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Optimization of experimental conditions for the separation of small and large starch granules by gravitational field-flow fractionation

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Abstract

Separation of small and large barley starch granules by gravitational field-flow fractionation was investigated from the point of view of sample pre-treatment, amount of injected sample, and elution conditions. The sample pre-treatment study resulted in the conclusion that it is reasonable to soak the starch granules for at least 24 h prior to separation. The experiments with different amounts of injected sample show that it is possible to increase as well as decrease twofold the sample amount usually used without any change in retention ratios. The implementation of flow-rate gradients for elution of the starch granules reduced total separation time. However, the applied flow-rate gradients did not improve the resolution of peaks A and B compared with the generally used constant flow-rate. Thus, for barley starch granules, the constant flow-rates within the range from 0.8 to 1.0 ml/min seem to provide the best compromise of total separation time, peak resolution and instrumental expense.

Keywords: Field-flow fractionation; Optimization; Flow-rate gradients; Gradient elution; Starch

1. Introduction

Size characterization of starch granules from barley kernels is of importance for the food and brewing industries. During processing, certain fractions of starch granules are less susceptible to enzymatic digestion, which results in technological problems. From this point of view, a tool for the evaluation of barley starch granule distribution is desirable. Several techniques, such as light microscopy [1], sieving techniques [2], scanning electron microscopy [3,4], and Coulter counter [5] have been generally employed for this purpose. Recently, low-angle laser light scattering [6] seemed to provide the most precise information, however, the cost makes it unattainable for general use. Implementation of field-flow fractionation (FFF) for monitoring of barley starch granule distribution [7–10] fitted the needs of low cost and short separation time.

Starch is formed in green plants as the final product of photosynthesis. The starch granules are deposited in plant generative organs (grain, bulb). The shape and size of the starch granules are characteristic of the plant origin [3]. Chemical and physical starch properties are influenced by plant origin and also by vegetative conditions and maturity. Undamaged starch granules are insoluble in cold water, but they can imbibe water reversibly. They can swell slightly, and then return to their original size on drying [11]. Most starches are composed of two homopolysaccharides – amylose and amylopectin, in approximate mass percentages of 15–30% and 85–70%, respectively [3,12,13].

Starch is one of the barley components important for its malting quality. Malting barley cultivars

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contain starch granules of bimodal distribution. Large starch granules of type A are of diameter range 10–40 μm and small starch granules of type B are of diameter range 1–10 μm. The total mass ratio of granules A/B was determined as 9:1, while the total number ratio is 1:9 [14]. The values of these ratios differ depending on the barley starch population [15] and are related to the malting quality of barley. B granules are less susceptible to enzymatic degradation during malting and mashing and gelatinize at higher temperatures and over a wider temperature range than A granules [16]. The distribution of protein within the endosperm cells and the binding of the protein matrix with small starch granules may also influence the malting characteristics of the grain. The matrix proteins can affect accessibility of the substrate to enzymes, either by binding directly to starch granules or to the cell walls [17]. The amount of starch-associated protein in poor malting quality cultivars is high [18]. B granules tend to be deeply embedded in the endosperm protein matrix [19]. Only about half of the B granules (i.e., 5% of the total starch) are degraded during malting and mashing. The remaining B granules can form hazes and cause technological problems (e.g., filtration, stability) in brewery [14]. It means that small starch granules are undesirable for malting and that the ratio of granules A/B is one of the important features of malting quality of barley. It was observed [17] that good-malting cultivars reached faster and more uniform breakdown of cell walls and digestion of endosperm reserves than poor-malting cultivars.

Gravitational FFF (GFFF) is the experimentally simplest and cheapest among the family of FFF techniques. GFFF is based on the combination of a non-uniform flow velocity profile of carrier liquid and the gravitational field applied perpendicularly to the flow [20]. Gentle experimental conditions (low pressure and the option of weak force field) and the possibility to use isotonic buffer solutions as carrier liquids provide GFFF with unique separation capabilities for biological particulate samples [21–27]. GFFF has potential not only for separation but also for micropreparation of the purified fractions [26].

This work is focused on three experimental aspects of the separation of barley starch granules by GFFF: sample preparation, the influence of injected amount of the sample and flow-rate programming. We paid attention to these experimental topics, because they were supposed to provide further possibilities for optimization of the separation in terms of time and resolution.

2. Experimental

2.1. Material

Starch granules were isolated and purified in our laboratory according to the previously reported procedure [28]. The final products of the isolation were acetone-dried starch granules. The barley kernels for the isolation were provided by the Institute of Brewery and Malting, Brno, Czech Republic.

