V. Psota, I. Bohačenko, J. Chmelík and J. Hartmann

Comparison of GFFF and LALLS methods for determination of large and small starch granules in spring barley grain (*Hordeum vulgare* L.)

A newly developed method GFFF (Gravitational Field-Flow Fractionation) and the well known method LALLS (Low Angle Laser Light Scattering) were used to assess content of starch granules in the grain endosperm of seven varieties of spring barley (*Hordeum vulgare* L.). As a distribution criterion, the ratio of starch granules larger than 8 µm (type A) and smaller than 8 µm (type B) was chosen. Both methods assigned the varieties Akcent and Forum to the variety set with the highest ratio of large and small starch granules. Other studied varieties (Amulet, Kompakt, Olbram, Scarlett, and Tolar) formed a statistically indiscernible variety set with a lower ratio of large and small starch granules. Statistically highly significant correlation between the GFFF and LALLS methods was proved (r = 0.782).

Descriptors: barley, starch granules, size distribution, LALLS, GFFF

1 Introduction

Starch in barley caryopses occurs in a form of large (type A, 8–30 µm) and small (type B, 2-8 µm) granules. [14] Generally, the small granules represent ca 90 % of the total amount of the granules but only 10 % of the starch total weight. [1] During the technological process of the beer production, most granules are degraded to fermentable sugars which are converted by yeasts to alcohol. Due to their compactness, however, ca 5 % of starch granules remains inaccessible for enzymatic degradation. The small starch granules tend to embed firmly into the protein matrix and interior of the cell walls. [16] Besides the loss of yield at alcohol production, these spherical particles can cause further technological problems. They can create starch haze and block filter beds in lauter tuns. [23]

A number of methods have been developed for measuring the starch granule distribution. [5, 10] The main techniques used are Image Analysis, the Coulter Counter method and the Low Angle Laser Light Scattering method (LALLS). Another method for measuring starch granule size distribution is the newly developed method of Gravitational Field-Flow Fractionation (GFFF). [3, 4, 9, 21]. These techniques allow to acquire more information on this aspect of starch. New data can then be utilized in breeding programs and for assessment of specific lots of barley or other cereals.

With respect to study of the barley caryopsis structure and composition, future research trends in the following areas have been outlined [11]:

- Identification of factors affecting and controlling the ratio of large and small starch granules in barley endosperm with the aim to breed cultivars containing minimum amount of small granules but maximum content of total starch.
- Identification of factors affecting the relationship between the content and distribution of small starch granules and mealiness and malting potential.

Study of barley caryopsis proteom has followed the research of the starch itself [6, 7] with the aim to describe composition of enzymes and izoenzymes, some of them are denoted as biochemical markers of certain processes.

2 Materials and methods

2.1 Varieties

Starch granule size distribution was observed in the following set of seven two-row malting varieties of spring barley [19, 19, 20]: Akcent, Amulet (Selgen, CZ), Forum, Olbram (Monsanto ČR, CZ), Kompakt (Hordeum, SK), Scarlett (Saatzucht J.Breun, D) and Tolar (Plant Select, CZ).

Seed samples were obtained from three testing stations of the Central Institute for Supervising and Testing, Czech Republic (CISTA): Věrovany (sugar beet production area), Pusté Jakartice (sugar beet production area) and Krásné Údolí (forage production area) (Table 1). Samples were graded and fractions above 2.5 mm were used for further observations.

2.2 Determination of starch granule size distribution by means of the LALLS and GFFF methods

Determination of the starch granule size distribution was carried out in two different locations. The Food Research Institute in Prague carried out sample preparation and subsequent measurements of starch granule size distribution by means of the LALLS method. Sample preparation and starch granule size distribution by the means of the GFFF method were carried out at the Institute of Analytical Chemistry in Brno.

Already published methods were used for sample preparation and measurement. [18, 5]. Starch granules were divided into two groups. The starch granules with the size of 0–8 µm, with the peak of the curve in the area of 3 µm were considered as the small starch

Authors: Ing. Vratislav Psota, Research Institute of Brewing and Malting, Plc., Malting Institute, Mostecká 7, CZ-614 00 Brno, Czech Republic;
Ing. Ivan Bohačenko, Food Research Institute, CZ-102 31 Radiová 7, Prague, Czech Republic;
RNDr. Josef Chmelík, Institute of Analytical Chemistry, CZ-611 42 Veveří 97, Brno, Czech Republic;
Ing. Jiří Hartmann, Central Institute for Supervising and Testing in Agriculture, CZ-656 06 Hroznová 2, Brno, Czech Republic.

Tables see Appendix.

granules (B). Those with the size of 8.1–100 μm and the curve peak in the area of 20 μm were considered as large granules (A). This distribution resulted from the natural course of the bimodal curve of the starch granule size distribution. The smallest amount of starch granules was recorded in the area of 8 μm.

