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Fractionation of starch hydrolysates into dextrins with narrow molecular mass distribution and their detection by high-performance anion-exchange chromatography with pulsed amperometric detection

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Abstract

Low levels of high-molecular-mass dextrins in starch hydrolysates can be detected by high-performance anion-exchange chromatography with pulsed amperometric detection in spite of their low responses by dialysis of the starch hydrolysate and fractionation of the resulting adialysate with ethanol (final concentration 30–80% at 6 °C). In doing so, dextrin fractions with a relatively narrow molecular mass distribution were obtained.

Keywords: Dialysis; Starch; Dextrins; Carbohydrates; Glucooligosaccharides

1. Introduction

Today, high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD) is the method of choice for analysis of glucooligosaccharides with a degree of polymerization (DP) in a range of 1 to 80, further referred to as dextrins [1]. Techniques such as high-performance liquid chromatography with either metal-loaded cation-exchange columns or amino-bonded silica columns coupled with refractive index (RI) detection only inadequately resolve carbohydrate polymers of DP>30 according to molecular mass (M_r), due to the incompatibility of RI detection with gradient elution but also due to lack of suitable columns [1–3]. The same holds for gel permeation chromatography (GPC) that cannot resolve individual polysaccharides and therefore provide only partial information on chain length distribution [4]. Hydrophilic interaction chromatography [5], capillary electrophoresis [6] and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry [7] are less suitable as well, respectively, because of dextrin solubility problems in the acetonitrile–water eluent (DP>35), derivatization problems (DP>13) and lower reproducibility.

In HPAEC–PAD, apart from molecular conformation, small differences in pK_a values (12–14 range) are the basis for separation of linear and branched dextrins at high pH [8]. In PAD, dextrins are easily oxidized at the gold working electrode at high pH

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and low potential, resulting in a very specific and sensitive carbohydrate detection [4].

However, loss of sensitivity of the gold working electrode and decreasing responses (on a mass basis) for high-M_r dextrins complicate the analysis [9–11].

In the particular case of dextrin mixtures containing low levels of high-M_r dextrins, such as in many starch hydrolysates, poor if any resolution of the high-M_r dextrins is observed. A full characterisation of the composition of starch hydrolysates by HPAEC–PAD is therefore difficult unless high-M_r dextrins are concentrated by dialysis and by fractionation of the corresponding adialysate with organic solvent. Organic solvent fractionation has frequently been applied to study the fine structure of amylopectin. α-Dextrins, obtained after α-amylolysis, are fractionated with methanol [12–14] or ethanol [15] to reveal the unit structures of amylopectin. Starch hydrolysate fractionation, however, has only been described by Defloor et al. [16]. The authors obtained three different dextrin populations after ethanol [50 and 75% (v/v)] precipitation and, at a given ethanol concentration, the size of the dextrin fraction depended upon the initial concentration of the starch hydrolysate.

In this study, starch hydrolysate adialysates (10%, w/v) were fractionated by 10% stepwise increases of organic solvent. The influence of organic solvent, temperature and starch hydrolysate composition on the fractionation procedure were investigated. By doing so, an analytical method for detection of low levels of high-M_r dextrins in HPAEC–PAD profiles was developed. We here report on the outcome of this work.

Table 1
Composition (%/100% carbohydrate material) of starch hydrolysates SH18, SH42 and SH43 with dextrose equivalents of 18, 42 and 43 DE

Saccharide		SH18	SH42	SH43
Fructose		0.2	–	0.3
Glucose	DP 1	1.7	2.1	18.8
	DP 2	5.3	50.2	13.5
	DP 3	9.0	17.6	11.6
	DP 4	5.4	2.2	9.6
	DP 5	5.4	0.4	7.7
	DP 6	9.6	0.7	6.2
	DP 7	8.2	1.2	5.0
	DP 8	4.4	1.9	4.0
	DP 9	2.7	2.3	3.3
	DP>9	48.3	21.5	20.0

DP=Degree of polymerization. Data provided by the supplier.

2. Experimental

2.1. Materials

Three wheat starch hydrolysates, SH18, SH42 and SH43, with compositions as outlined in Table 1 and dextrose equivalents (DEs) of 18, 42 and 43 DE, respectively, were supplied by Amylum (Aalst, Belgium). Standards were glucose (M_r $0.18\cdot10^3$), maltose ($0.34\cdot10^3$), maltotriose ($0.50\cdot10^3$), maltotetraose ($0.67\cdot10^3$), maltopentaose ($0.83\cdot10^3$), maltohexaose ($0.99\cdot10^3$) and maltoheptaose ($1.15\cdot10^3$), all from Sigma–Aldrich (Bornem, Belgium). Shodex P-82 pullullan standards were from Showa Denko (Tokyo, Japan) with M_r values of $404\cdot10^3$, $212\cdot10^3$, $112\cdot10^3$, $47.3\cdot10^3$, $22.8\cdot10^3$, $11.8\cdot10^3$ and $5.9\cdot10^3$. Sodium hydroxide was from Baker (Deventer, The Netherlands) and sodium acetate (analytical-reagent grade) from Merck Eurolab (Leuven, Belgium). All other reagents were of at least analytical grade and supplied by Sigma–Aldrich.

