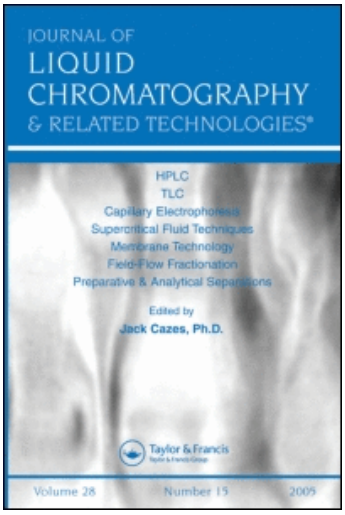

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Relationship Between the Ratio of Large and Small Starch Granules Determined by Gravitational Field-Flow Fractionation and Malting Quality of Barley Varieties

Josef Chmelík[a]; Karel Mazanec[a]; Ivan Bohačenko[b]; Vratislav Psota[c]
[a] Institute of Analytical Chemistry, Brno, Czech Republic [b] Food Research Institute, Prague, Czech Republic [c] Research Institute of Brewing and Malting, PLC, Brno, Czech Republic

To cite this Article Chmelík, Josef , Mazanec, Karel , Bohačenko, Ivan and Psota, Vratislav(2007) 'Relationship Between the Ratio of Large and Small Starch Granules Determined by Gravitational Field-Flow Fractionation and Malting Quality of Barley Varieties', Journal of Liquid Chromatography & Related Technologies, 30: 9, 1289 — 1301
To link to this Article: DOI: 10.1080/10826070701274767
URL: http://dx.doi.org/10.1080/10826070701274767

Journal of Liquid Chromatography & Related Technologies®, 30: 1289–1301, 2007

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070701274767

Relationship Between the Ratio of Large and Small Starch Granules Determined by Gravitational Field-Flow Fractionation and Malting Quality of Barley Varieties

Josef Chmelík and Karel Mazanec
Institute of Analytical Chemistry, Brno, Czech Republic

Ivan Bohačenko
Food Research Institute, Prague, Czech Republic

Vratislav Psota
Research Institute of Brewing and Malting, PLC, Brno, Czech Republic

Abstract: The ratio of large and small barley starch granules was determined by gravitational field-flow fractionation (GFFF) under optimized conditions. The optimization was aimed to reach the elution of both large and small starch granules in the focusing elution mode and long-term reproducibility. Kernels of twelve spring and winter barley varieties grown in three testing stations during three years were used for isolation and size determination of starch granules, i.e., monitoring was conducted with 108 samples. The two-row winter varieties had the highest ratios of large and small starch granules A/B. On the other hand, the two-row varieties of spring malting barley had the lowest ratios A/B. The ratios of large and small starch granules A/B of six-row winter varieties were between the previous groups. The obtained relationship between the ratio of large and small starch granules and malting properties for this set of barley varieties is opposite to previous expectations, assuming that spring malting barley varieties have higher ratios of large and small starch granules than winter ones. GFFF was shown as a suitable technique for the determination of the ratio A/B starch granules. From the point of view of the results obtained in this study, it is evident that not only the ratio A/B of

Address correspondence to Josef Chmelík, Institute of Analytical Chemistry, Veveří 97, Brno CZ-602 00, Czech Republic. E-mail: chmelik@iach.cz

large and small starch granules is important for malting properties, but other factors are also important, e.g., activity of hydrolytic enzymes.

Keywords: Gravitational field-flow fractionation, Starch granules, Determination, Size distribution, Malting quality, Barley varieties

INTRODUCTION

Starch (a major reserve carbohydrate in plants) is stored in various granular forms. Size and shape of starch granules is characteristic of botanical origin. Their shapes vary from perfect spheres to oval, rounded, and polyhedral[1] and the sizes from hundreds nanometers to hundreds micrometers (for a recent review see Reference[2]).

Barley starch granules have a bimodal particle size distribution. The oval large starch granules A have diameters in the range from 10 to 40 μm and they prevail in weight.[3] They are easily accessible to hydrolytic enzymes during malting and mashing and, thus, they are the main source of carbohydrates for beer production.[4] On the other hand, the spherical small starch granules B have diameters from 1 to 10 μm and they prevail in number.[3] They are deeply embedded into a protein matrix and endosperm cell walls of barley kernels, they gelatinize at higher temperatures and over wider temperature range than the granules A, and that is why the granules B are less susceptible to enzymatic degradation during malting and mashing.[4] Tillet and Bryce[3] assume that only about one half of the small starch granules is degraded in the course of malting and kilning, which means that approximately 5% of the total starch content is not degraded to fermentable saccharides that are important for the consequent conversion to alcohol. The remaining small starch granules B participate in haze formation and cause technological problems during beer production, especially during filtration.

