

# Comparison of the GFFF and LALLS Methods for the Measurement of Starch Granule Size Distribution in Spring Barley Caryopses

V. Psota,<sup>1,5</sup> I. Boháčenko,<sup>2</sup> J. Hartmann,<sup>3</sup> M. Budinská,<sup>4</sup> and J. Chmelík<sup>4</sup>

## ABSTRACT

*J. Inst. Brew.* 108(2), 200–203, 2002

A newly developed method GFFF (Gravitational Field-Flow Fractionation) and the well known method LALLS (Low Angle Laser Light Scattering) were used to assess starch granule size distribution of ten varieties of spring barley. As a distribution criterion, the ratio of starch granule content larger than 8 µm (type A) and smaller than 8 µm (type B) was chosen. Both methods divided the observed set in a similar way. Varieties Akcent, Forum and Atribut formed a variety set with the highest ratio of large and small starch granules. Varieties Scarlet and Kompakt had intermediate ratios. The remaining five varieties Amulet, Novum, Olbram, Tolar and Krona had the lowest ratios of large and small starch granules. Statistical analysis showed that there was a highly significant correlation between the GFFF and LALLS methods.

**Key words:** Barley, GFFF, LALLS, starch granule size distribution.

## INTRODUCTION

Detailed knowledge of the endosperm structure of barley caryopsis contributes to an improved understanding of the malting process. The main component of barley endosperm is starch in the form of large and small granules. Size 8–10 µm is generally accepted as a boundary between large (type A) and small (type B) starch granules<sup>8</sup>. Small starch granules tend to embed into the protein matrix<sup>12,13</sup>, and this can influence the rate and extent of endosperm modification during malting. Also in the process of beer production they can contribute to wort turbidity<sup>1</sup>.

A number of methods have been developed for measuring the starch granule size distribution<sup>5</sup>. The main techniques used are Image Analysis of microscopic measurements, 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)<sup>3,4</sup>.

Using the above-mentioned techniques enabled us to obtain more information on this aspect of starch. The information obtained can be used in breeding programmes and in the quality evaluation of particular barley lots and other cereals.

## MATERIALS AND METHODS

Starch granule size distribution was observed with the malting varieties of spring barley<sup>14,16,17</sup> listed in Table I. Seed samples were obtained from testing stations of ÚKZÚZ (the Central Institute for Supervising and Testing of the Czech Republic). Observations covered a three-year period. In every harvest year the above-mentioned varieties were grown at four testing stations. Growth conditions influenced all varieties in the same way. For the purposes of this study, the testing stations were selected in which, from the malting point of view, nitrogen content in barley caryopses of the observed varieties approximated optimal values. Seed samples were graded and fractions above 2.5 mm were used for further observations.

### Sample preparation and determination of starch granule size distribution by means of the LALLS and GFFF methods

Separation of starch granules from barley grains and determination of starch granule size distribution was carried out independently in two different locations. The Food Research Institute in Prague carried out sample preparation<sup>15</sup> and the subsequent measurement of starch granule size distribution by means of the LALLS method<sup>15</sup>. Sample preparation<sup>5</sup> and starch granule size distribution were carried out by means of the GFFF method at the Institute of Analytical Chemistry in Brno<sup>7</sup>. Both methods of sample preparation were based on the fundamental work of MacDonald and Stark<sup>10</sup>.

### Statistical evaluation

Results were statistically evaluated by means of scattering analysis, correlation and regression analysis (linear, pair and step analysis). To determine an optimal shape of multiple linear regression, the function method of 'Stepwise Backward Selection' was used.