2.2. Preparation of freeze–dried adialysates

Solutions of SH18, SH42 and SH43 (10%, w/v) were dialysed against deionised water for 60 or 96 h at 6 °C, with three water refreshments per 24 h, with Medicell International (London, UK) dialysis membranes (M_r cut-off $12\cdot10^3$–$14\cdot10^3$, diameter 44.4 mm). Adialysates were frozen with liquid nitrogen and freeze–dried.

2.3. Fractionation of freeze–dried adialysates

Based on Defloor et al. [16], methanol (99.5%), ethanol (95%), isopropanol (99%) and three temperatures (6, 22 and 28 °C) were evaluated to optimise the fractionation procedure. In what follows, the procedure is described for methanol (99.5%) at a temperature of 22 °C. Fractionations with the other solvents and temperatures were carried out in a similar way.

Freeze–dried adialysates were dissolved in water

at a concentration of 10% (w/v) by stirring for 1 h. Methanol was slowly (total time 10 min) added under continuous stirring to obtain a final concentration of 10% (22 °C). The mixture was stirred for an additional 10 min and kept for 1 h at 22 °C. Because no precipitate formed we added more methanol (to a final concentration of 20%) and repeated the above procedure until the first precipitate was formed (for methanol, a 30% concentration was required). Then, the precipitated material was recovered by centrifugation (22 °C, 10 000 *g*, 30 min), suspended in methanol, and transferred to a flask. The solvent was removed by rotary evaporation and the resulting precipitate redissolved in 200 ml water, frozen with liquid nitrogen and freeze–dried. The obtained fraction is referred to as F30, the dextrin population that precipitated at a methanol concentration of 30%. The supernatant was further saturated with methanol (10% stepwise increases) until a final methanol concentration of 80%, with intermediate removal of the precipitated fractions. This was done by repeating the above procedure. The methanol in the final supernatant was removed by rotary evaporation, resulting in a final fraction F>80. Seven dextrin fractions were hence obtained: F30, F40, F50, F60, F70, F80 and F>80.

2.4. Degree of polymerization

The average DP of the fractions, was calculated from the ratio of total (reducing and non-reducing) glucose residues to reducing glucose residues [17,18]. The RSD was below 3.0%. Peracetylated monosaccharides or alditols (1 μl) were separated on a Supelco SP-2380 column (30 m×0.32 mm I.D., 0.2 μm film thickness) (Supelco, Bellefonte, PA, USA) in a Hewlett-Packard chromatograph (Agilent 6890 series, Wilmington, DE, USA) equipped with an autosampler, splitter injection port (split ratio 1:20) and flame ionising detector. The carrier gas was He (column head pressure 63 kPa). Separation