The knowledge of the ratio of large and small starch granules A/B offers new attitude to the quality assessment of malting barley varieties. Spring barley varieties have better malting properties than winter ones. Because of incomplete digestion of small starch granules, it is assumed that malting barley varieties should have higher ratios of starch granules A/B than non-malting barley varieties. In a set of 10 malting barley varieties, the granule size distribution was determined to be predominantly dependent on the variety (67%), the effects of the locality (12%) and the year (6%) were significantly lower. However, the set of barley varieties used in that study was rather narrow to draw any definite conclusions about relationships between the ratio of starch granules A/B and the malting quality of barley varieties.[5] Therefore, the present study involved several nonmalting varieties in addition to the spring malting varieties.

Recent studies on starch granules focused, above all, on their physical and chemical properties, such as content and mutual relation between amylase and

amylopectin, gelatinization, etc. Data on the relationship between starch granule size composition and the basic technological features are limited. The effect of starch granule distribution on the technological quality is influenced by the relations between the starch granules and protein matrix, protein matrix quality, quality of cell walls,[6] and enzymatic apparatus quality. Significant correlations between malt extract and surface of small starch granules were found.[7,8] Various methods were used for determination of the ratio of starch granules A/B, e.g., Coulter counter,[9] low angle laser light scattering (LALLS),[10] image analysis,[11] or field-flow fractionation (FFF).[12]

FFF is a group of elution separation methods where an external force field acts perpendicularly to a carrier liquid flow with a non-uniform velocity profile. The principle of FFF and its applications were described in detail elsewhere.[13–15] FFF appears to be not only promising rapid technique for this purpose, but it offers some other advantages. It is a separation method that enables to separate particles in relatively short time and to collect fractions for further size characterization (e.g., scanning electron microscopy) or for investigation of the properties of large and small starch granules independently. Another advantage of FFF is small consumption of samples. Gravitational field-flow fractionation (GFFF) used in this work is the experimentally simplest FFF technique with high resolution of nano- and microparticles.[16,17] Gentle experimental conditions (low pressure and weak force field) and the possibility to use isotonic buffer solutions as carrier liquids make GFFF uniquely suited for separations and purifications of biological samples (e.g., blood and stem cells,[18–20] yeasts,[21–23] and starch granules.[12,24–27]

Two FFF techniques, utilizing sedimentation field, were successfully applied to separation of starch granules[15,16] in the 1990's. Moon and Giddings[15] employed sedimentation field-flow fractionation (SdFFF) for particle size analysis of starch granules from wheat, durum wheat, corn, oat, tapioca, and potato. The technique was shown to be suitable for size characterization of starch granules because of good resolution and high speed of separation. Another advantage of SdFFF is a possibility of compensation for different densities of particles by using different acceleration values during calibration and sample measurement. However, this technique requires a rather expensive instrumentation because a continuous-flow centrifuge generates the applied force.

Chmelik et al.[16] used GFFF for separation of barley starch granules. Separation in GFFF is based on a combination of Earth's gravitational field and a non-uniform flow velocity profile of a carrier liquid in the separation channel (Figure 1). The Earth's gravity causes settlement of particles toward the channel bottom resulting from their effective weight and forms specific concentration profiles of sample components. In FFF, the samples are eluted in different elution modes in dependency on the strength of the field applied, properties of the particles and the flow rate of the carrier liquid.[14] There can be other forces acting on particles in the channel, e.g., electrostatic interactions between particles or particles and channel wall,

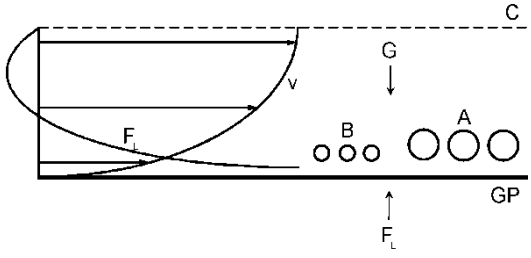

Figure 1. Scheme of the focusing elution mode: G - Eart's gravity force, F_L - hydrodynamic lift forces, A - large starch granules, B - small starch granules, C - channel center, v - flow velocity profile, GP - glass plate channel wall.