<sup>1</sup>The Research Institute of Brewing and Malting, Mostecká 7, 614 00 Brno, Czech Republic

<sup>2</sup>Institute of Chemical Technology, Radiová 7, 102 31 Prague, Czech Republic

<sup>3</sup>Central Institute for Supervising and Testing in Agriculture, Hroznová 2, 656 06 Brno, Czech Republic

<sup>4</sup>Institute of Analytical Chemistry AS CR, Veveří 97, 611 42 Brno, Czech Republic

<sup>5</sup>Corresponding author. E-mail: psota@brno.beerresearch.cz

## RESULTS AND DISCUSSION

Standard procedures of sample preparation before measuring starch granule size distribution were highly effective. However they did not guarantee complete extraction of starch granules from the original protein matrix or the separation of all contaminating particles. Aspects of good extraction and purification have been emphasized in the majority of studies dealing with starch granule size distribution in plant materials. In most of the work cited, considerable attention was paid to perfecting methods used for starch granule preparation<sup>5,7,10,15</sup>.

Results are presented in Tables II and III. Samples with high values for ratios of large and small starch granules have more large starch granules than small starch granules, compared to samples with lower ratios<sup>15</sup>. Results obtained with the LALLS method are elaborated either in the form of distribution or cumulative curves. For calculations presented in this publication, cumulative curves were used which indicate correlations of percentage volume of the given starch fraction and the size of starch granules contained in it.

GFFF confirmed a bimodal distribution of barley starch. The first eluted peak corresponds to A-granules and the second to B-granules. This was 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 with other samples.

From a more detailed evaluation of both methods using the scattering method it is obvious that both methods divide the given set of varieties in very similar ways (Table II). It is evident that these methods separated Akcent, Forum, and Atribut as the varieties with the highest ratio of large and small starch granules. The LALLS method separated Kompakt and Scarlett as the varieties that were significantly different from the previous group of varieties and from the remaining varieties. The GFFF method did not separate the variety Kompakt and particularly the variety Scarlett from the remaining varieties. Differences among remaining varieties (Krona, Tolar, Novum, Amulet and Olbram) were so small that the LALLS method ranged them into one statistically significant group. This was confirmed by the GFFF method which placed the remaining varieties in a different order, Amulet, Novum, Olbram, Tolar, and Krona. This group was significantly different from the first three varieties, Akcent, Forum, and Atribut.

The results show clearly that starch granule size distribution is strongly correlated with variety. The proportions of individual size fractions of starch granules are likely to be influenced by varietal factors. This has been confirmed by a number of authors<sup>2,6,9,15,18</sup>.

At the observed variety set, the influence of varieties on the total variability of the ratio of small and large

TABLE I. Varieties with nitrogen content in a barley caryopsis.

Varieties	Breeder	Location	Nitrogen content (%)		
			Average	Min. value	Max. value
Akcent	Selgen, a. s.	Czech Republic	1.73	1.57	1.84
Amulet	Selgen, a. s.	Czech Republic	1.74	1.57	1.95
Atribut	Selgen, a. s.	Czech Republic	1.78	1.62	1.98
Forum	Monsanto ČR s. r. o.	Czech Republic	1.66	1.49	1.79
Kompakt	Hordeum, spol. s r. o.	Slovak Republic	1.66	1.47	1.84
Krona	Semundo Saatzucht GmbH	Germany	1.74	1.54	2.00
Novum	Hordeum, spol. s r. o.	Slovak Republic	1.71	1.54	1.87
Olbram	Monsanto ČR s. r. o.	Czech Republic	1.78	1.60	1.95
Scarlett	Saatzucht J.Breun GdbR	Germany	1.71	1.46	1.84
Tolar	Plant Select	Czech Republic	1.68	1.54	1.87

TABLE II. Ratio of large and small starch granules determined by the LALLS and GFFF methods.