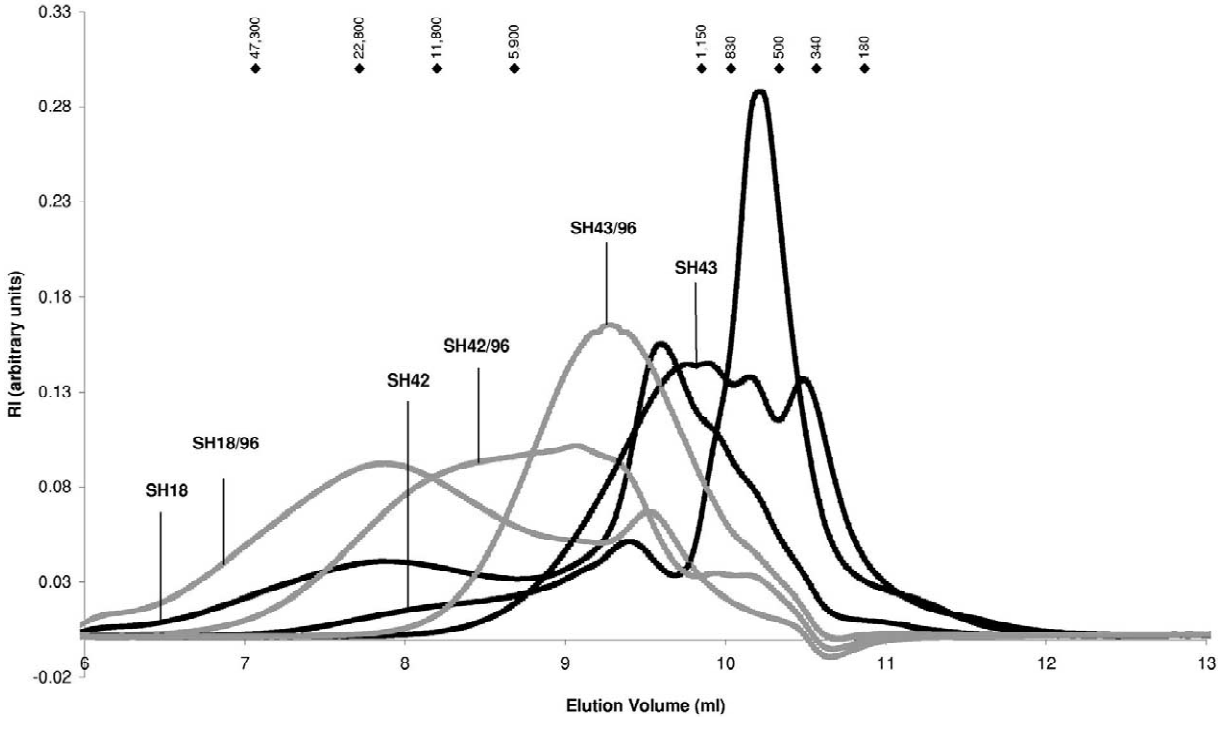

Fig. 1. Shodex SB-803 gel permeation chromatograms of non dialysed and resulting adialysates SH18, SH42 and SH43 following 96 h of dialysis.

was at 210 °C, injection and detection were at 270 °C.

2.5. Gel permeation chromatography

All dextrin fractions were solubilised in 0.3% NaCl (4 mg/ml) by stirring (60 min). The solutions were filtered (0.45 μm) and an aliquot (20 μl) was separated on a Shodex GPC column (Showa Denko) SB-803 (fractionation range $0–100\cdot10^3$; 300×8 mm) and SB-804 (fractionation range $0–1000\cdot10^3$; 300×8 mm) by elution with 0.3% NaCl (0.5 ml/min at 30 °C). The refractive index of the eluate was monitored using an RI detector Model 8110 (VDS Optilab, Berlin, Germany). The Shodex columns were calibrated with dextrin and pullullan standards.

2.6. High-performance anion-exchange chromatography with pulsed amperometric detection

HPAEC–PAD was performed with a Dionex DX-500 chromatography system (Sunnyvale, CA, USA) equipped with an ED-40 electrochemical detector, a GP-50 gradient pump and an AS-3500 autosampler. Samples (4 mg/ml deionised water) were filtered (0.25 μm) and injected (25 μl) onto a CarboPac PA-100 anion-exchange column (250×4 mm) in combination with a CarboPac PA-100 guard column. The potentials and time periods for the pulsed amperometric detection were: E_1, +0.05 V (t_1=400 ms); E_2, +0.60 V (t_2=200 ms); E_3, −0.15 V (t_3=400 ms). The flow-rate was 1.0 ml/min and the acetate gradient system included three eluents. Eluents were: (A) 100 m*M* sodium hydroxide, 10 m*M* sodium acetate; (B) 100 m*M* sodium hydroxide, 500 m*M* sodium acetate; (C) 500 m*M* sodium hydroxide. The system was equilibrated with 100% eluent A for 10 min before each run. The gradient was: 0–180 min, linear gradient from 0 to 100% eluent B, 180–184 min, linear gradient to 100% eluent C, 184–188 min, 100% eluent C.

3. Results and discussion

3.1. Dialysis of starch hydrolysates

Average DP values of the non dialysed starch hydrolysates were 5.5 for SH18, 2.4 for SH42 and 2.3 for SH43. Dialysis for 60 and 96 h resulted in SH18/60 and SH18/96 adialysates of average DP 13.6 and 21.5, respectively. SH42/96 and SH43/96 adialysates following dialysis for 96 h had average DP values of 13.0 and 8.3, respectively. Recoveries after 96 h dialysis were 40, 15 and 12% for the starch hydrolysates SH18, SH42 and SH43, respectively.