Van der Waals and solvophobic forces, etc. However, the most important forces, which influence separation of particles, are hydrodynamic lift forces. On contrary to Earth's gravity, they lift particles away from the channel bottom to faster streamlines and form narrow focused zones (Figure 1). This elution mode is called focusing[14] or lift-hyperlayer.[13]

In the 2000's, FFF techniques were applied to characterization of starch granules more frequently.[12,16–34] Comparisons of FFF data to those determined by other techniques showed very good correlations in the case of starch granules.[2,12,27,30,33,35]

This paper presents GFFF as a simple, cheap and rapid technique for separation of large A and small B starch granules, which allows determination of the ratio A/B of starch granules. Optimization of separation conditions was investigated previously from the point of view of sample pre-treatment, amount of injected sample, elution conditions, swelling effect, and recovery of starch granules.[26,27,36] The optimized procedure was successfully applied to separation of the starch granules isolated from twelve barley varieties. The aim of this work is not development of a method for quantitative determination of the ratio of large and small starch granules, but optimize the GFFF protocol in order to get a reliable tool for relative characterization of starch granules from different varieties. The goal is to apply GFFF at optimized separation conditions to determine the ratios of starch granules A/B of twelve barley varieties grown in three locations and to compare the 3-year results to malting properties of the barley varieties.

EXPERIMENTAL

Plant Material and Sample Preparation

Kernels of twelve spring and winter barley varieties (*Hordeum vulgare* L.), obtained from three testing stations of the Central Institute for Supervising

Table 1. An overview of the spring and winter barley varieties with the malting quality indices

Varieties	Spring winter	6/2 row	Malting quality index	Country of origin	Maintainer parentage
Tolar	S	2	6	CZ	Plant Select spol s r. o. HE 4710/HWS 78267-83
Heris	S	2	5	CZ	Plant Select spol s r. o. HE 4431/CE 431
Orthega	S	2	2	D	Lochow-Petkus GmbH Ceb.7931/Pompadour// S.77323/Golf
Jersey	S	2	7	NL	Cebeco Zaden B. V. Apex/Alexis
Prestige	S	2	7	GB	PBI Cambridge Ltd. Cork/Chariot
Scarlett	S	2	6	D	Saatzucht J. Breun GdbR Amazone/Breun St. 2730 e//Kym
Luran	W	6	2	CZ	Selgen, a. s. LU 27/LU 16
Luxor	W	6	2	CZ	Selgen, a. s. LU 27/LU 16
Nelly	W	6	3	D	W.von Borries-Eckendorf GmbH&Co. Tapir/76079/3/Birgit/ Banteng//Gerbel
Vilna	W	2	2	NL	Plant Select spol s r. o. Intro//Cebeco 87262/ Tamara
Camera	W	2	2	GB	Nickerson Seeds Ltd. NRPB 87-5685c*Stamm 41
Tiffany	W	2	4	D	Saatzucht J. Breun GdbR Labea/Marinka

Note: MQI: 9 = the best quality; 1 = without malting quality (Psota, Kosař 2002).

and Testing in Agriculture of the Czech Republic from 2001 to 2003 (Table 1), were used for isolation of starch granules, i.e., monitoring was conducted with 108 samples. The set included 6 two-row varieties of spring malting barley (Tolar, Orthega, Heris, Jersey, Prestige and Scarlett), 3 two-row winter varieties (Tiffany, Camera and Vilna) and 3 six-row winter varieties (Luran, Luxor and Nelly).[37,38] All kernel samples were graded and the fractions over 2.5 mm were used in this study; these fractions were also used for malting and subsequent assessment of the malting quality index.[39] The kernels were graded and fractions over 2.5 mm were used in this study. A

combination of classical approaches (incl. crushing of barley kernels by a roll crusher, steeping in 0.02M HCl, repeated rubbing and filtering through sieve 0.08 mm) and present knowledge (treatment with ß-glucanase and cellulase, alkalization at pH 10.0 and centrifugation of crude starch suspension through the layer of CsCl) was used for isolation of starch granules. The procedure is described in detail elsewhere.[40] Starch granules were suspended in 10^{-3}% sodium dodecyl sulphate (SDS) (Fluka, Germany) at the concentration of 10 mg/mL and soaked for at least 24 h. Then the samples were sonicated for 1 h prior to the FFF experiment.

Gravitational Field-Flow Fractionation

GFFF data showed very good correlations to those determined by other techniques in the case of starch granules;[2,12,27,30,33,35] therefore, it was used in the present study. The arrangement of GFFF channel is shown in Figure 2. The separation channel was cut in 0.150 mm-thick foil (spacer) and inserted between two glass plates. The channel dimensions were $360 \times 20 \times 0.150$ mm. The carrier liquid and the sample were introduced into the channel via an inlet capillary situated at the channel head, and taken out via an outlet capillary located at the end of the channel. The high-pressure pump HPP 4001 (Laboratory Instruments, Prague, Czech Republic) was used to introduce the carrier liquid into the channel. UV/VIS Spectra 100 (Spectra Physics, San Jose, USA) operated at 470 nm was used as a detector. Data were collected by a PC equipped with a digitalization card.