2.2. Method

The separation channel was cut in a 150-μm spacer, which was placed between two float glass plates and clamped between two Plexiglas blocks. The width and length of the channel were 20 and 350 mm, respectively. A HP 1100 (Hewlett-Packard) pump was employed for sample elution including flow-rate programming. A UV spectrophotometric detector (Development Workshop Academy of Sciences of the Czech Republic, Prague, Czech Republic) equipped with a Z-shaped cell with an optical path of 5 mm and operated at 254 nm was used. Milli-Q water was used as carrier liquid.

For the sample preparation studies, the dried granules were suspended (10 mg/ml) in deionized water and sonicated for 1 h. Prior to each injection, the ultrasound stirring was repeated for 3 min. Volumes of 10-μl of the sample were injected at the defined time intervals into the channel directly through a septum-equipped injector using a Hamilton syringe. After a 2-min stop-flow time, the flow-rate of 0.8 ml/min was switched on and the sample was eluted through the channel into the detector. For all the other measurements, the starch granules were suspended and soaked for at least 24 h. After that, the sample was sonicated for 1 h prior to the FFF experiment. The stop-flow time was always 2 min.

The parameters for the evaluation of different elution conditions are summarized in Table 1, where t_{eA} is the elution time of A granules, t_{eB} is the

Table 1
Parameters for the evaluation of various elution conditions

Flow-rate (ml/min)	t_{eA} (min)	t_{eB} (min)	t_t (min)	R^*
0.5	6.0	11.6	13.5	2.6
0.8	3.7	6.9	8.7	2.3
1.0	2.8	5.3	6.9	2.3
1.5	1.8	3.2	4.4	1.8
2.0	1.3	2.3	3.1	1.8
Linear gradient	3.6	5.3	6.4	2.1
Step gradient	4.0	6.4	7.8	2.3
Concave parabolic gradient	3.0	4.7	6.1	2.1
Convex parabolic gradient	4.6	6.6	7.5	2.2

elution time of B granules, t_t is the total separation time and R^* is the peak resolution, defined arbitrarily by the following equation:

$$R^* = \frac{t_{eB} - t_{eA}}{\alpha + \beta}$$

where the parameters α and β express the adjacent half-widths of the peaks A and B taken at one half of their heights.

For the measurement of the dependence of retention ratio on the injected amount of sample, the flow-rate was 0.8 ml/min and the used volumes and concentrations of the sample are reported below. For the elution study, 10 μl of suspension (10 mg/ml) was injected and run. The tested constant flow-rates were 0.5, 0.8, 1.0, 1.5 and 2.0 ml/min. The courses of the applied flow-rate gradients are described by the plots (Fig. 2).

3. Results and discussion

3.1. Sample preparation

The retention ratios of starch particles slightly increase within the first 24 h of soaking. After this period, these values do not significantly change. We concluded that, in order to ensure standard experimental conditions, it is reasonable to soak the suspended sample for at least 24 h prior to the injection. However, this time period might depend on the starch variety.

3.2. The dependence of retention ratio on injected amount of the sample

In order to specify the influence of the injected amount of sample on retention data, the following volumes and concentrations were applied: 10 and 20 μl of the 5 mg/ml suspension, 5, 10, 15 and 20 μl of the 10 mg/ml suspension and 10 μl of the 20 mg/ml suspension. Within the range of these measured concentrations and volumes, retention ratio did not show any tendency to change. All the values of retention ratio were in the frame of the standard deviation usually obtained for experiments with repeating injection of constant volume and constant concentration.

3.3. Flow-rate programming

Elution of starch granules was performed by GFFF under the conditions of focusing mode, which is confirmed by the flow-rate dependence of the retention time presented in Fig. 1. Two counteracting forces, gravitational force and hydrodynamic lift force (HLF), form an analyte zone with the maximum concentration at the position where the resulting force acting on the analyte equals zero [29]. The action of HLF increases with increasing particle radius; therefore the zone of larger particles is focused in higher velocity streamlines than the zone of smaller particles. This causes the large particles to elute first. It means that in the fractograms all the peaks A correspond to the large A starch granules and all the peaks B correspond to the small B starch granules.

The fractograms in Fig. 1 illustrate the influence of different flow-rates on the separation of starch granules. All the flow-rates were constant and within the range from 0.5 to 2.0 ml/min. The best resolution was observed at the flow-rate of 0.5 ml/min (Fig. 1e). Resolution became worse as the flow-rate increased. In contrast, total separation time advantageously shortened with the increasing flow-rate.

With regard to the results measured in this work, under the conditions described above, the flow-rates of 0.8 ml/min and 1.0 ml/min seem to be the most suitable for elution of starch granules when considering the impacts of both separation time and resolution of peaks A and B.

