JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Enrichment of Higher Molecular Weight Fractions in Inulin

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Inulin (general formulas GF_n and F_m, with G = anhydroglucose and F = anhydrofructose) naturally occurs as a homologous series of oligo- and polysaccharides with different chain lengths. For reasons of growing interest in the food and pet food industries, the short chain inulins have to be separated from their long chain analogues because their properties (digestibility, prebiotic activity and health promoting potential, caloric value, sweetening power, water binding capacity, etc.) differ substantially. To study these properties in relation to the number average degree of polymerization (DP_n), ultrafiltration, specific crystallization from aqueous solution, and precipitation from solvent/water mixtures were used to enrich native chicory and dahlia inulin in the higher molecular weight fractions. Depending on the membrane module used, the DP_n of chicory inulin ($DP_n = 8.1$) and dahlia inulin ($DP_n = 29$) could be increased by ultrafiltration to a maximum value of, respectively, 22 and 43. With crystallization from aqueous solutions (25 °C), similar results were obtained but at a much higher yield. Finally, long chain inulin could be precipitated from aqueous solutions in the presence of high concentrations of methanol, ethanol, and acetone. Acetone demonstrated to be the best solvent system to increase the DP_n, followed by ethanol and methanol. However, for safety reasons and food purposes, ethanol was evaluated to be the best choice. With ethanol, the DP_n could be raised to 25 for chicory inulin and up to 40 for dahlia inulin.

KEYWORDS: Inulin; chicory; dahlia; number average degree of polymerization; DP_n; ultrafiltration; crystallization; solvent precipitation

INTRODUCTION

Inulin obtained from several Compositae (Jerusalem artichokes, artichokes, chicory, dahlias, and dandelions) is a subject of interest in many food and "agrification" research programs (*1*−*5*). Recently also, enzymatic in vitro synthesis processes starting from sucrose have been developed, and several bacteria and fungi are examined for their potential to synthesize fructans that are in almost all cases of the levan type (*4*). The only bacterial species known to produce an inulin type fructan is *Streptococcus mutans*. Inulin naturally occurs as a homologous series of oligo- and polysaccharides of different lengths made up of β-2,1-linked anhydrofructofuranosyl units (F) that are in most cases terminated with an anhydroglucopyranosyl residue (G). This anhydroglucose unit is linked to the reducing end of the fructan chain by means of an α-1,2 bond. Inulin (with less than 2% branching, mainly branches of β-2,6-linked anhydrofructose units) can thus generally be represented, depending on the terminal carbohydrate unit, by the general formulas GF_n and F_m. Therein, G represents a glucose unit, F represents a fructose unit, n is an integer representing the number of fructose units linked to the terminal glucose unit, and m is an integer representing the number of fructose units linked to each other in the carbohydrate chain. The numbers $n + 1$ and m, respectively, indicate the degree of polymerization (DP) of the inulin molecule. As inulin is a polydisperse mixture of oligomers with varying DPs, inulin samples are characterized by the number average degree of polymerization, DP_n.

For reasons of growing interest in the food and pet food industries, the short chain inulins have to be separated from their long chain analogues, because their properties (digestibility, prebiotic activity and health promoting potential, caloric value, sweetening power, water binding capacity, etc.) differ substantially (*5*−*7*). To get insight into the unique properties of polymeric inulin in relation to its DP, several fractionation procedures are examined for their potential to enrich high molecular weight fractions. A series of scientifically described methods known to increase DP of polysaccharides are studied as follows: ultrafiltration, specific precipitation/crystallization from aqueous solutions (*3*), and specific precipitation through addition of a solvent, which decreases inulin solubility (*8*−*10*). Comparisons are made in the average DP obtained, the process yield, and the process complexity.

MATERIALS AND METHODS

Materials. Standard grade chicory inulin (Standard grade inulin is inulin obtained from plants or plant parts through conventional

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10.1021/jf030590v CCC: $27.50
Published on Web 05/22/2004

manufacturing techniques, including extraction, purification, and isolation, without any process to increase the DP of the native inulin. As a consequence of the manufacturing process, the DP of standard grade inulin is usually about 1−1.5 lower than the DP of the corresponding native inulin) (tradename Raftiline ST) was purchased from ORAFTI, a business unit within the Tiense Suikerraffinaderij (Belgium), and was used as received. Standard grade dahlia inulin was obtained from the Sigma Chemical Co. and used as provided.