The samples were injected at the stopped flow by using a Hamilton micro-syringe. Just after injection, a loading flow rate of 0.2 mL/min was applied for

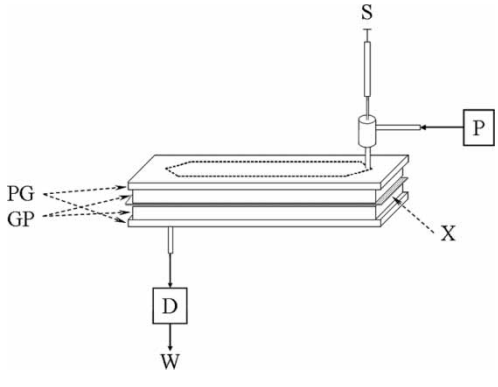

Figure 2. The scheme of an experimental arrangement for GFFF. P - pump, S - syringe for sample injection into the channel through the inlet capillary, PG - Plexiglas blocks, GP - glass plate channel walls, X - spacer, D - detector, R - PC based recorder, W - waste. The dashed line shows the shape of the channel cut in the spacer.

10 s. Then a relaxation time (stopped-flow period) was 1.5 min. After this time period, a linear flow rate of 0.8 mL/min was applied.

RESULTS AND DISCUSSION

Because samples in GFFF can be eluted in different elution modes in dependency on experimental conditions,[14] it is necessary to find such conditions that ensure elution of starch granules in a mode where large and small starch granules are well separated. Such an elution mode is called focusing or lift-hyperlayer,[13,14] where the starch granules form narrow focused zones above the channel bottom (Figure 1). In this elution mode, starch granules do not interact with the channel bottom; therefore, it is not necessary to modify the channel bottom in order to minimize particle-wall interactions.[41] On the other hand, focusing elution mode is sensitive to overloading and particle-particle interactions.[42,43] The recovery of starch granules was studied experimentally. Recovery was calculated as a ratio of the sum of all peak areas in the fractogram of separated starch granules and the peak area corresponding to the same amount of starch granules injected directly to the detector at the same flow rate. The results showed that the recovery was 0.92, i.e., 92%, in this arrangement, which is high enough for our purpose.

An important step to find optimal elution conditions for separation is measurement of dependence of the retention ratio R on the flow rate. The results are shown in Figure 3. The separations were performed at the flow rates in the range from 0.2 to 1.0 mL/min. It is apparent (see Figure 3) that

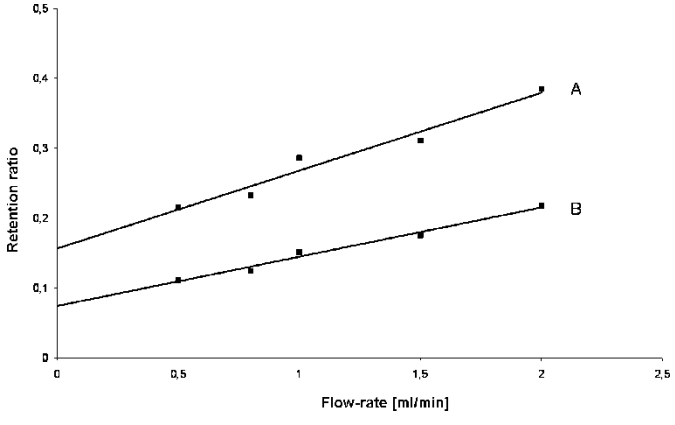

Figure 3. The dependence of retention ratio R of large and small starch granules on the flow rate. Separation of starch granules were performed at flow rates 0.2, 0.5, 0.8 and 1 mL/min. Other experimental conditions: channel dimensions (0.15 mm × 360 mm × 20 mm), concentration of starch suspensions - 10 mg/mL, injected volume 10 μL, stopped - flow time 1.5 min.

retention ratios R of both starch granules increase with the increasing flow rate. This result indicates that the elution mode is a focusing one, where two opposite oriented forces act on particles during separation. The gravitational force, causing a settlement of particles to the channel bottom and the antagonistically acting hydrodynamic lift forces that lift particles away from the channel bottom up to the faster streamlines. This means that the retention ratio is related to the size of particles because the sedimentation force is proportional to the third power of the particle diameter and the lift forces to the fourth power of the particle diameter.[15] This results in the earlier elution of the large starch granules A because hydrodynamic lift forces act more strongly on the larger particles that are eluted before the smaller ones.[26]