Ratio of large and small starch granules (LALLS) Method: 95% LSD <sup>1</sup>					Ratio of peak areas A/B (GFFF) Method: 95% LSD <sup>2</sup>				
Varieties	n	Average	S.E.	Homogeneous groups	Varieties	n	Average	S.E.	Homogeneous groups
Olbram	12	2.443	0.05	a	Krona	12	0.496	0.10	a
Amulet	12	2.452	0.06	a	Tolar	12	0.553	0.04	a b
Novum	12	2.452	0.11	a	Olbram	12	0.567	0.07	a b
Tolar	12	2.510	0.12	a	Novum	12	0.578	0.14	a b
Krona	12	2.527	0.06	a	Amulet	12	0.628	0.06	b c
Kompakt	12	2.668	0.10	b	Scarlett	12	0.633	0.06	b c
Scarlett	12	2.843	0.04	c	Kompakt	12	0.665	0.06	c
Atribut	12	3.027	0.04	d	Atribut	12	0.808	0.05	d
Forum	12	3.102	0.11	d	Forum	12	1.296	0.07	e
Akcent	12	3.302	0.12	e	Akcent	12	1.467	0.15	f

<sup>1</sup>LSD(t) (0.05) = 0.11972. Average values indicated by various letters are statistically different (P = 0.05). S.E. = standard error.

<sup>2</sup>LSD(t) (0.05) = 0.08584. Average values indicated by various letters are statistically different (P = 0.05). S.E. = standard error.

TABLE III. Analysis of variance for ratio of large and small starch granules by LALLS and GFFF methods.

LALLS					
Source of variation	Sum of squares	d.f	Mean square	F-ratio	Significant level
Year	0.702	2	0.351	16.073	0.0000
Locality	1.359	6	0.227	10.370	0.0000
Variety	10.757	9	1.195	54.703	0.0000
Residual	2.229	102	0.022		
GFFF					
Source of variation	Sum of squares	d.f	Mean square	F-ratio	Significant level
Year	0.457	2	0.228	20.333	0.0000
Locality	0.251	6	0.042	3.731	0.0021
Variety	12.164	9	1.352	120.318	0.0000
Residual	1.146	102	0.011		

TABLE IV. Correlation and regression analysis for ratio of large and small starch granules by LALLS and GFFF methods.

Regression analysis - linear model: $Y = a + bx$					
Dependent variable: LALLS			Independent variable: GFFF		
Parameter	Estimate	Standard error	T value	Prob. level	
$\alpha$ (Intercept)	2.206	0.053	41.524	0.000	
$\beta$ (Slope)	0.749	0.064	11.713	0.000	
Analysis of Variance					
Source of variation	Sum of squares	d.f.	Mean square	F-ratio	Sig. level
Model	7.967	1	7.967	137.189	0.000
Residual	6.852	118	0.058		
Total (Corr.)	14.819	119			
Correlation analysis					
Correlation Coefficient = 0.733		R = squared = 53.76%			
Stand. Error of Est. = 0.241					
95% confidence interval for parameter $\alpha$ (Intercept) (2.101 < $\alpha$ < 2.311)					
95 % confidence interval for parameter $\beta$ (Slope) (0.622 < $\beta$ < 0.875)					

starch granules in barley caryopses, measured with the LALLS method, reached 65%, localities 13%. Influence of the year moved about 8%. Results obtained by the means of the GFFF method confirmed the highly significant effect of variety (84%). In contrast, influence of the years (5%) and localities (2%) was small (Table V). In the literature there are no studies that describe the levels of influences of varieties, localities and years on the ratio of large and small starch granules. Significant effects of environment were detected. However the influence of the combination of environment x variety on

some features of starch granules was found to be significant<sup>11</sup>.

There was significant correlation between GFFF and LALLS methods (Table IV). However with regard to laboratory practice, the difference between the results of both methods is too high for comparative use because the coefficient of the determination reaches only 53.8%. Although these methods are based on different principles it is important that the results achieved indicate similar trends in starch granule size distribution.

The method of isolation of starch granules carried out at two laboratories using different methods could contribute to the difference between the results obtained by the LALLS and GFFF methods. Although different sample isolation procedures decrease the conformity of the results obtained by the LALLS and GFFF, they in fact support the findings on the barley varieties. It means that both measuring methods showed very similar distribution of varieties into three sub-groups based on the ratio of large and small starch granules. We can assume that after further amendments of the method of sample preparation and after amendments of the GFFF method, it will be possible to use the GFFF method as a valuable screening method for measuring starch granule size distribution, as a complimentary method to the more accurate and complex LALLS method.