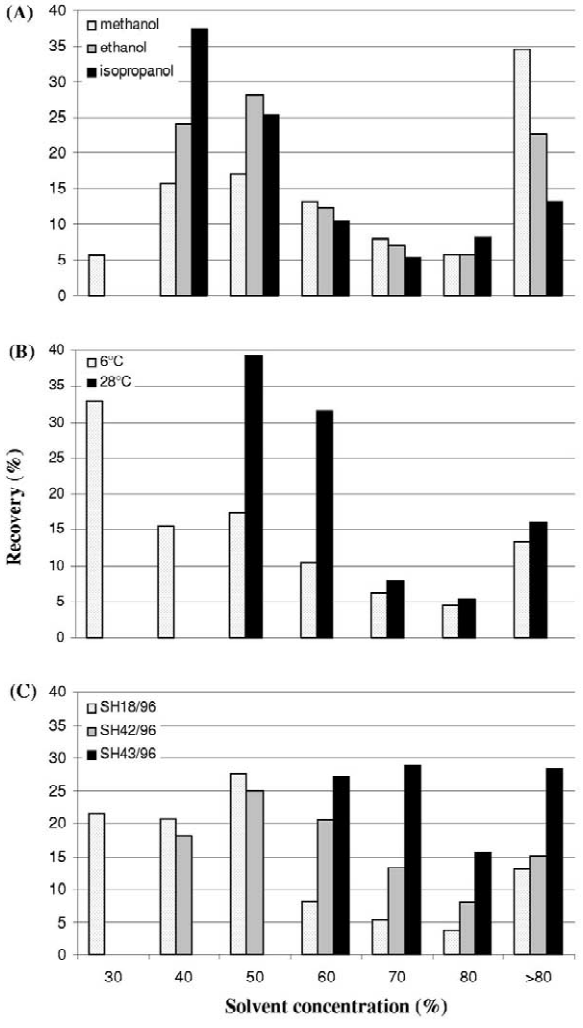

Fig. 2. Recoveries (%) of the different dextrin fractions obtained after organic solvent fractionation of SH18/60 adialysate (10%, w/v) with methanol, ethanol and isopropanol at 22 °C (A), of SH18/96 adialysate (10%, w/v) with ethanol at 6 °C and 28 °C (B), and of SH18/96, SH42/96 and SH43/96 adialysates (10%, w/v) with ethanol at 6 °C (C).

A distinct shift to higher-M_r was observed for all three dialysed starch hydrolysates in the GPC profiles of the non dialysed samples and the resulting adialysates (96 h dialysis, Fig. 1).

3.2. Optimisation of a fractionation procedure

3.2.1. Fractionation of SH18/60 with methanol, ethanol and isopropanol at 22 °C

Three batches of 300 ml SH18/60 adialysate (10%, w/v) were fractionated with methanol, ethanol or isopropanol at 22 °C.

At a methanol concentration of 30%, a small yield of a first precipitate was formed in SH18/60 adialysate (5.7%, Fig. 2). It had an average DP of 36.5 (Table 2). From the methanol fraction F40 onwards, the average DP decreased because more and more lower-M_r dextrins precipitated at higher methanol concentrations. The methanol fraction F> 80 (34.6% recovery) represented the low-M_r dextrins that remained in solution even at a methanol concentration of 80%. For the ethanol and isopropanol series, precipitation started at a solvent concentration of 40%. For the isopropanol F40 fraction, a significant quantity of precipitate was formed.

Organic solvent fractionation is based on the (gradual) lowering of polarity or the dielectric constant ε of a polymer solution by adding an organic solvent. This results in a (gradual) precipitation of the polymers of decreasing M_r and radius of gyration [15]. As the ε values of methanol, ethanol and isopropanol are, respectively, 32.6, 24.3 and 18.3 at 25 °C [19], we expected a larger decrease in polarity in the order methanol—ethanol—isopropanol for increasing organic solvent concentrations and, as a consequence, a sooner and more profound precipitation of high-M_r dextrins in the opposite order. This is clearly the case for fractions F40, because recovery data decrease in the expected order: isopropanol—ethanol—methanol. As more high-M_r dextrins precipitated at low isopropanol concentrations, the observed recovery and average DP data for the final methanol, ethanol and isopropanol fractions F>80 are logical.

Table 2 indicates, however, that the first precipitates of the methanol (F30 fraction) and ethanol (F40 fraction) series had a lower average DP in comparison to the subsequent fractions. This can be explained by the fact that only a small level of high-M_r dextrins precipitated first and that, hence, contribution of co-precipitated low-M_r dextrins in the average DP determination was large, especially since precipitates were not washed. When the yield of precipitated high-M_r dextrins was higher, such as in the case of the isopropanol F40 fraction, this effect was levelled off.

Another explanation, as postulated by Zhu and Bertoft [13], could be that some high-M_r dextrins did not precipitate at lower methanol or ethanol concentrations and were therefore recovered together with lower-M_r dextrins when more methanol or ethanol was added. Fractionation was apparently not only based on the size of the dextrins but also on their structure (radius of gyration).