The optimized separation conditions were used for determination of the ratio A/B of barley starch granules. The bimodal distribution of barley starch granules was found for all twelve studied varieties with minima on the distribution curves around 8 μm (Figure 4). This value, determined previously by LALLS,[12] was used as a border between the large and small starch granules. It means that the ratio A/B is defined as a ratio of the starch granules larger than 8 μm to the starch granules smaller than 8 μm. In the case of GFFF, the ratio A/B is defined as a ratio of peak areas of the starch granules A and B. However, it is necessary to realize that the ratio is obtained as the ratio of the detector responses and not as the ratio of the real numbers of large and small starch granules. Nevertheless, very good separations of large and small starch granules for twelve barley varieties shown in Figure 4 allow calculation of the ratios A/B (Table 2).

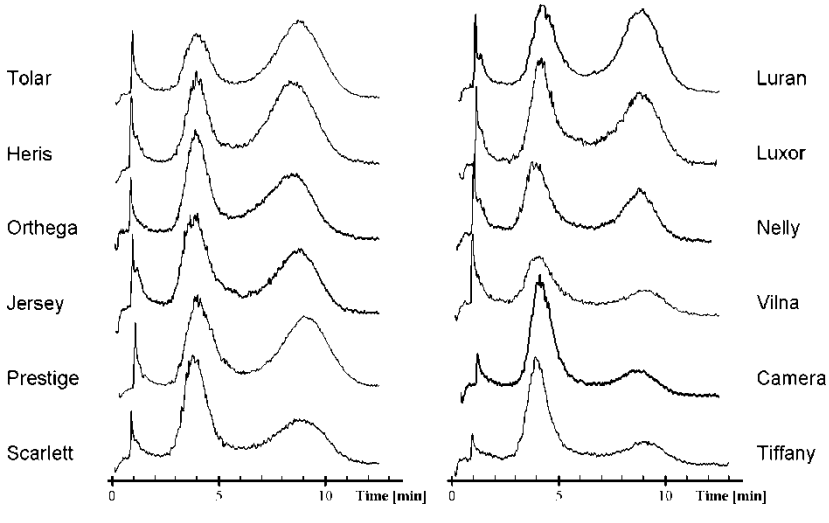

Figure 4. GFFF separations of starch granules isolated from twelve barley varieties at the flow rate of 0.8 mL/min. The other conditions were the same as described in Figure 3.

Table 2. The ratios A/B of the large and small starch granules and standard deviations of twelve barley varieties from three locations, the average values of these ratios for each variety from all regions during the period 2001–2003

Cultivar	Winter/ spring row	A/B			
		2001	2002	2003	Average
Tolar	Spring 2	0,46 ± 0,03 1,25 ± 0,08 1,23 ± 0,07	0,47 ± 0,07 0,45 ± 0,04 0,55 ± 0,06	0,61 ± 0,07 0,58 ± 0,13 0,75 ± 0,04	0,71
Heris	Spring 2	0,51 ± 0,10 1,37 ± 0,06 0,69 ± 0,03	1,04 ± 0,09 0,55 ± 0,04 0,69 ± 0,03	1,01 ± 0,08 0,71 ± 0,04 0,99 ± 0,08	0,84
Orthega	Spring 2	0,59 ± 0,08 1,29 ± 0,09 0,98 ± 0,11	1,18 ± 0,07 0,89 ± 0,08 1,28 ± 0,04	0,50 ± 0,08 0,56 ± 0,09 0,97 ± 0,14	0,92
Jersey	Spring 2	0,60 ± 0,03 1,47 ± 0,09 1,52 ± 0,10	1,03 ± 0,03 0,62 ± 0,13 0,76 ± 0,14	1,39 ± 0,04 0,91 ± 0,03 1,25 ± 0,13	1,06
Prestige	Spring 2	1,05 ± 0,07 1,55 ± 0,02 1,52 ± 0,11	1,10 ± 0,05 0,84 ± 0,04 0,87 ± 0,13	0,75 ± 0,10 0,74 ± 0,03 1,21 ± 0,06	1,07
Scarlett	Spring 2	1,40 ± 0,04 1,74 ± 0,05 1,29 ± 0,07	1,12 ± 0,04 0,57 ± 0,04 0,93 ± 0,08	0,89 ± 0,06 1,04 ± 0,04 0,72 ± 0,06	1,08
Luran	Winter 6	1,03 ± 0,11 1,07 ± 0,14 1,27 ± 0,18	1,07 ± 0,15 1,27 ± 0,19 1,04 ± 0,33	0,96 ± 0,14 0,98 ± 0,08 1,22 ± 0,05	1,10
Luxor	Winter 6	0,99 ± 0,07 1,12 ± 0,04 1,29 ± 0,08	1,22 ± 0,09 1,39 ± 0,24 1,07 ± 0,02	1,02 ± 0,11 0,90 ± 0,05 1,54 ± 0,11	1,17
Nelly	Winter 6	1,83 ± 0,24 0,73 ± 0,05 0,83 ± 0,06	1,80 ± 0,28 1,29 ± 0,07 1,22 ± 0,20	1,09 ± 0,12 1,10 ± 0,11 1,33 ± 0,17	1,25
Vilna	Winter 2	1,19 ± 0,21 0,98 ± 0,13 0,97 ± 0,09	1,85 ± 0,41 1,81 ± 0,32 1,75 ± 0,39	1,59 ± 0,06 1,54 ± 0,04 1,47 ± 0,14	1,46
Camera	Winter 2	1,26 ± 0,17 1,07 ± 0,08 1,07 ± 0,05	1,65 ± 0,09 1,53 ± 0,19 1,87 ± 0,14	2,05 ± 0,37 2,84 ± 0,26 1,25 ± 0,04	1,62
Tiffany	Winter 2	1,53 ± 0,15 1,21 ± 0,11 1,45 ± 0,08	1,90 ± 0,18 1,80 ± 0,26 1,86 ± 0,23	2,97 ± 0,18 2,54 ± 0,15 2,74 ± 0,05	2,00

