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Fractionation of maltodextrins by ethanol

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Abstract

Potato starch and waxy maize starch derived maltodextrins were fractionated by ethanol precipitation. Fractional precipitation was effective in separating the maltodextrins into fractions of different size as evidenced by their gel permeation profiles and their degrees of polymerisation. At a given ethanol concentration, the size of the precipitated material clearly depends upon the initial concentration of the maltodextrins in solution. © 1998 Elsevier Science B.V.

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1. Introduction

Maltodextrins are starch hydrolysates with a dextrose equivalent of less than 20 and consisting of α-D-glucose units linked by (1→4) glycosidic linkages (primarily) as well as by (1→6) linkages [1]. For an investigation of the relationship between the structure of maltodextrins and their possible activity in retarding bread firming, we needed considerable amounts of maltodextrins varying in size and structure. Indeed, commercial maltodextrins are polydisperse and need to be fractionated according to molecular size before their functionality in retarding bread firming [2] can be investigated.

Ethanol precipitation of saccharide polymers has been described earlier for amylodextrins [3] and for arabinoxylans [4]. Methanol has been used [5] to fractionate amylopectin α-dextrins.

We applied here a similar technique to commercial maltodextrins and showed that they can be fractionated into fractions of different molecular size. In order to obtain fractions differing in branching degree, commercial maltodextrins derived from a regular starch on the one hand and from a waxy starch (containing virtually no amylose) on the other hand were included in the study.

2. Experimental

2.1. Materials

Paselli MD6 (potato starch derived maltodextrins) and Star-Dri 5 (waxy maize starch derived maltodextrins) were from Avebe (Antwerpen, Belgium) and Amylum (Aalst, Belgium), respectively. The average degrees of polymerisation (DP) of the samples were 24 and 18, and 28 and 21 respectively when evaluated by the chemical and ^{1}H NMR methods respectively (see below).

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2.2. Fractionation at different concentrations

Paselli MD6 (PAS) (1.0, 10 or 20 g) was dissolved in 100 ml of water by stirring at 200 rpm for 30 min. Because 20 g of the material did not dissolve completely in 100 ml water, it was centrifuged (room temperature, 30 min, 31 000 *g*) to remove opalescency.

Aliquots of ethanol were then added under continuous stirring to obtain a final concentration of 50% (v/v) ethanol. The mixture was stirred for an additional 10 min and kept overnight at room temperature. The precipitated material (P) was recovered by centrifugation (room temperature, 30 min, 10 000 *g*) and dried by washing with ethanol and diethyl ether over a crucible with a sintered glass filter (porosity 4). It was finally dissolved in water and recovered by freeze drying. It is referred to as P/50/1/Pas, P/50/10/Pas, and P/50/20/Pas, respectively. The supernatants were concentrated to 75% (v/v) ethanol and the precipitated materials were recovered in a similar manner. They are referred to as P/75/1/Pas, P/75/10/Pas, and P/75/20/Pas, respectively. Ethanol was removed from the second supernatant (SN) by rotary evaporation. It was lyophilised to obtain the SN/1/Pas, SN/10/Pas and SN/20/Pas materials, respectively.

2.3. Large scale fractionation

Paselli MD6 or Star-Dri 5 (300 g) were mixed at high speed with 1500 ml water[1]. The mixtures were centrifuged at 14 000 *g* (room temperature, 30 min) to remove opalescency. Fractionation by ethanol precipitation was carried out as described above. The precipitates at 50 and 75% ethanol concentration for Paselli MD6 and Star-Dri 5 are referred to as P/50/300/Pas, P/75/300/Pas, P/50/300/Star, and P/75/300/Star, respectively. The second supernatants are referred to as SN/300/Pas and SN/300/Star, respectively.

[1]The solubilisation was much easier for the Star-Dri 5 sample than for Paselli MD6. The use of a high speed mixer did not alter the maltodextrin population.

2.4. Gel permeation chromatography

Samples (15 to 30 mg) were solubilised in 1.0 ml 1.0 *M* KOH by continuous stirring overnight at 4°C. Water (9.0 ml) was added and the solution was filtered (Gelman Sciences, nylon Acrodisc 0.2 μm). An aliquot (200 μl) was separated on a Superose 12 HR (Pharmacia Biotech, Uppsala, Sweden) gel permeation column (30×1.0 cm) by elution with 0.1 *M* KOH at a flow-rate of 0.4 ml/min. Fractions (0.4 ml) were collected and analysed for total carbohydrates with the phenol–sulphuric acid method [6].