Fig. 3 shows the HPAEC–PAD profiles of the methanol fractions. Unresolved peaks at high re-

Table 2
Average degrees of polymerization (DPs) of dextrin fractions obtained after organic solvent fractionation of SH18/60 with methanol, ethanol and isopropanol at 22 °C (a), of SH18/96 at 6 °C and 28 °C with ethanol (b), of SH18/96, SH42/96 and SH43/96 adialysates with ethanol at 6 °C (c)

Dextrin fraction	(a) SH18/60 at 22 °C			(b) SH18/96 with ethanol		(c) With ethanol at 6 °C		
	Methanol	Ethanol	Isopropanol	6 °C	28 °C	SH18/96	SH42/96	SH43/96
F30	36.5	–	–	64.1	–	45.7	–	–
F40	69.8	33.0	57.4	65.6	–	78.7	42.7	–
F50	57.0	52.5	41.2	52.1	82.0	56.7	37.8	–
F60	46.0	33.8	22.7	33.1	50.9	30.6	25.2	16.0
F70	34.4	19.5	12.9	20.5	26.4	20.0	15.9	12.5
F80	24.8	11.7	8.0	12.4	14.6	12.5	11.3	9.1
F>80	6.4	5.0	4.8	5.6	5.8	5.7	3.9	4.4

SH*xx*/*yy*: Starch hydrolysate *xx* following dialysis for *yy* h.

