1991). Data on harvest, grading and TGW were taken from the Central Institute for Supervising and Testing.

#### 2.4 Sample preparation for starch granule size distribution assessment by the LALLS method

Preparation of a sample and subsequent measurement of starch granule size distribution by the LALLS method was provided by the Food Research Institute in Prague. Sample preparation and measurement procedure by the LALLS method have been published recently (*Psota et al.* 2000, *Chmelík et al.* 2001). The starch granules were split into two groups for these study purposes. The granules with size 0–8 µm and curve peak in the area of 3 µm were regarded small starch granules (B). The granules with size 8.1–100 µm and curve peak in the area of 20 µm were regarded large starch granules (A). This classification resulted from the natural split of the bimodal curve of starch granule size distribution. The lowest amount of starch granules was caught in the area of 8 µm.

#### 2.5 Statistical processing

Results were processed statistically using a correlation and regression analysis (linear pair and step analysis) and a method of residual maximum likelihood (*Patterson, Thompson* 1974), by a program REML of the University of Edinburgh (*Robinson et al.* 1995). Differences among effects of years, stations and varieties were tested by a multiple t-method. The effect of varieties and the environment (years and localities) on the followed parameters was quantified based on variance of component.

### 3 Results and discussion

#### 3.1 Level and variability of parameters

The literature presents various values of starch granule size distribution (*MacGregor, Fincher* 1993). These values were obtained by measuring different barley varieties grown under different conditions. Starch was isolated with the help of different techniques and the actual measurement of the starch granule size was carried out by different methods. Reported values are therefore very different.

When comparing the results presented in this publication with other authors' data (e.g. *MacGregor, Fincher* 1993), principally the same interval ranges of starch granule size in barley caryopses can be observed. The main size fraction of starch granules occur in the areas of 0–10 µm with peak around 3 µm and 10–30 µm with peak around 20 µm. In the literature, however, also three size fractions of starch granules were described. For example in the species *Hordeum distichum* L. fractions of small (2.3 µm), middle (7.5 µm) and large (20 µm) starch granules were detected (*Takeda et al.* 1999). In the varieties followed in this publication the smallest amount of the starch granules was caught in a size fraction of 8 µm. From this reason this fraction was taken as a boundary between small and large starch granules. Starch granule size distribution found by the LALLS method corresponds to the literature data. For the small starch granules, maximum was detected in the area around 3 µm, for the large granules around 20 µm. Existence of differences in content of the large and small starch granules among barley varieties was found earlier (*Goering et al.* 1973, *Oliveira et al.* 1994, and *Psota et al.* 2000).

Regarding the weight percentage of the large starch granules, the data found by us differ partially from those presented in the literature. It is usually presented that the large starch granules form approx. 9% of starch weight. In the given set of ten malting varieties of spring barley, the large starch granules formed 71–77% of starch weight. Some reasons are presented above. Way of calculation can be another reason of the difference. The

data on starch granule size distribution presented in this publication were acquired from the cumulative curves (or their tabular expression), giving the dependence of a percentage volume portion of the given starch fraction on the size of the starch granules contained in it. Provided that all starch granules are of the same specific density, the given volume percentage can be taken, with some reservation, as weight percentage.

In the followed set, the varieties had the biggest share in the total variability of content of both small (0.0–8.0 µm) and large (8.1–100.0 µm) starch granules in barley caryopses, roughly from 67% or from 70.0% (Tables 1, 2), which is also documented by a difference between the variety with the highest and lowest content of small (large) starch granules in caryopses which made % points (Table 3). Impact of localities and years was significantly lower and participated in the aforementioned variability only from 12% or from 6% (Tables 1, 2). The achieved results thus confirmed a significant variety dependency of starch granule size distribution (Tables 1, 2).

Significant differences among 14 genotypes were detected also in some parameters of starch granules (surface area, volume, proportion of A to total starch by volume, ratio B/A) (*Ellis et al.* 1992). Outer environment had an evidential effect on the presented parameters of starch granules too (*Oliveira et al.* 1994), which is in accordance with our above mentioned results.