Among the set of the studied varieties, there are three very good malting varieties of two-row spring barley (Tolar, Jersey, and Prestige), two good malting varieties (two-row spring variety Scarlett and two-row winter variety Tiffany), and the other varieties have feed quality. The results comparing twelve barley varieties show the significant differences in the ratios of large and small starch granules A/B among them (Table 2). The set of the varieties was split into three groups according to the ratios of large and small starch granules A/B obtained by GFFF. The two-row winter varieties Tiffany, Camera, and Vilna belonged to a group of varieties with the highest ratios A/B. Conversely, the two-row varieties of spring malting barley Tolar, Orthega, Heris, Jersey, Prestige, and Scarlett formed a group with the lowest ratios A/B. The six-row winter varieties Luran, Luxor, and Nelly were between the previous groups. Thus, our study did not confirm an assumption that the malting spring barley varieties have higher ratios of large and small starch granules A/B of starch granules in comparison to the winter varieties. The difference is apparent from Table 2, where the ratios A/B and their standard deviations from three locations for three years are presented, as well as the average values of the ratios A/B from all regions are shown for particular varieties. Although the standard deviations of the ratios of large and small starch granules A/B are not negligible (Table 2), the tendency shown by our results is evident.

This apparent discrepancy can be explained by an assumption that, not only the ratio of large and small starch granules A/B is important for proper malting properties, but other factors are also important, e.g., activity of hydrolytic enzymes. Proteomic studies comparing the enzyme systems of different barley varieties will be necessary to elucidate this problem.[44,45] Another cause of the discrepancy might be connected to different composition of starch granules of various barley varieties. In order to understand the malting quality of different barley varieties, it is also important to perform both qualitative and quantitative analysis of starch degradation products in malt and beer by combinations of various separation and mass spectrometric techniques.[46–49]

ACKNOWLEDGMENT

The results were obtained in the framework of solution of the project NAZV QD1005 from Ministry of Agriculture of the Czech Republic.

REFERENCES

1. Jane, J.L.; Kasemsuwan, T.; Leas, S.; Zobel, H.; Robyt, J.F. Anthology of starch granule morphology by scanning electron-microscopy. Starch/Stärke **1994**, *46*, 121–129.