Determination of the DP_n. To determine the average DP of the standard grade and fractionated inulin samples, their glucose, fructose, and sucrose contents were quantified. Glucose and fructose in the samples were determined both before and after hydrolysis with perchloroacetic acid (PCA). For that purpose, an enzyme test kit of the Boehringer Mannheim GmbH company (No. 716260) was used (*11*, *12*). Before the hydrolysis with PCA, the sucrose content in the samples was calculated from the difference of the D-glucose concentrations before and after enzymatic inversion of that sucrose with β-fructosidase. Analysis of D-glucose always proceeded according to the same principle: hexokinase, a first enzyme in the kit, catalyzes the phosphorylation of D-glucose to glucose-6-phosphate, which with the aid of glucose-6-phosphate dehydrogenase and nicotinamide adenine dinucleotide phosphate (NADP) is further specifically oxidized to D-gluconate-6-phosphate. According to the stoichiometry of the last reaction, the photospectrometrically quantified amount of reduced nicotinamide adenine dinucleotide phosphate (NADPH) is representative for the amount of D-glucose. D-Fructose was always determined subsequently to the determination of D-glucose. With the same hexokinase, D-fructose undergoes phosphorylation to fructose-6-phosphate, which is further converted to glucose-6-phosphate with phosphoglucose isomerase. Further oxidation to d-gluconate-6-phosphate as described upstairs generates a supplementary amount of NADPH that is stoichiometric with the amount of D-fructose. By calculating the fructose-to-glucose ratio (number of fructose units per number of glucose units), the average DP was obtained.

$$\mathrm{DP} = \text{number of F units per G unit} + 1 \text{ G unit}$$

$$\mathrm{DP} = C_{\mathrm{fructose}}/C_{\mathrm{glucose}} + 1$$

The procedure to determine the DP_n is based on the principles of end group analysis. DP_n is the value that corresponds to the total number of saccharide units (G and F units) in a given inulin sample divided by the total number of inulin molecules, without taking into account the monosaccharides glucose (G), fructose (F), and the disaccharide sucrose (GF), which are possibly present in the sample.

Ultrafiltration. During ultrafiltration, inulin fractions having average DPs less than a predetermined value pass through said membrane as permeate, and inulin fractions having average DPs greater than said predetermined value (NMWCO = nominal molecular weight cutoff) are collected as retentate. Two ultrafiltration modules YM-2 and YM-3 of Amicon-Millipore with NMWCO of, respectively, 2 and 3 kDa and one cellulose/regenerated cellulose hollow fiber cartridge Nephross Andante H.F. of Organon Technika with a NMWCO of 5 kDa were tested on their applicability to enrich high molecular weight fractions in inulin. The ultrafiltration membranes were first rinsed with 1 L of demineralized water, and then, 100 mL of 0.5% w/v chicory inulin (raftiline) solutions ($DP_n = 8.1$) (a concentration of 7.5% can cause precipitation of inulin through oversaturation) was subjected to diafiltration for 5.5 h at a rate of 210 mL/min and a pressure of 0.4−0.6 bar. Diafiltration is an operating mode of ultrafiltration where water (in this case 60 L) was added to the concentrate to maintain the original volume. The ultrafiltration step was followed by a concentration step (for 1 h). Several fractions obtained were then freeze-dried and weighed. The DP_n of these fractions was determined. Because of the successful enrichment of chicory inulin (raftiline) ($DP_n = 8.1$) with the Nephross Andante H.F. hollow fiber cartridge, the diafiltration experiment with this hollow fiber cartridge (5 kDa NMWCO) was repeated for 100 mL of both a 0.5% w/v chicory inulin (raftiline) solution ($DP_n = 8.1$) and a 0.5% w/v dahlia inulin solution ($DP_n = 29-30$) for a 24 h period.

Crystallization/Precipitation Reactions in Water. Because inulin molecules with a high DP are less soluble than those with a lower DP, the former will precipitate before the latter. Moreover, during sinking in a solution of inulin, the heavier molecular weight inulin molecules will push the lower molecular weight inulin molecules upward, tending to segregate the inulin by molecular weight. Therefore, when a 10% w/v solution of dahlia inulin ($DP_n = 29-30$) in demineralized water was boiled for 20 min (to kill any bacteria present and to destroy all of the enzymatic activity), half of this solution was diluted with the same amount of demineralized water. Both the 5 and the 10% w/v inulin solutions were then stored at room temperature for 6 days. Crystallization/precipitation occurred, and the clear supernatant was siphoned off. The precipitate was stored at −18 °C and then freeze-dried. The DP_n of the obtained material was determined.