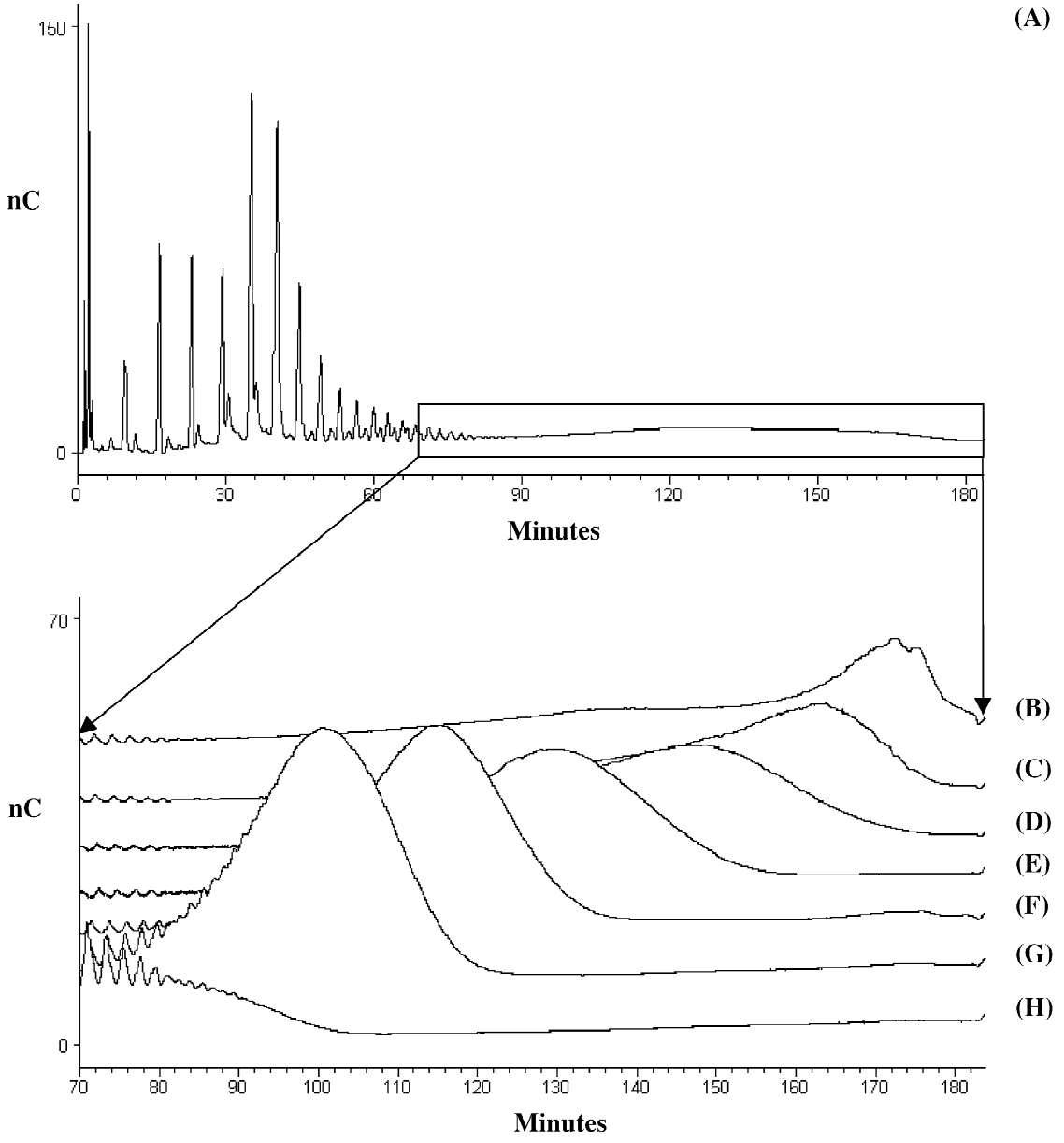

Fig. 3. HPAEC–PAD profiles of the dextrin fractions obtained after fractionation of (A) SH18/60 adialysate (10%, w/v) with methanol at 22 °C. (B) F30, (C) F40, (D) F50, (E) F60, (F) F70, (G) F80 and (H) F>80.

tention time (170 min) appeared for the first time in profile F30 and migrated slowly into a more concentrated ''bump'' at shorter retention times and lower-M_r for all higher methanol concentrations. In the GPC profiles for methanol fractions, from F30 to F>80, a migration was also observed from low (high-M_r dextrins) to higher elution volumes (low-M_r dextrins) (Fig. 4). HPAEC–PAD and GPC profiles confirmed the contamination of high-M_r dextrin fractions by lower-M_r dextrins. HPAEC–PAD and GPC of the ethanol and isopropanol series resulted in similar observations.

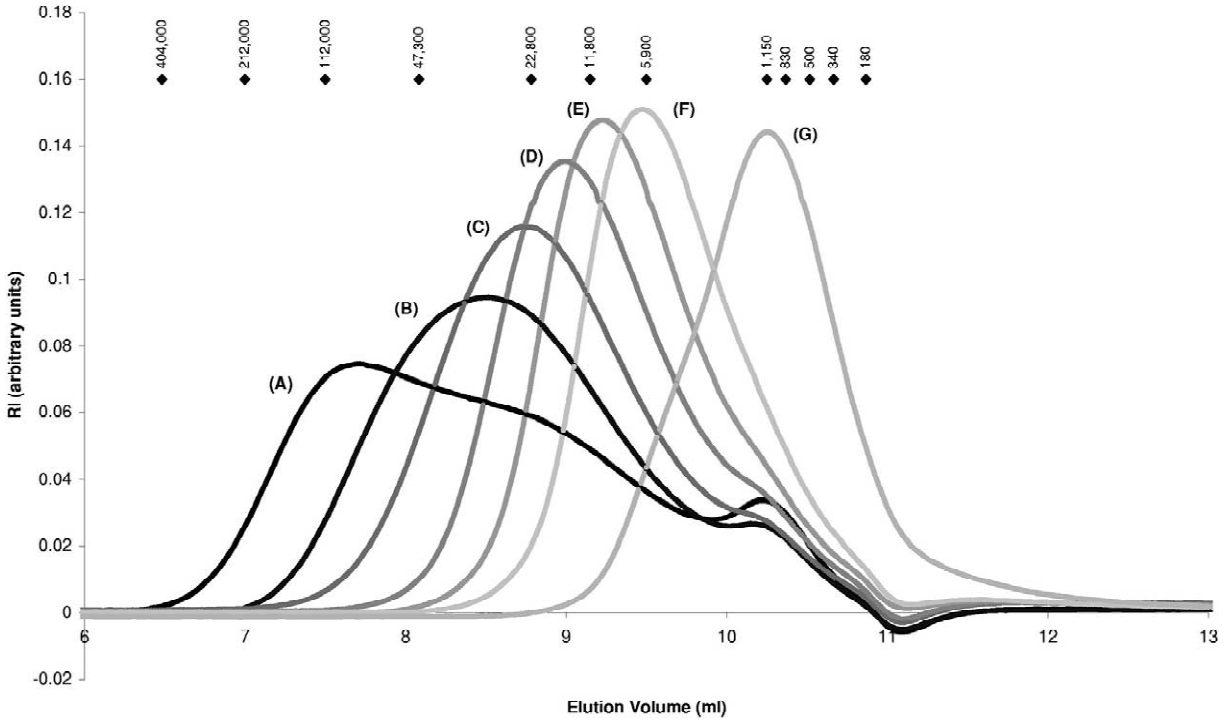

Fig. 4. Shodex SB-804 gel permeation chromatograms of dextrin fractions obtained after fractionation of SH18/60 adialysate (10%, w/v) with methanol at 22 °C. (A) F30, (B) F40, (C) F50, (D) F60, (E) F70, (F) F80 and (G) F>80.