Blue Dextran 2000 (Pharmacia Biotech, Uppsala, Sweden) and maltose (UCB, Belgium) were used to evaluate the void and total volumes of the column.

2.5. High-performance anion-exchange chromatography

SN/300/Pas and SN/300/Star (30 mg) were dissolved in 100 ml deionised water. Paselli MD6, Star-Dri 5, P/50/300/Pas, P/50/300/Star, P/75/300/Pas and P/75/300/Star (25 mg) were solubilised in 0.25 ml of 1.5 *M* NaOH by stirring during 10 min at room temperature. Deionised water (2.25 ml) was added. An aliquot (500 μl) was diluted with 2.0 ml deionised water. The samples were filtered through a 0.2-μm membrane filter.

High-performance anion-exchange chromatography (HPAEC) was with a Dionex DX 500 system (Dionex, Sunnyvale, USA) equipped with a pulsed amperometric detection (PAD) system. The working and the reference electrodes of the PAD were gold and silver–silver chloride, respectively. The pulse potentials and durations applied were: $E_1=0.05$ V ($t_1=200$ ms); $E_2=0.75$ V ($t_2=200$ ms); $E_3=-0.15$ V ($t_3=400$ ms). The sampling rate of the detector was 1 Hz. Aliquots of sample (25 μl) were separated on a Dionex CarboPac PA-100 column (250×4 mm) with a CarboPac PA-100 guard column at a flow-rate of 1.0 ml/min. Eluents were deionised water, 1.0 *M* Na-acetate and 0.5 *M* NaOH. All were prepared with deionised water (0.06 μS cm^{-1}). The applied elution (0–31.5 min) and clean up (31.5–39.5 min) programme is shown in Table 1.

Glucose, maltose, maltotriose and a maltooligosac-

Table 1
Elution programme applied for HPAEC

Time (min)	Eluent A (%)	Eluent B (%)	Eluent C (%)
0.0	80	0	20
2.5	80	0	20
30.5	30	50	20
31.5	0	80	20
32.5	80	0	20
33.5	0	0	100
38.5	0	0	100
39.5	80	0	20

Eluent A: deionised water; eluent B: 1.0 *M* sodium acetate; and eluent C: 0.5 *M* NaOH. A linear gradient is applied over a specified time. The system is equilibrated for 12 min before every run. The linear gradient is started 2.5 min after injection of the sample.

charide mixture of DP 4–10 (Sigma M-3639) were used as references.

2.6. Degree of polymerisation

The average DP was determined by analysis of the quantity of total sugar in triplicate (phenol–sulfuric acid method [6]) per quantity of reducing sugar (measured by the reduction of ferri-ions in quadruplicate [7]). Average standard deviation for the degree of polymerisation was 1.1.

2.7. Evaluation of the branching of the maltodextrin fractions obtained from Paselli MD6

A maltodextrin solution was prepared by solubilisation of 10 mg in 1.0 ml 1.0 *M* NaOH (continuous stirring, 3 h, 4°C), neutralisation, and dilution of the solution to 10 ml. To 0.5 ml of this maltodextrin solution, 9.5 ml sodium acetate buffer (pH 5.5, 10.53 m*M*) and 2 units pullulanase (P5420, Sigma, St. Louis, MO, USA) were added. This mixture was incubated (continuous stirring, 15.30 h, 28°C), immersed in a water-bath (15 min, 100°C), centrifuged (20 min, 1500 *g*, room temperature) and adjusted to pH 6.5–7.0. The average degree of polymerisation of the debranched maltodextrins was determined as above. Average standard deviation for the average chain-length determination was 1.4.

Because the pullulanase enzyme was suspended in 3.2 *M* ammonium sulfate solution, standard curves and a sample control were run in the presence of the same amount of ammonium sulfate.

2.8. H NMR spectroscopy

^{1}H NMR spectra were recorded with a 300 MHz Fourier transform (FT) spectrometer (Bruker AMX 300, Karlsruhe, Germany). The analysis was performed at 85°C. Pulse repetition time was 5 s. Samples were solubilised in $^{2}H_2O$ (99%, 1.0 ml/mg) and lyophilised. This step was repeated once and the sample was solubilised again for analysis. Peak assignments were on the basis of the data by Gidley [8] thus relating signals at δ 5.40, 5.00, 5.27, and 4.68 ppm to H-1 of (1→4)- and (1→6)-α-linked units, and the H-1 of the α and β forms of reducing units, respectively.

The ratio of the relative intensity of the peaks at δ 5.40 and 5.00 ppm to that of the peaks at δ 5.27 and 4.68 ppm allowed for a second estimation of the average degree of polymerisation.