Table 1. Results of Diafiltration Experiments Performed with a 0.5% w/v Chicory Inulin (Raftiline) Solution ($DP_n = 8.1$) Using Different NMWCO Membranes

membrane type	NMWCO	removal of	start amount (mg)	final amount (mg)	yield (%)	DP
YM-2	2000	DP < 12	500	460	92	12
YM-3	3000	DP < 18	500	311	62	12–15
Nephross Andante H.F.	5000	DP < 30	500	35	7	15–16

Precipitation of Inulin in Solvent/Water Mixtures Containing Varying Amounts of Methanol, Ethanol, or Acetone. Fractionated precipitation of inulin from an aqueous solution is possible by gradually decreasing the solubility of inulin in the solution through the addition of a lower alcohol or another solvent. The longer polysaccharide chains precipitate before the shorter ones. After cooking a 10% w/v chicory inulin (Raftiline) solution for 15 min, the solution was filtered and diluted to 1% w/v. To 20 mL samples of this 1% w/v chicory inulin (Raftiline) solution, methanol 99.8%, p.a., ethanol abs. 99%, and acetone 99.5%, p.a., were added in amounts of 10, 20, 40, or 60 mL, respectively. The solvent/water solutions were placed in a heating bath at 65 °C for 3 days. To avoid solvent evaporation, the recipients containing the inulin/water/solvent solution were hermetically sealed. After this period during which the precipitate was allowed to settle, the supernatant was siphoned off and the precipitate was recovered by centrifugation (11 000 rpm). The precipitates were repeatedly washed with a 40/60 water/solvent mixture. The precipitates formed were then dissolved in warm demineralized water. The resulting solutions were frozen at −18 °C and freeze-dried. As ethanol was demonstrated to generate the best results, the same experiments were repeated with ethanol for 20 mL solutions of 1, 5, 10, 15, 20, and 25% w/v of chicory inulin (Raftiline) and dahlia inulin at 65 °C for a 24 h time period.

RESULTS AND DISCUSSIONS

Ultrafiltration (Diafiltration). As expected, ultrafiltration experiments with membranes of the lowest cutoff (see **Table 1**) give the smallest effect on the DP_n. Only glucose, fructose, sucrose, and some of the inulo-oligosaccharides with DP < 10 are removed. Moreover, the diafiltration process is complicated by the fouling of the membrane. When the NMWCO of the membranes increases, small amounts of the medium chain inulins (10 < DP < 20) are also removed, resulting in a higher DP at a somewhat lower yield. Membranes with a NMWCO of 5 kDa give the largest increase in DP, but the yield is very low. As a consequence of the last result, the fraction of high molecular inulin chains in chicory inulin (Raftiline) is estimated as very small.

When starting with a native plant inulin (e.g., dahlia inulin) having a greater DP (see **Table 2**), the average DP can be raised more quickly. With dahlia inulin, an acceptable high fraction of high molecular weight inulin can be obtained, but for this ultrafiltration experiment, the yield remains low. The use of ultrafiltration for the separation of inulin involves thus some

Table 2. Results for the Diafiltration Experiments Performed on Nephross Andante H.F. (NMWCO 5 kDa) for 0.5% w/v Chicory Inulin (Raftiline) and Dahlia Inulin Solution (24 h Experiment)

inulin	start amount (mg)	final amount (mg)	yield (%)	DP_n (start)	DP_n (end)
chicory inulin	500	16.5	3.3	8.1	22
dahlia inulin	500	51	10.2	29–30	43

Table 3. Results of the Precipitation/Crystallization Reactions with 5 and 10% w/v Solutions of Dahlia Inulin in Water (6 Days of Storage at 25 °C)

inulin	start amount (g)	end amount (g)	yield (%)	DP_n (start)	DP_n (end)
5% w/v dahlia inulin	2.5	0.74	29.6	29	42
10% w/v dahlia inulin	5	1.24	24.8	29	40

serious disadvantages. The monosaccharides, disaccharides, and short chain oligosaccharides are obtained in the permeate in extremely dilute solutions and can only be recovered with a high input of energy (evaporation, freeze-drying). Moreover, in agreement with Kunz et al. (*14*), the membranes are expensive, tend to foul readily, and thus have short working lives.

Crystallization of Inulin from Aqueous Solutions. The solubility of the polymeric carbohydrates decreases with decreasing temperature and increasing chain length. Through addition of seed crystals, mainly long chain inulins are precipitated resulting in a relative high average DP of the isolated precipitates. Because of the higher degree of saturation, this precipitation proceeds faster in the 10% w/v inulin solution than in the 5% w/v inulin solution. As a consequence, after 6 days of storage at room temperature, the supernatant of the 10% w/v inulin solution was nearly clear and a dense precipitate was formed. In contrary, a turbid supernatant above the precipitate was observed in the 5% w/v inulin solution. Through variation of the inulin concentration, it is thus possible to influence the DP_n of inulin samples under crystallization. Although an acceptable DP_n (see **Table 3**) is obtained, the yield remains low because, according to Kunz et al. (*14*), a large proportion of the higher inulins remains in the mother liquor and is thus lost. In agreement with Smits et al. (*2*), the method is evaluated as rather laborious, economically not attractive, and hardly suitable for manufacture at an industrial scale.