High-M_r dextrins were thus fractionated and visualized in HPAEC–PAD chromatographic profiles. In further fractionations of SH18 adialysate, we used ethanol because a good distribution of the dextrin fractions was obtained at ethanol concentrations between 40 and 80%, in contrast to what was observed with methanol and isopropanol. These solvents resulted in large F>80 and F40 fractions.

3.2.2. Fractionation of SH18/96 with ethanol at 6 and 28 °C

The SH18/60 adialysate still had a large level of low-M_r dextrins in the GPC profile (not shown). This caused contamination of the first precipitates by low-M_r dextrins. Therefore, a SH18/96 adialysate with a smaller proportion of low-M_r dextrins (Fig. 1) was preferred in further experiments. The influence of temperature on the fractionation procedure was investigated. As the precipitation behaviour at 22 °C with ethanol was already known (cf. Section 3.2.1 for SH18/60 adialysate), two batches of 300 ml SH18/96 (10%, w/v) were fractionated with ethanol at a lower (6 °C) and higher (28 °C) temperature. The first precipitates were formed at ethanol concentrations of 30 and 50%, for 6 and 28 °C, respectively (Fig. 2). The same trend was seen for the average DP values (Table 2). The cumulative recoveries as a function of ethanol concentration (Fig. 5) show that higher temperatures obviously increased solubility. However, once precipitation started, it occurred more extensively, in line with observations by Bertoft and Spoof [12] during fractionation of amylopectin α-dextrins with methanol. The same migration of either a bump to shorter retention times (HPAEC–PAD) or a peak to higher elution volumes (GPC) was seen for the first time at an ethanol concentration of 30% (6 °C) or 50% (28 °C) (results not shown).

A temperature of 6 °C was chosen in all further fractionations to fractionate in a broad range of ethanol concentrations.

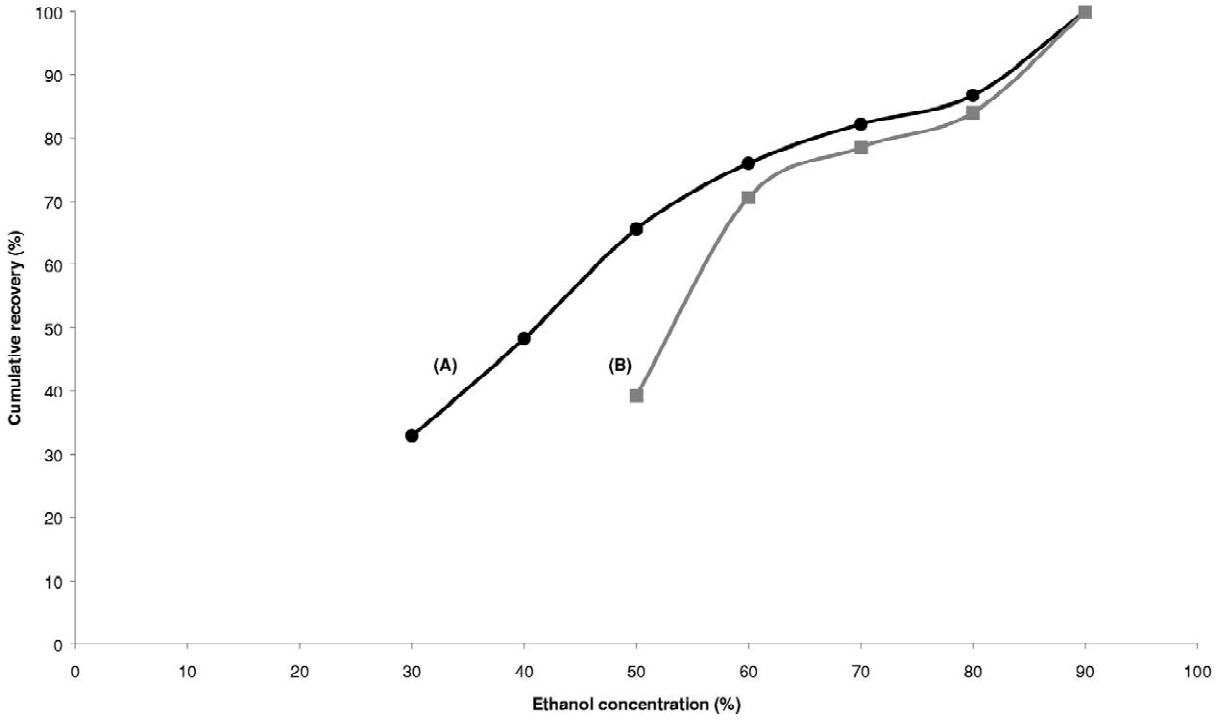

Fig. 5. Cumulative recoveries (%) for ethanol precipitation of SH18/96 adialysate (10%, w/v) at 6 °C (A) and 28 °C (B).