3. Results

3.1. Fractionation

Yields of the respective samples are listed in Table 2. A more concentrated solution of Paselli MD6 resulted in a larger recovery of maltodextrins precipitating upon increasing the ethanol concentration to

Table 2
Percentages and degrees of polymerisation (DP) of the different fractions obtained in the fractionation of different concentrations of Paselli MD6 or in a large-scale fractionation of Paselli MD6 and Star-Dri 5 (fractionation scheme as described in Sections 2.2 and 2.3, respectively)

	Fraction	Recovery (%)	Average DP
At different concentrations[a]			
1.0	P/50/1/Pas	62.2	251
	P/75/1/Pas	21.0	42
	SN/1/Pas	16.8	7
10	P/50/10/Pas	67.6	115
	P/75/10/Pas	19.4	24
	SN/10/Pas	13.0	6
20	P/50/20/Pas	72.1	73
	P/75/20/Pas	17.2	15
	SN/20/Pas	10.7	5
Large scale			
Paselli MD6	P/50/300/Pas	74.1	66 (66)
	P/75/300/Pas	14.8	17 (18)
	SN/300/Pas	11.1	5 (5)
Star-Dri 5	P/50/300/Star	68.3	61 (68)
	P/75/300/Star	15.9	19 (21)
	SN/300/Star	15.8	4 (5)

Values between brackets are calculated from the ^{1}H NMR-spectra as described in the Section 3.4.
[a] in g per 100 ml water.

50% (v/v). As a result, less P/75- and SN-material were recovered.

In the large scale fractionation of Paselli MD6 and Star-Dri 5, the yield of P/50/300/Pas was higher than that of P/50/300/Star, while the yield of SN/300/Pas was smaller than that of SN/300/Star.

3.2. Gel permeation chromatography (GPC)

The gel permeation profiles of maltodextrin fractions obtained by fractionation of Paselli MD6 (1.0 g/100 ml) as described above, are shown in Fig. 1. Similar profiles were obtained for Star-Dri 5 and its fractions. It is clear that the ethanol precipitation method is effective in fractionating the commercial maltodextrin into samples of different size. Apart from this, elution profiles are shifted to smaller average molecular sizes when the solution of Paselli MD6 used for fractionation is more concentrated. This follows from the fact that the solubility of dextrins decreases with their molecular mass and from solubility product considerations. This is illustrated in Table 2 by the average DP of the respective fractions, and in Fig. 2 for the samples recovered from the 75% ethanol precipitate. Fractions of comparable average degree of polymerisation were obtained for the large scale fractionation of Paselli MD6 and Star-Dri 5, respectively (Table 2).

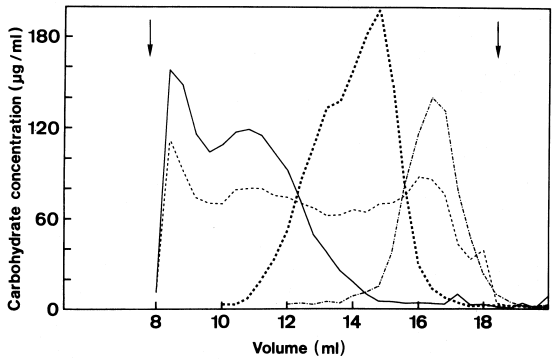

Fig. 1. Gel permeation profiles on a Superose 12 HR gel permeation column of the maltodextrin samples P/50/1/Pas (———), P/75/1/Pas (· · ·), and SN/1/Pas (– · – · –), obtained by fractionation of Paselli MD6 (– – –, 1.0 g /100 ml) as described in Section 2.2. Elution volume of Blue Dextran 2000 and of maltose are indicated by arrows to illustrate the void and total volumes of the column.

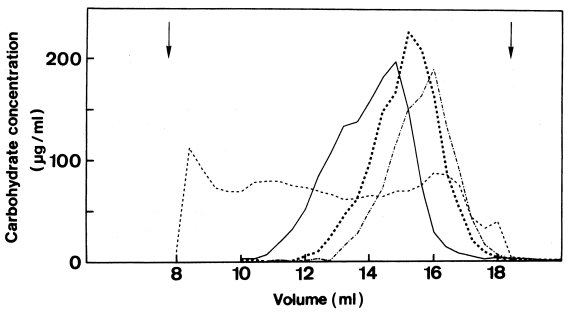

Fig. 2. Gel permeation profiles on a Superose 12 HR gel permeation column of the maltodextrin samples P/75/1/Pas (———), P/75/10/Pas (· · · · ·) and P/75/20/Pas (– · – · –), obtained by fractionation of Paselli MD6 (– – –). Abbreviations as described in Section 2.2. Elution volume of Blue Dextran 2000 and of maltose are indicated by arrows to illustrate the void and total volumes of the column.