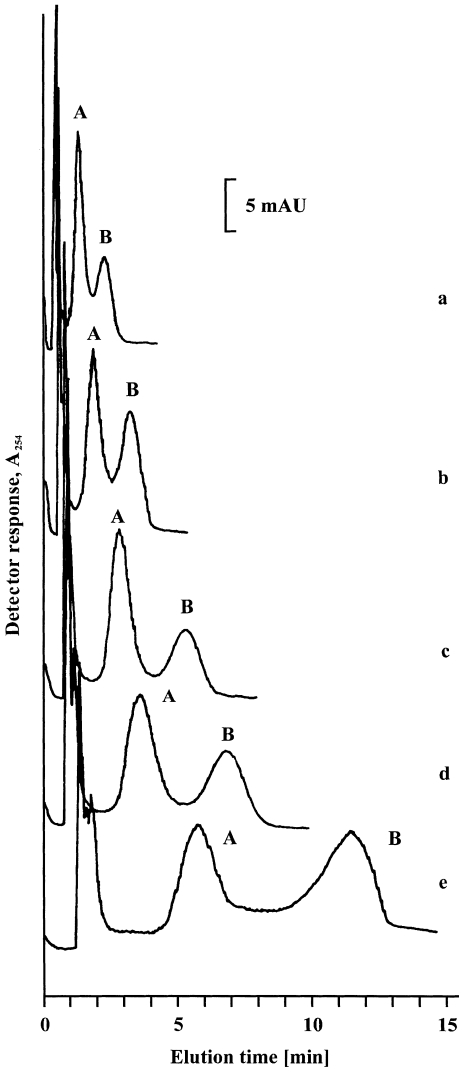

Fig. 1. The influence of different constant flow-rates on the separation of starch granules. Fractograms refer to the flow-rates of (a) 2.0 ml/min, (b) 1.5 ml/min, (c) 1.0 ml/min, (d) 0.8 ml/min, and (e) 0.5 ml/min. Peak A = starch A granules and peak B = starch B granules.

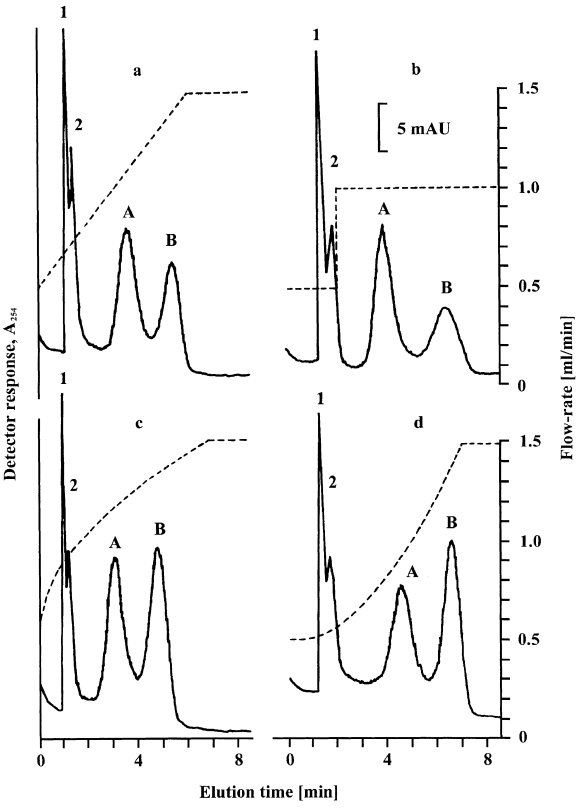

Fig. 2. The influence of different flow-rate gradients on the separation of starch granules. The courses of the applied gradients are described by the plots: (a) linear gradient, (b) step gradient, (c) concave parabolic gradient, (d) convex parabolic gradient. Peak A = starch A granules and peak B = starch B granules. The breakthrough peak is denoted 1 and the peak corresponding to the void volume is denoted 2.

Fig. 2 shows the influence of flow-rate programming applied to the elution of starch granules. The flow-rate gradients were used in order to combine advantages of low and high flow-rates, i.e., higher resolution and shorter elution time, respectively. In this case, the best resolution was achieved with the step gradient (Fig. 2b). The implementation of the concave parabolic gradient (Fig. 2c) resulted in a considerably shorter total separation time. A relatively short separation time and satisfactory resolution were achieved in the case of the linear flow-rate gradient (Fig. 2a). The parameters for the data evaluation are reported in Table 1.

All the gradients used for our measurement confirmed the assumption of the advantages of a combination of low and high flow-rates. On the other hand, there are some disadvantages of using these gradients: the rather expensive instrumentation (a gradient pump) and problems with drifted baselines of the fractograms. We concluded that it is not necessary to use gradients for separation of A and B starch granules. However, the flow-rate gradients have been successfully used for optimization of separation of

silica gel particle mixture [30]. We suppose there is a possibility to employ flow-rate programming for more complex mixtures.