The GFFF method is based on division of particles with the size 1–100 μm in the through-flow channel by means of hydrodynamic and gravitation power. GFFF confirmed a bimodal distribution of barley starch. The first eluted peak corresponds to A-granules and the second to B-granules. This was also confirmed by light microscopy. The ratio of the peak areas corresponding to A- and B-granules can be used as a preliminary measure of the number (or mass) when calculated as A/B ratios. It is important to note that the determined value of the peak area ratio does not correspond to any real ratio of A/B. This value only shows whether one sample of isolated barley starch granules contains relatively more or less of one type of starch granules in comparison to other samples.

The LALLS method is based on the analysis of light scattering occurring when a ray of monochromatic light falls at the boundary of two substances with different refraction index. Results obtained with the LALLS method are elaborated either in the form of distribution or cumulative curves. For calculations presented in this publication we used cumulative curves which indicate correlations of percentage volume of the given starch fraction and the size of starch granules contained in it.

2.3 Statistical evaluation

The minimal significant difference (LSD t) was used for comparison of the pair of varieties. Varieties among which statistically significant difference (LSD T) was not proved are marked with the same letter (“a“ to “e“) and they form a homogenous group.

3 Results and discussion

Literature presents various values of the starch granule size distribution [12]. These values were obtained from measurements of various barley varieties grown under diverse conditions. Starch was isolated with versatile techniques and different methods were employed for the actual measurement of starch granule size. Therefore, the data presented in the literature are very heterogenous.

Comparison of the achieved results with the data from other authors (e.g. [12]) shows basically the same interval ranges of the starch granule sizes in barley caryopses. The principal size fraction of starch granules is given in the areas of 0–10 μm with the peak round 3 μm and 10–30 μm with the peak round 20 μm. But even three size fractions of starch granules have been described in the literature. For example in *Hordeum distichum* L., fractions of small (2.3 μm), medium (7.5 μm) and large (20 μm) starch granules were detected. [22]

In the varieties studied in this manuscript, the minimum amount of starch granules was found on the distribution curve at the value 8 μm. For this reason, this value was taken as a boundary between the large and small starch granules.

Existence of differences in the content of large and small starch granules in different varieties was described previously. [8, 15, 18]

Significant differences among 14 genotypes were found also in some parameters of starch granules (surface area, volume, proportion of A to total starch by volume, ratio B/A). Also environment significantly affected the given parameters of starch granules [15], which agrees with our results presented above.

Both methods separated the studied set of barley varieties according to the ratio between large and small starch granules in a similar way (Table 2). The varieties Akcent and Forum exhibited the highest ratio of large and small starch granules. The varieties Akcent and Forum differed statistically significantly one from another and at the same time they differed from the remaining observed varieties (Scarlett, Kompakt, Olbram, Amulet, and Tolar). Proportions between the individual size fractions of starch granules are likely to vary with respect to a species or variety. This has been confirmed by a number of authors (e.g. [2, 8, 13, 18, 22]).

Although the LALLS and GFFF methods are based on different principles (light scattering x separation based on specific weight), statistically highly significant correlation dependence ($r = 0.782$) between their results was confirmed.

The GFFF method could be used by laboratories in barley breeding industry for the assessment of ratio of large and small starch granules from the endosperm of barley and other cereals.

Acknowledgement

This work was founded by grant from the Ministry of Education, Youth and Sports of the Czech Republic (OK 392).

4 Literature

1. Bathgate, G. N.; Palmer, G. H.: A reassessment of the chemical structure of barley and wheat starch granules. Staerke **24** (1972), pp. 336-341
2. Baum, B. R.; Bailey, L. G.: A survey of endosperm starch granules in the genus *Hordeum*: a study using image analytic and numerical taxonomic techniques. Can. J. Bot. **65** (1987), pp. 1563-1569.
3. Chmelík, J.; Krumlová, A.; Čáslavský, J.: Characterization of starch materials from barley by gravitational field flow fractionation and matrix assisted lasser desorption/ ionization mass spectrometry. Chem. Pap. **52** (1998), pp. 360-361.
4. Chmelík, J.; Psota, V.: Characterization of starch materials from barley. Proceedings of the European Brewery Convention Congress, Cannes, (1999), Fachverlag Hans Carl GmbH, Nürnberg, pp. 421-428.
5. Chmelík, J.; Krumlová, A.; Budinská, M.; Kruml, T.; Psota, V.; Bohačenko, I.; Mazal, P.; Vydrová, H.: Comparison of Size Characterization of Barley Starch Granules Determined by Electron and Optical Microscopy, Low Angle Laser Light Scattering and Gravitational Field-Flow Fractionation. J. Inst. Brew., **107** (2001): pp. 11-17.
6. Chmelík, J.; Řehulka, P.; Mayrhofer, C.; Allemaier, G.: Proteomics – A new and fast tool for identification of barley varieties. Kvasny Prum. **47** (2001): pp.159-163.
7. Chmelík, J.; Řehulka, P.; Střelcová, M.; Kubáň, V.; Mayrhofer, C.; Allmaier, G.: Proteomic analysis of different extracts from barley grains. Rostlinná Výroba. **48** (2002): pp. 261-264.
8. Goering, K. J.; Fritts, D. H.; Eslick, R. F.: A study of starch granule size and distribution in 29 barley varieties. Staerke **32** (1973), pp. 297-302.
9. Janoušková, J.; Budinská, M.; Plocková, J.; Chmelík, J.: Optimization of experimental conditions for the separation of small and large starch granules by gravitational field-flow fractionation. Journal of Chromatography A, 914 (2001), pp. 183-187.
10. Lindenboom, N.; Chang, P. R.; Tyler, R. T.: Analytical, Biochemical and Physicochemical aspects of starch granule size, with emphasis on small granule starches: a review. Starch/Stärke, **56** (2004), pp. 89-99.