Based on these results, it was possible to split this set into three groups (Table 3). The first group was formed by the varieties with the lowest ratio of small starch granules and at the same time with the highest ratio of large starch granules. These characteristics were exhibited by the variety Akcent followed by the varieties Forum and Atribut. The varieties Scarlett and Kompakt formed a transient group in the given set of varieties. The remaining varieties Krona, Tolar, Amulet, Olbram, and Novum had the highest ratio of small starch granules and the lowest ratio of large starch granules. There were no significant differences among these varieties and thus they jointly formed a group statistically different from the previous varieties. Statistically significant differences in starch granule size distribution between the varieties Kompakt and Novum were found earlier (*Psota et al.* 2000).

#### 3.2 Relations among parameters

Recently attention has been paid first of all to physical and chemical properties of starch granules such as content and mutual proportion between amyloza and amylopectin, gelatinization, etc. (*Tang et al.* 2001, *Tang et al.* 2000, *Myllarinen et al.* 1998, *Vasanthan, Bhaty* 1996, *Tester et al.* 1993, *Tester, Morrison* 1992). However, factual information on the relation of the large and small starch granule content to the basic technological parameters is still insufficient.

Correlation analysis between the ratio of small or large starch granules and selected technological parameters in the followed set enabled the following statement (Table 4).

Increase of the ratio of the small starch granules in non-malted caryopses of spring barley was in the followed set of varieties accompanied with  $\beta$ -glucan decrease ( $r = -0.32^{**}$ ).

With the increasing ratio of the small starch granules, values of germination index ( $r = -0.18^*$ ) and homogeneity of germination ( $r = -0.27^{**}$ ) were declining (Table 4).

The increasing ratio of the small starch granules influenced consequently also some parameters of wort and malt. A higher ratio of the small starch granules was accompanied with decrease

of values of: relative extract at 45° C ( $r = -0.28^{**}$ ),  $\beta$ -glucan content in wort ( $r = -0.29^{**}$ ) and in malt ( $r = -0.32^{**}$ ), wort viscosity ( $r = -0.20^*$ ). The samples with the increasing ratio of the small starch granules exhibited a longer time of saccharification ( $r = 0.24^{**}$ ) and higher modification and homogeneity values, ( $r = 0.19^*$ ) and ( $r = 0.25^{**}$ ) respectively by the Carlsberg method (Table 4).

A higher ratio of the small starch granules was in relation to lower content of  $\beta$ -glucans in a caryopsis. This relation was shown also in malt and wort. Lower  $\beta$ -glucan content in malt and wort corresponded to a better level of homogeneity and modification by the Carlsberg method and with lower values of wort viscosity.

A higher ratio of the small starch granules negatively influenced rate and homogeneity of germination and at the same time prolonged wort saccharification time, which partly corresponded to reduction of values of relative extract at 45° C.

Low coefficients of determination ( $r^2 \times 100$ ) indicate that relations between the classical technological parameters and starch granule size distribution are of a complex nature and can give only a rough image about a subsequent malting quality. Effect of starch granule size distribution on the technological quality is partly veiled by relations among starch granules and protein matrix, protein matrix quality and cell wall quality (Brennan *et al.* 1996, 1997). Neither the already mentioned study (Oliveira *et al.* 1994) provided an unambiguous evidence of the relation between starch granule parameters and some technological parameters (kernel weight, plump kernels, malt extract, ratio of soluble to total protein,  $\alpha$ -amylase). The significant relation was found only among wort extract and surface area and volume of the small starch granules. Also Ellis *et al.* (1992) recorded an evidential correlation between hot water extract, surface area of the small starch granules and further they found that with an increasing size of the small starch granules, increase of grain milling energy is connected as well.

The chosen set can be one of the reasons of the detected low ratio of statistical dependency. The set contains ten malting varieties with the good quality and with optimum nitrogenous substance content. If a relation of content of the small and large starch granules to the selected malting or distilling technological parameters is followed, larger spectrum of varieties will have to be assigned from the qualitative point of view.