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References

[1] T.J. Schoch, E.C. Maywald, in: R.L. Whistler, E.G. Paschall, J.N. BeMiller, H.J. Roberts (Eds.), Starch Chemistry and Technology, Vol. 2, Academic Press, New York, 1967, p. 637.
[2] A.D. Evers, C.T. Greenwood, D.D. Muir, C. Venables, Stärke 2 (1974) 42.
[3] J. Jane, T. Kasemsuwan, S. Leas, H. Zobel, J.F. Robyt, Starch/Staerke 46 (1994) 121.
[4] A.M.L. McDonald, J.R. Stark, J. Inst. Brew. 94 (1988) 125.
[5] J.B. South, W.R. Morrison, J. Cereal Sci. 12 (1990) 43.
[6] H.J. Cornell, A.W. Hoveling, A. Chryss, M. Rogers, Starch/Staerke 46 (1994) 203.
[7] M.H. Moon, J.C. Giddings, J. Food Sci. 58 (1993) 1166.
[8] J. Chmelík, A. Krumlová, J. Čáslavský, Chem. Pap. 52 (1998) 360.
[9] P. Reschiglian, A. Zattoni, G. Berardi, C. Fuentes, K.-G. Wahlund, A. Krumlová, J. Chmelík, presented at the 8th International Symposium on Field-Flow Fractionation, Paris, 6–8 September 1999, lecture No. 28.
[10] C. Contado, P. Reschiglian, S. Faccini, A. Zattoni, F. Dondi, J. Chromatogr. A 871 (2000) 449.
[11] J.N. BeMiller, R.L. Whistler, in: O.R. Fennema (Ed.), Food Chemistry, Marcel Dekker, New York, Basel, 1996, p. 157.
[12] W. Banks, C.T. Greenwood, J. Sloss, Carbohydr. Res. 11 (1969) 399.
[13] A.W. MacGregor, G.B. Fincher, in: A.W. MacGregor, R.S. Bhatty (Eds.), Barley Chemistry and Technology, American Association of Cereal Chemists, St. Paul, MN, 1993, p. 297.
[14] I.J.L. Tillett, J.H. Bryce, European Brewery Convention, Proceedings of the 24th Congress, Oslo, 1993, lecture No. 4.
[15] K.J. Goering, D.H. Fritts, R.F. Eslick, Stärke 25 (1973) 297.
[16] A.W. MacGregor, European Brewery Convention, Proceedings of the 23rd Congress, Lisbon, 1991, lecture No. 3.
[17] C.S. Brennan, M.A. Amor, N. Harris, D. Smith, I. Cantrell, D. Griggs, P.R. Shewry, J. Cereal Sci. 26 (1997) 83.
[18] C.S. Brennan, N. Harris, D. Smith, K.R. Shewry, J. Cereal Sci. 24 (1996) 171.
[19] G.H. Palmer, J. Inst. Brew. 78 (1972) 326.
[20] J.C. Giddings, Science 260 (1993) 1456.
[21] A. Bernard, B. Paulet, V. Colin, P.J.P. Cardot, Trends Anal. Chem. 14 (1995) 266.
[22] P.J.P. Cardot, J. Gerota, M. Martin, J. Chromatogr. 568 (1991) 93.
[23] E. Urbánková, A. Vacek, N. Nováková, F. Matulík, J. Chmelík, J. Chromatogr. 583 (1992) 27.
[24] R. Parsons, V. Yue, X. Tong, P.J.P. Cardot, A. Bernard, J.P. Andreux, K. Caldwell, J. Chromatogr. B 686 (1996) 177.
[25] A. Bernard, C. Bories, P.M. Loiseau, P.J.P. Cardot, J. Chromatogr. B 664 (1995) 444.
[26] E. Urbánková, A. Vacek, J. Chmelík, J. Chromatogr. B 687 (1996) 449.
[27] A. Merino, C. Bories, J.-C. Gantier, P.J.P. Cardot, J. Chromatogr. 572 (1991) 291.
[28] A. Krumlová, J. Plocek, T. Kruml, V. Psota, I. Bohacenko, J. Chmelík, in: Proceedings of the International Conference: Cereals for Human Health and Preventive Nutrition, Brno, 7–11 July 1998, p. 196.
[29] J. Chmelík, J. Chromatogr. A 845 (1999) 285.
[30] J. Plocková, J. Chmelík, Jr., F. Matulík, J. Chmelík, presented at the 8th International Symposium on Field-Flow Fractionation, Paris, 6–8 September 1999, poster No. 10.