3.3. Fractionation of SH18/96, SH42/96 and SH43/96 with ethanol at 6 °C

For SH18/96, SH42/96 and SH43/96 (10%, w/v), precipitation started at ethanol concentrations of 30, 40 and 60%, respectively (Fig. 2). The average DP values of all first fractions indicated the precipitation of high-M_r dextrins of the respective starch hydrolysate adialysates (Table 2). HPAEC–PAD profiles (not shown) again showed an envelope of unseparated material at higher retention times for ethanol concentrations 30% (SH18/96), 40% (SH42/96) and 60% (SH43/96) that migrated further to shorter retention times for higher ethanol concentrations. The same trends were seen for the corresponding GPC profiles (not shown) but the M_r range in which fractionation occurred became smaller in the order SH18/96—SH42/96—SH43/96. Fractionation of starch hydrolysates with high levels of high-M_r dextrins leads to a broader M_r range wherein the different dextrin fractions are distributed.

Comparison of recovery (Fig. 2) and average DP (Table 2) data of the SH18/96 fractions and those obtained after fractionation with ethanol at 6 °C in the previous section (cf. Section 3.2.2) indicate the same trend. The first precipitate was formed at an ethanol concentration of 30%. However, fractions F30 and F40 yielded no similar recovery and average DP data. Although we tried to keep it constant, the smallest change in the addition rate of ethanol had a large impact on the precipitation behaviour (recovery) and precipitate composition (average DP).

4. Conclusions

Dialysis and organic solvent fractionation of three starch hydrolysates yielded dextrin fractions with relatively narrow M_r distributions. Dialysis efficiently separated low-M_r from high-M_r dextrins. Ethanol (at 22 °C) gave a broad and more balanced distribution of dextrin fractions with different average DP between organic solvent concentrations 40 and

80%. Higher fractionation temperatures increased solubility and subsequently delayed precipitation of high-M_r dextrins. Also, starch hydrolysate compositions influenced the fractionation range. The higher the level of high-M_r dextrins, the broader the M_r range of the different dextrin fractions.

In conclusion, we were able to fractionate starch hydrolysates into dextrin fractions with a relatively narrow M_r distribution which allow for detection of low levels of high-M_r dextrins in HPAEC–PAD profiles. In ongoing research, better resolution of the high-M_r dextrins will be aimed for.

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References

[1] I. Hanashiro, J.-I. Abe, S. Hizukuri, Carbohydr. Res. 283 (1996) 151.
[2] D.A. Martens, W.T. Frankenberger Jr., Chromatographia 29 (1/2) (1990) 7.
[3] K. Koizumi, T. Utamura, Y. Okada, J. Chromatogr. 321 (1985) 145.
[4] A. Henshall, Cereal Foods World 41 (1996) 419.
[5] A.S. Feste, I. Khan, J. Chromatogr. 630 (1993) 129.
[6] T. Kazmaier, S. Roth, J. Zapp, M. Harding, R. Kuhn, Fresenius J. Anal. Chem. 361 (1998) 473.
[7] S. Broberg, K. Koch, K. Andersson, L. Kenne, Carbohydr. Polym. 43 (2000) 285.
[8] T. Nagamine, K. Komae, J. Chromatogr. A 732 (1996) 255.
[9] K. Koch, R. Andersson, P. Åman, J. Chromatogr. A 800 (1998) 199.
[10] K. Koizumi, M. Fukuda, S. Hizukuri, J. Chromatogr. 585 (1991) 233.
[11] K. Koizumi, Y. Kubota, T. Tanimoto, Y. Okada, J. Chromatogr. 464 (1989) 365.
[12] E. Bertoft, L. Spoof, Carbohydr. Res. 189 (1989) 169.
[13] Q. Zhu, E. Bertoft, Carbohydr. Res. 288 (1986) 155.
[14] E. Bertoft, Q. Zhu, H. Andtfolk, M. Jungner, Carbohydr. Polym. 38 (1999) 349.
[15] T. Frigard, R. Andersson, P. Åman, Carbohydr. Polym. 47 (2002) 169.
[16] I. Defloor, V. Vandenreyken, P.J. Grobet, J.A. Delcour, J. Chromatogr. A 803 (1998) 103.
[17] C.M. Courtin, H. Van den Broeck, J.A. Delcour, J. Chromatogr. A 866 (2000) 97.
[18] I.M. Sims, A. Thomson, U. Hubl, N.G. Larsen, R.H. Furneaux, Carbohydr. Polym. 45 (2001) 285.
[19] R.C. Wheast (Ed.), CRC Handbook of Chemistry and Physics, 58th ed., CRC Press, Cleveland, OH, 1977–1978.