2. Lindeboom, 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 **2004**, *56* (3–4), 89–99.
3. Tillet, I.J.L.; Bryce, J.H. In *The Regulation of Starch Granule Size in Endosperm of Developing Barley Grains*; Proc. European Brewery Conv. Cong, Oslo, Norway, 1993, 45–51.
4. Mac Gregor, A.W. In *The Effect of Barley Structure and Composition on Malt Quality*; Proc. European Brewery Conv. Cong, Lisbon, Portugal, 1991, 37–42.
5. Psota, V.; Bohačenko, I.; Chmelík, J.; Hartmann, J. Starch granule size distribution in caryopses of selected malting varieties of spring barley. Monatssch. für Brauwissen. **2004**, *57*, 8–12.
6. Brennan, C.S.; Harris, N.; Smith, D.; Shewry, P.R. Structural differences in the mature endosperms of good and poor malting barley cultivars. J. Cereal Sci. **1996**, *24*, 171–177.
7. Oliveira, A.B.; Rasmusson, D.C.; Fulcher, R.G. Genetic-aspects of starch granule traits in barley. Crop Sci. **1994**, *34*, 1176–1180.
8. Ellis, R.P.; Camm, J.P.; Morrison, W.R. A rapid test for malting quality in barley, HGCA Proj. Rept. No. 63 **1992**.
9. South, J.B.; Morrison, W.R. Isolation and analysis of starch from single kernels of wheat and barley. J. Cereal Sci. **1990**, *12*, 43–51.
10. 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 **2000**, *10*, 433–436.
11. Baum, B.R.; Baily, L.G. A survey of endosperm starch granules in the genus hordeum—A study using image analytic and numerical taxonomic techniques. Can. J. Botany **1987**, *65*, 1563–1569.
12. 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. **2001**, *107*, 11–17.
13. Giddings, J.C. Field-flow fractionation—Analysis of macromolecular, colloidal, and particulate materials. Science **1993**, *260*, 1456–1465.
14. Chmelík, J. Different elution modes and field programming in gravitational field-flow fractionation I. A theoretical approach. J. Chromatogr. A **1999**, *845*, 285–291.
15. Reschiglian, P.; Zattoni, A.; Roda, B.; Michelini, E.; Roda, A. Field-flow fractionation and biotechnology. Trends Biotechnol. **2005**, *23*, 475–483.
16. Pazourek, J.; Wahlund, K.G.; Chmelík, J. Effect of flowrate and ionic strength on retention of nonporous micron-sized silica gel particles in gravitational field-flow fractionation. J. Liq. Chromatogr. **1996**, *8*, 331–338.
17. Pazourek, J.; Chmelík, J. Optimization of the separation of micron-sized latex-particles by gravitational field-flow fractionation. Chromatographia **1993**, *35*, 591–596.
18. Cardot, P.J.P.; Gerota, J.; Martin, M. Separation of living red-blood-cells by gravitational field-flow fractionation. J. Chromatogr. **1991**, *568* (1), 93–103.
19. Urbánková, E.; Vacek, A.; Nováková, N.; Matulík, F.; Chmelík, J. Investigation of red-blood-cell fractionation by gravitational field-flow fractionation. J. Chromatogr. **1992**, *583*, 27–34.
20. Urbánková, E.; Vacek, A.; Chmelík, J. Micropreparation of hemopoietic stem cells from the mouse bone marrow suspension by gravitational field-flow fractionation. J. Chromatogr. B **1996**, *687*, 449–452.

21. Sanz, R.; Torsello, B.; Reschiglian, P.; Puignou, L.; Galceran, M.T. Improved performance of gravitational field-flow fractionation for screening wine-making yeast varieties. J. Chromatogr. A **2002**, *966*, 135–143.
22. Sanz, R.; Battu, S.; Puignou, L.; Galceran, M.T.; Cardot, P.J.P. Sonication effect on cellular material in sedimentation and gravitational field flow fractionation. J. Chromatogr. A **2003**, *1002*, 145–154.
23. Garcia, M.T.; Sanz, R.; Galceran, M.T.; Puignou, L. Use of fluorescent probes for determination of yeast cell viability by gravitational field-flow fractionation. Biotechnol. Progr. **2006**, *22*, 847–852.
24. Moon, M.H.; Giddings, J.C. Rapid separation and measurement of particle-size distribution of starch granules by sedimentation steric field-flow fractionation. J. Food Sci. **1993**, *58*, 1156–1171.
25. Chmelík, J.; Krumlová, A.; Čáslavský, J. Characterization of starch materials from barley by gravitational field-flow fractionation and matrix-assisted laser desorption/ionization mass spectrometry. Chem. Pap.-Chem. Zvesti **1998**, *52*, 360–361.
26. 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. J. Chromatogr. A **2001**, *914*, 183–187.
27. 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. Anal. Chim. **2002**, *92*, 457–467.
28. Farmakis, L.; Sakellaraki, J.; Koliadima, A.; Gavril, D.; Karaiskakis, G. Size analysis of barley starch granules by sedimentation/steric field flow fractionation. Starch/Stärke **2000**, *52*, 275–282.
29. Contado, C.; Reschiglian, P.; Faccini, S.; Zattoni, A.; Dondi, F. Continuous split-flow thin cell and gravitational field-flow fractionation of wheat starch particles. J. Chromatogr. A **2000**, *871*, 449–460.
30. Contado, C.; Dondi, F. Barley starch granules subject to SPLITT cell fractionation and Sd/StFFF size characterization. Starch/Stärke **2001**, *53*, 414–423.
31. Farmakis, L.; Karaiskakis, G.; Koliadima, A. Investigation of the variation of the mass ratios for large and small starch granules with the pH and ionic strength of the dispersing medium by sedimentation/steric field-flow fractionation. J. Liq. Chromatogr. Rel. Technol. **2002**, *25*, 2135–2152.
32. Cledat, D.; Battu, S.; Mokrini, R.; Cardot, P.J.P. Rice starch granule characterization by flow cytometry scattering techniques hyphenated with sedimentation field-flow fractionation. J. Chromatogr. A **2004**, *1049*, 131–138.
33. Morelon, X.; Battu, S.; Salesse, C.; Begaud-Grimaud, G.; Cledat, D.; Cardot, P.J.P. Sedimentation field flow fractionation monitoring of rice starch amylolysis. J. Chromatogr. A **2005**, *1093*, 147–155.
34. Salesse, C.; Battu, S.; Begaud-Grimaud, G.; Cledat, D.; Cook-Moreau, J.; Cardot, P.J.P. Sedimentation field flow fractionation monitoring of bimodal wheat starch amylolysis. J. Chromatogr. A **2006**, *1129*, 247–254.
35. Psota, V.; Bohačenko, I.; Hartmann, J.; Budinská, M.; Chmelík, J. Comparison of the GFFF and LALLS methods for the measurement of starch granule size distribution in spring barley caryopses. J. Inst. Brew. **2002**, *108*, 200–203.
36. Dokulilová, M.; Plocková, J.; Chmelík, J. In *Several Aspects of the Gravitational Field-Flow Fractionation of Barley Starch Granules*; 12th Intl. Symp. Field-Flow Fractionation, FFF 2005, Brno, Czech Republic, 2005, P6.