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## Appendix

**Table 1** Estimated components of variance

	Year	Locality	Variety	Residual
Starch granule fraction 0.00 – 8.00 µm (LALLS)				
	0.423	0.797	4.539	1.028
%	6.200	11.700	66.900	15.200
SE	0.466	0.515	2.181	0.144
Starch granule fraction 8.01 – 100.00 µm (LALLS)				
	0.383	0.821	4.524	1.021
%	5.700	12.200	67.000	15.100
SE	0.426	0.529	2.174	0.143

**Table 2** Analysis of variance for starch granule fraction 0.00 – 8.00 µm (LALLS)

Source of variation	Sum of squares	d.f.	Mean square	F-ratio	Sig. level
Year	26.88567	2	13.442833	13.153	0.0000**
Locality	61.77592	6	10.295986	10.074	0.0000**
Variety	499.43408	9	55.492676	54.297	0.0000**
Residual	104.24525	102	1.022012		

**Analysis of variance for starch granule fraction 8.01 – 100.00 µm (LALLS)**

Source of variation	Sum of squares	d.f.	Mean square	F-ratio	Sig. level
Year	24.54092	2	12.2704580	12.091	0.0000**
Locality	63.13967	6	19.5232780	10.369	0.0000**
Variety	497.78175	9	55.3090830	54.500	0.0000**
Residual	103.51433	102	1.0148464		

**Table 3** Starch granule fraction 0.00 – 8.00 µm (LALLS)  
Method: 95% LSD

Varieties	n	Average	
Akcent	12	23.398690	a
Forum	12	24.482024	b
Atribut	12	24.865357	b
Scarlett	12	26.015357	c
Kompakt	12	27.282024	d
Krona	12	28.382024	e
Tolar	12	28.582024	e
Amulet	12	28.973690	e
Olbram	12	29.015357	e
Novum	12	29.082024	e

Comments:

LSD (t) (0,05) = 0.81881

Average values indicated by various letters are statistically significantly different

**Starch granule fraction 8.01 – 100.00 µm (LALLS)**  
Method: 95% LSD

Varieties	n	Average	
Novum	12	70.795952	a
Olbram	12	70.929286	a
Amulet	12	70.970952	a
Tolar	12	71.387619	a
Krona	12	71.462619	a
Kompakt	12	72.645952	b
Scarlett	12	73.870952	c
Atribut	12	75.062619	d
Forum	12	75.445952	d

**Table 4** Correlation between small and large starch granules – technological parameters

	SSG	LSG
Protein content in caryopsis	0.0370	-0.0367
Extract yield	0.0250	-0.0289
Relative extract (45°C)	-0.2840 **	0.2861 **
Kolbach number	-0.1110	0.1124
Diastatic power	-0.0100	0.0163
Apparent final attenuation	-0.0160	0.0119
Friability	0.0980	-0.0941
β-glucan in wort	-0.2860 **	0.2789 **
Protein in malt	0.0247	-0.0233
Total N in malt	0.0220	-0.0206
Total sol. N in wort	-0.1232	0.1267
Total sol. N in malt	-0.1396	0.1430
Glycid extract	0.0766	-0.0813
Viscosity	-0.1962 *	0.1940 *
Colour of wort	-0.0472	0.0493
Saccharification rate	0.2352 **	-0.2294 *
Glassy corns	0.0518	-0.0526
Homogeneity (friabilimeter)	0.0995	-0.0928
Length of acrospire	0.0890	-0.0853
Germination energy	-0.0738	0.0688
Germination index	-0.1824 *	0.1850 *
Germination homogeneity	-0.2722 **	0.2679 **
1000-kernel weight	-0.1270	0.1251
Modification (Carlsberg)	0.1936 *	-0.1845 *
Homogeneity (Carlsberg)	0.2552 **	-0.2493 **
β-glucan in barley	-0.3223 **	0.3113 **
β-glucan in malt	-0.2287 *	0.2237 *
Starch in barley	0.1626	-0.1653

Note:

SSG – small starch granules (0.00 – 8.01 µm)

LSG – large starch granules (8.01 – 100.00 µm)