3.3. High-performance anion-exchange chromatography

Satisfactory resolution of the peak areas of the HPAEC separations was obtained upto a 20 min retention time (Fig. 3). The chromatograms were interpreted by evaluation of the peak area of the respective signals. The retention times of the DP 1–10 references ranged from 4.6 to 16.3 min.

Star-Dri 5 and its different fractions contained more glucose and maltose than Paselli MD6 and its fractions. Differences in maltotriose content were less pronounced. It is clear from Fig. 3 and Table 3 that during the fractional precipitation of Paselli MD6, the fractions obtained successively are enriched in saccharides of a lower DP. Similar HPAEC results were obtained for Star-Dri 5 (not shown).

3.4. Degree of polymerisation

Average DP values of the different samples are illustrated in Table 2. An effective fractionation of commercial maltodextrin was obtained by ethanol precipitation, and a higher concentration of the starting solution of Paselli MD6 resulted in a lower average DP of the fractions.

The average degree of polymerisation calculated from the ratio of the relative intensity of the ^{1}H NMR signals at δ 5.40 and 5.00 ppm to that of the peaks at δ 5.27 and 4.68 ppm agreed very well (Table 2) with the values obtained by analysis of the quantity of total sugar [6] per quantity of reducing sugar [7].

3.5. Evaluation of the branched nature of the maltodextrin fractions obtained from Paselli MD6

Average chain-lengths of the debranched samples are listed in Table 4. The average chain-length of Paselli MD6 was 16. The average chain-length and the average degree of polymerisation of the samples indicated that (1→6)-α-branch points occur in Paselli MD6 as well as in samples recovered from the P/50/1/Pas, and the P/75/1/Pas samples (Tables 2 and 4). These results were confirmed by the ^{1}H nuclear magnetic resonance spectra (not shown).

3.6. H NMR spectroscopy

The intensity of the peak at δ 5.00 ppm (H-1 of (1→6)-α-linked glucose units) relative to the intensity of the peak at δ 5.40 ppm (H-1 of (1→4)-α-linked units) of the different samples are given in Table 5. As was to be expected from the origin of the samples, Star-Dri 5 and its fractions respectively showed a higher branching than Paselli MD6 and its fractions.

4. Conclusions

In conclusion, the method described here allows for a straightforward fractionation of maltodextrins of varying average DP. The technique can easily be scaled up for isolation of large quantities of maltodextrin fractions with a narrower size distribution than the starting material than can e.g. chromatography.