37. Psota, V.; Jurečka, D. Barley varieties registered in the Czech Republic in the year 2003. Kvasny Prum. **2003**, *49*, 142–146.
38. Psota, V.; Jurečka, D. Registration of barley varieties in the Czech Republic in 2004. Kvasny Prum. **2004**, *50*, 158–161.
39. Psota, V.; Kosař, K. Malting quality index. Kvasny Prum. **2002**, *48*, 142–148.
40. Bohačenko, I.; Chmelík, J.; Psota, V. Determination of the contents of A and B starch in barley using low angle laser light scattering. Czech J. Food Sci. **2006**, *24*, 11–18.
41. Plocek, J.; Konečný, P.; Chmelík, J. Modification of glass channel walls for separation of biological particles by gravitational field-flow fractionation. J. Chromatogr. B **1994**, *656*, 427–431.
42. Pazourek, J.; Urbankova, E.; Chmelik, J. Experimental-study on the separation of silica-gel supports by gravitational filed-flow fractionation. J. Chromatogr. A **1994**, *660* (1–2), 113–118.
43. Pazourek, J.; Chmelik, J. Experimental-study on the separation of silica-gel supports by gravitational filed-flow fractionation. 2. Sample preparation, stop-flow procedure and overloading effect. J. Chromatogr. A **1995**, *715* (2), 259–265.
44. Chmelík, J.; Řehulka, P.; Střelcová, M.; Kubáň, V.; Mayrhofer, C.; Allmaier, G. Proteomic analysis of different extracts from barley grains. Rostlinná výroba **2002**, *48*, 261–264.
45. Bak-Jensen, K.S.; Laugesen, S.; Roepstorff, P.; Svensson, B. Two-dimensional gel electrophoresis pattern (pH 6-11) and identification of water-soluble barley seed and malt proteins by mass spectrometry. Proteomics **2004**, *4*, 728–742.
46. Mauri, P.; Minoggio, M.; Simonetti, P.; Gardana, C.; Pietta, P. Analysis of saccharides in beer samples by flow injection with electrospray mass spectrometry. Rapid Comm. Mass Spectrom. **2002**, *16*, 743–748.
47. Cabálková, J.; Žídková, J.; Přibyla, L.; Chmelík, J. Determination of carbohydrates in juices by high-performance liquid capillary electrophoresis, chromatography, and matrix-assisted laser desorption/ionization-time of flight-mass spectrometry. Electrophoresis **2004**, *25*, 487–493.
48. Araujo, A.S.; da Rocha, L.L.; Tomazela, D.M.; Sawaya, A.C.H.F.; Almeida, R.R.; Catharino, R.R.; Eberlin, M.N. Electrospray ionization mass spectrometry fingerprinting of beer. Analyst **2005**, *130*, 884–889.
49. Laštovičková, M.; Chmelík, J. Simple and fast method for recognition of reducing and non-reducing neutral carbohydrates by MALDI-TOF MS. J. Agric. Food Chem. **2006**, *54* (14), 5092–5097.

Received January 12, 2007
Accepted February 13, 2007
Manuscript 6048