TABLE V. Estimation components of variance of large and starch granules by LALLS and GFFF methods.

Ratio of small and large starch granules (LALLS)				
	Year	Locality	Variety	Residual
abs.	0.012	0.020	0.098	0.022
%	7.9	13.1	64.5	14.5
SE	0.012	0.013	0.047	0.003
The ratio of peak areas A/B (GFFF)				
	Year	Locality	Variety	Residual
abs.	0.007	0.003	0.112	0.011
%	5.3	2.3	84.1	8.3
SE	0.008	0.002	0.053	0.002

## CONCLUSIONS

Starch granule size distribution was observed at the set of the spring barley by two different methods, the newly developed method GFFF and the well-known LALLS method. Highly significant correlation was established between the GFFF and the LALLS methods of preparation of starch granules from caryopses for measurement with the dissimilar methods. However, in spite of these differences both methods separated the observed set of varieties into similar groupings. We can assume that after further adjustments of the starch granule preparation procedure and after modification of the GFFF method, it will be possible to use the latter for the measurement of starch granule size distribution.

## ACKNOWLEDGEMENTS

Results were obtained within the projects INCO-COPERNICUS (EC, No. ERB 3512, PL 979070) and the Grant Agency of Academy of Sciences of the Czech Republic (A4031805).

## REFERENCES

1. Bamforth, C. W. and Barclay, A. H. P., Malting technology and the uses of malt. In: Barley Chemistry and Technology. A. W. MacGregor and R. S. Bhaty, Eds., Am. Assoc. Cereal Chem: St Paul, MN., 1993, p. 297.
2. Baum, B. R. and Bailey, L. G., *Canadian Journal of Botany*, 1987, **65**, 1563.
3. Chmelík, J., Krumlová, A. and Čáslavský, J., *Chemical Papers*, 1998, **52**, 360.
4. Chmelík, J. and Psota, V., Proceedings of the European Brewery Convention Congress, Cannes, IRL Press: Oxford, 1999, p.421.
5. Chmelík, J., Krumlová, A., Budinská, M., Kruml, T., Psota, V., Boháčenko, I., Mazal, P. and Vydrová, H., *Journal of the Institute of Brewing*, 2001, **107**, 11.
6. Goering, K. J., Fritts, D. H. and Eslick, R. F., *Stärke*, 1973, **32**, 297.
7. Janoušková, J., Budinská, M., Pločková, J. and Chmelík, J., *Journal of Chromatography*, 2001, A **914**, 183.
8. May, L. H. and Buttrose, M. S., *Australian Journal of Biological Sciences*, 1959, **12**, 146.
9. Mäkelä, M. J., Korpela, T. and Laakso, S., *Starch/Stärke* 1982, **34**, 329.
10. McDonald, A. M. and Stark, J. R., *Journal of the Institute of Brewing*, 1988, **94**, 125.
11. Oliveira, A. B., Rasmusson, D. C. and Fulcher, R. G., *Crop Science*, 1994, **34**, 1176.
12. Palmer, G. H., *Journal of the Institute of Brewing*, 1972, **78**, 326.
13. Palmer, G. H., *Ferment*, 1993, **6**, 105.
14. Psota, V. and Jurečka, D., *Kvasny Prumysl*, 2000, **46**, 155.
15. Psota, V., Boháčenko, I., Pytela, J., Vydrová, H. and Chmelík, J., *Rostlinna Výroba*, 2000, **46**, 433.
16. Psota, V. and Jurečka, D., *Kvasny Prumysl*, 2001, **47**, 154.
17. Psota, V., Kosař, K. and Jurečka, D., *Monatsschrift für Brauwissenschaft*, 2001, **54**, 9.
18. Takeda, Y., Takeda, C., Mizukami, H. and Hanashiro, I., *Carbohydrate Polymers*, 1999, **38**, 109.

(Accepted for publication May 2002)