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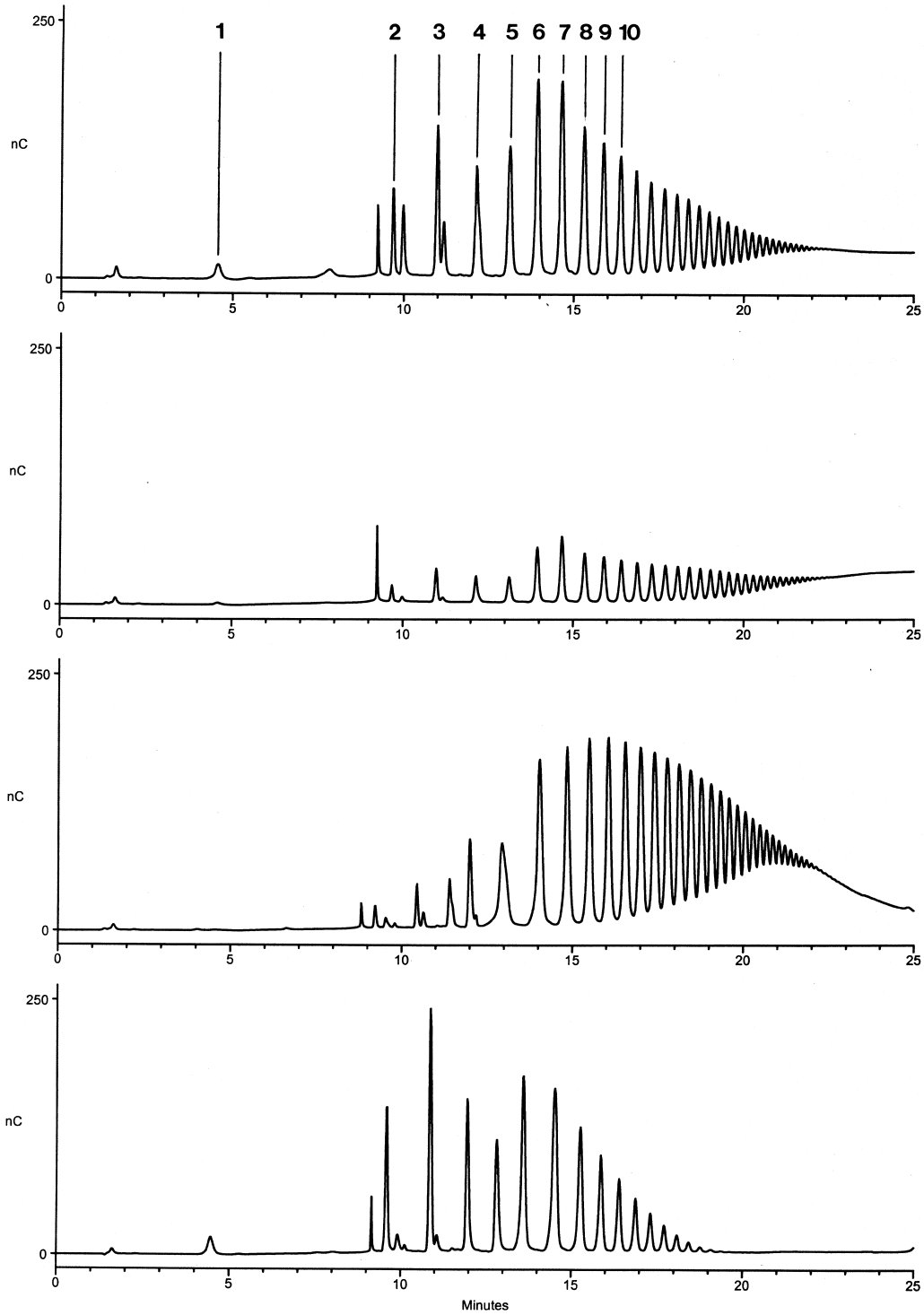

Fig. 3. High-performance anion-exchange chromatograms of Paselli MD6, P/50/300/Pas, P/75/300/Pas and SN/300/Pas (from top to bottom). Integers on top of peaks refer to the DP of the eluted saccharides.

Table 3
Distribution[a] and proportion[b] of oligosaccharides in Paselli MD6 and Star-Dri 5 and their respective fractions as determined by HPAEC

Sample	Distribution				Proportion
	Glucose	Maltose	Maltotriose	DP 4-10	DP 1-10/Total
Paselli MD6	1.8	4.3	8.1	85.8	54.8
P/50/300/Pas	1.1	4.2	7.0	87.7	46.9
P/75/300/Pas	0.1	1.5	2.3	96.1	36.9
SN/300/Pas	2.2	7.6	12.5	77.7	79.4
Star-Dri 5	14.4	7.2	8.6	69.8	61.3
P/50/300/Star	6.5	6.6	6.4	80.5	51.2
P/75/300/Star	2.8	4.8	4.4	88.0	32.4
SN/300/Star	15.5	10.5	9.9	64.1	77.3

[a] in %, peak area of the (mono)saccharide(s) relative to the total peak area of oligosaccharides (DP≤10).
[b] in %, total peak area of oligosaccharides (DP≤10) relative to the total area of peaks giving satisfactory resolution (i.e. upto a 20 min retention time).

Table 4
Average chain-length (CL) of the debranched samples obtained in the fractionation of different concentrations of Paselli MD6 (fractionation scheme and abbreviations as outlined in Section 2.2)

Paselli MD6 dissolved in 100 ml (g)	Fraction	Average CL
1.0	P/50/1/Pas	18
	P/75/1/Pas	16
	SN/1/Pas	8
10	P/50/10/Pas	10
	P/75/10/Pas	18
	SN/10/Pas	7
20	P/50/20/Pas	17
	P/75/20/Pas	15
	SN/20/Pas	8

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Table 5
Relative intensity of the peak at δ 5.00 ppm [H-1 of (1→6)-α-linked units] for the ^{1}H NMR spectrum

Sample	Relative peak intensity (δ 5.00 ppm)
Paselli MD6	0.0302
P/50/300/Pas	0.0422
P/75/300/Pas	0.0103
SN/300/Pas	0.0000
Star-Dri 5	0.0558
P/50/300/Star	0.0599
P/75/300/Star	0.0507
SN/300/Star	0.0113

Peak intensity at δ 5.40 ppm [H-1 of (1→4)-α-linked units] is 1.00.

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