Precipitation Reactions of Chicory and Dahlia Inulin in Water/Solvent Mixtures. In addition to the above-mentioned crystallization of inulin from water solutions, there are also precipitation reactions of chicory (Raftiline) and dahlia inulin performed in solvent/water mixtures. Concomitant with the change in solvent concentration, the inulin concentration is also varied (see **Table 4**).

These results show that the best yields are obtained with acetone followed by ethanol and methanol. This is in agreement with Wolff et al. (*9*) who demonstrated that acetone is more effective in precipitating carbohydrates than ethanol, with methanol being the least effective. With increasing amounts of solvent added, DP_n of inulin first seems to increase, but from a certain concentration on with more added solvent, this DP_n value is systematically decreased. The initial addition of solvent induces the precipitation of the longest inulin chains at a maximum of about 66% v/v for ethanol and a somewhat lower value (probably about 55−60% v/v) for acetone. However, further addition of these solvents in excess gives a rapid increasing precipitation of the shorter inulin molecules, resulting then in a decrease of that DP_n.

Table 4. Results of the Precipitation Reactions Performed with 0.2 g of Chicory Inulin (1% w/v Raftiline Solution at Start, $DP_n = 8.1$) in Different Solvent/Water Solutions during 3 Days at 65 °C

solvent	inulin (% w/v)	amount precipitate (g)	yield (%)	DP_n (end)
33% v/v methanol	0.7			
50% v/v methanol	0.5			
66% v/v methanol	0.33	0.006	3	14
75% v/v methanol	0.25	0.05	25	12
33% v/v ethanol	0.7			
50% v/v ethanol	0.5			
66% v/v ethanol	0.33	0.03	15	25
75% v/v ethanol	0.25	0.07	24.6	23
33% v/v acetone	0.7			
50% v/v acetone	0.5	0.0003	0.15	12
66% v/v acetone	0.33	0.072	36	23
75% v/v acetone	0.25	0.086	43	20

Table 5. DP_n and Yield for Chicory Inulin (Raftiline) in Several Ethanol/Water Mixtures[a]

	solvent concentration							
	33% v/v ethanol		50% v/v ethanol		67% v/v ethanol		75% v/v ethanol	
initial concn of chicory inulin	DP_n	yield (%)	DP_n	yield (%)	DP_n	yield (%)	DP_n	yield (%)
1% w/v	NP	0	NP	0	25	14.4	23	24.6
5% w/v	NP	0	NP	0	22	18	20	26.9
10% w/v	NP	0	NP	0	18.5	29	16	35.1
15% w/v	12	0.3	14	0.8	13	37.2	12	43.2
20% w/v	11	10	11	35	12	46.2	11	54.9
25% w/v	9.5	46	12	45	11	55.7	10	64.2

[a] NP = no precipitate.

Table 6. DP_n and Yield for Dahlia Inulin in Several Ethanol/Water Mixtures[a]

	solvent concentration							
	33% v/v ethanol		50% v/v ethanol		67% v/v ethanol		75% v/v ethanol	
initial concn of dahlia inulin	DP_n	yield (%)	DP_n	yield (%)	DP_n	yield (%)	DP_n	yield (%)
1% w/v	NP	0	NP	0	40	70	33	40
5% w/v	NP	0	36	36	32	82	31	74
10% w/v	33	24	31	82	31	89	30	89
20% w/v	29	100	32	95	31	93	30	95
25% w/v	30	97	29	92	30	92	29	95

[a] NP = no precipitate.

On repeating the same experiments with chicory inulin (Raftiline) and dahlia inulin in ethanol/water solutions at 65 °C for 24 h, the results in, respectively, **Tables 5** and **6** were obtained.

Low inulin concentrations favor the increase in DP_n but at rather low yield. If the initial inulin concentration is lower than 10% w/v, then ethanol must be present in a concentration of at least 60% v/v. At inulin concentrations higher than 10% w/v, the increase in DP_n is not favored by high ethanol concentrations. Using dahlia inulin with $DP_n = 29-30$ at a concentration

of 1% w/v, a long chain material with a $DP_n = 38-40$ is obtained using ethanol in a concentration of 67% v/v.

Long chain inulin can thus be precipitated under suitable conditions from an aqueous solution in the presence of high concentrations of organic solvents, such as methanol, ethanol, and acetone, and then isolated using a centrifuge or pressure filter. However, the use of inflammable organic solvents remains rather problematic in the food sector. Moreover, in agreement with Kunz et al. (*14*), the yield of desired long chain inulins is rather low due to losses caused by dissolution. Therefore, the process involves large volumes, and the dissolved components such as glucose, fructose, sucrose, and oligosaccharides, which have been separated off, have to be recovered from dilute solutions. Furthermore, the process is rather complex and laborious. For this reason, it is technically and economically not an attractive process for application on an industrial scale.

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Received for review August 8, 2003. Revised manuscript received February 10, 2004. Accepted February 26, 2004.

JF030590V