11. MacGregor A. W.: The effect of barley structure and composition on malting quality. In: Proc. Cong. EBC 23rd, Lisboa (1991), Fachverlag Hans Carl GmbH, Nürnberg.

12. MacGregor, A. W.; Fincher, G. B.: Carbohydrates of the barley grain, pp. 73-130. In: MacGregor, A. W., Bhatty, S. R. (eds.), Barley: Chemistry and Technology. AACC St.Paul, MN, USA (1993).

13. Mäkelä, M. J.; Korpela, T.; Laakso, S.: Studies of starch size and distribution in 33 barley varieties with a celloscope. Stärke **34** (1982), pp. 329-334.

14. May, L. H.; Buttrose, M. S.: Physiology of cereal grain. II. Starch granule formation in the developing barley kernel. Aust. J. Biol. Sci. **12** (1959), pp. 146-159.

15. Oliveira, A. B.; Rasmusson, D. C.; Fulcher, R. G.: Genetic aspects of starch granule traits in barley. Crop Science, **34** (1994), pp. 1176-1180.

16. Palmer, G. H.: Morphology of starch granules in cereal grains and malts. J. Inst. Brew. **78** (1972), pp. 326-332.

17. Psota, V.; Jurečka, D.: Registration of spring barley varieties in 2000. Kvasny Prum. **46** (2000), pp. 155-158.

18. Psota, V.; Bohačenko, I.; Pytela, J.; Vydrová, H.; Chmelík, J.: Determination of size distribution of barley starch granules using low angle laser light scattering. Rostlinná výroba **46** (2000), pp. 433-436.

19. Psota, V.; Jurečka, D. (2001): Registration of spring barley varieties in the Czech Republic in the year 2001. Kvasny Prum. **47** (2001), pp. 154-158.

20. Psota, V.; Kosař, K.; Jurečka, D.: Assortment of varieties of malting barley in the Czech Republic in the year 2000. Monatsschrift für Brauwissenschaft **54** (2001), pp. 9-12.

21. Reschiglian, P.; Zattoni, A.; Casolari, S.; Krumlová, A.; Budinská, M.; Chmelík, J.: Size characterization of barley starch granules by gravitational field-flow fractionation: A rapid, low-cost method to assess the brewing capability of different strains. Annali di Chimica **92** (2002), pp. 457-467.

22. Takeda, Y.; Takeda, C.; Mizukami, H.; Hanashiro, I.: Structures of large, medium and small starch granules of barley grain. Carbohydrate Polymers **38** (1999): 109-114.

23. Tillet, I. J. L.; Bryce, J. H.: The regulation of starch granule size in endosperm of developing barley grains. Proceedings of the EBC Congress, Oslo (1993), , Fachverlag Hans Carl GmbH, Nürnberg, pp. 45-52.

Received 22 September, 2006, accepted 15 April, 2007

Appendix

Table 1 Descriptions of locations

Location	Production region	Altitude (m)	Temperature (°C)	Rainfall (mm)	Soil
Věrovany	Sugar beet production region	207	8.6	605	Haplic Chernozem Loamy soil (medium)
Pusté Jakartice	Sugar beet production region	295	8.0	650	Orthic Luvisol Loamy soil (medium)
Krásné Údolí	Forage production region	647	6.1	605	Eutric Cambisol Loamy-sand (light)

Table 2 Multiple range analysis

for the ratio of large and small starch granules (LALLS)

Varieties	MQI	n	mean			
AKCENT	9	3	3.65	**a**		
FORUM	8	3	3.00		**b**	
SCARLETT	7	3	2.56			**c**
AMULET	6	3	2.48			**c**
KOMPAKT	9	3	2.48			**c**
OLBRAM	7	3	2.46			**c**
TOLAR	5	3	2.37			**c**

Comments: LSD(t) (0,05) = 0.20846
mean values indicated by various letters
MQI - Malting Quality Index

for the ratio of peak areas (GFFF)

Varieties	MQI	n	mean					
AKCENT	9	3	1.52	**a**				
FORUM	7	3	1.33		**b**			
KOMPAKT	7	3	0.85			**c**		
AMULET	6	3	0.79			**c**	**d**	
TOLAR	8	3	0.75			**c**	**d**	**e**
SCARLETT	6	3	0.69				**d**	**e**
OLBRAM	7	3	0.66					**e**

LSD(t) (0,05) = 0.1145588
are statistically different (P = 0,05)