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Studies on the physicochemical properties of inulin and inulin oligomers

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Abstract

Inulin, Raftiline® ST, Raftilose® P and oligofructose in aqueous solutions were investigated at 2.5, 5, 10, 15, 17.5, 20 and 25 g of solute in 100 g of water by physicochemical measurements in order to obtain molecular weights, reducing activities, apparent specific volumes, isentropic apparent specific compressibilities, and ^{1}H-NMR pulse relaxation times (T_1 values). Molecular weights have been found to range from 340 to 4620 g mol^{-1}. Raftilose® P is the only sample which shows reducing capacity and may be expected to be susceptible to the Maillard reaction. Apparent specific volumes lie within the sweet range 0.60–0.64 cm^3 g^{-1} and increase with degree of polymerisation (DP); this behaviour describes increasing displacement of water as molecular weight increases. Isentropic apparent specific compressibilities increase as concentration of solute, molecular weight and DP increase, showing reduced solute–water affinity. T_1 values decrease with increasing molecular weight and concentration as a result of increased order of protons and reduced water mobility. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Water has a predominant role in the transmission of taste stimuli to the gustative papillae. The importance of carbohydrate solution properties in the determination of taste quality has already been elaborated (Birch, Karim, Lopez & Morini, 1993). Apparent specific volume (ASV) and isentropic apparent specific compressibility [$K_{2(S)}$] appear to be appropriate parameters for comparing the packing characteristics of carbohydrates with different molecular weights. These parameters are related to partial molar volume and isentropic partial molar compressibility which are of great relevance to the evaluation of water-solute interactions (Galema & Høiland, 1991; Høiland, 1986a,b).

Inulin is the reserve carbohydrate present in the roots and tubers of many Compositae and other plant families, and which by a single enzyme — inulinase — can be hydrolysed to fructose and glucose, its components. Inulin and inulin oligomers have a growing interest in the food and petfood industries (Gupta, Kaur & Singh, 1993). In addition to common dietary fibre properties, fructans of low molecular weight have prebiotic activity. The term prebiotic refers to a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves the health of the host (Gibson & Roberfroid, 1995). Since the polydispersity and degree of polymerisation of inulin depend on the species, the harvest periods, and preparation methods, fundamental physicochemical properties of inulin are still obscure. No systematic comparison of the solution properties of inulin, Raftiline® ST, Raftilose® P or oligofructose has yet been reported. This study, therefore, examines all of the above mentioned carbohydrates and sucrose, considering each one at different concentrations in an attempt to characterise them by ASV, $K_{2(S)}$ and ^{1}H-NMR pulse relaxation time (T_1 value).

This study is useful for increasing the understanding of sweet taste mechanisms and providing new information on inulin and fructo oligosaccharides (FOS), dietary ingredients clinically proven as "functional foods".

2. Materials and methods

Sucrose and chicory inulin are Sigma products. Raftiline® ST or the standard form of chicory inulin, Raftilose® P or oligofructose produced by the partial

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enzymatic hydrolysis of chicory inulin, are Orafti products. Oligofructose is a product of Beghin–Meiji Industries and was produced by enzymatic synthesis from sucrose.

The carbohydrates were dried in a vacuum oven at 60°C over silica gel for 24 h except for the oligofructose, which was dried at 50°C. The water used for solution studies was HPLC grade. Solutions were made up by weight and sound velocity and density measurements were carried out at 20°C. Cryoscopic measurements were determined using an advanced milk Cryoscope 4L2 from Advanced Instruments Inc. with accuracy to ± 0.002°C. Molecular weights were calculated from cryoscopic values using the equation:

$$M = 1860c/T(100 - c) \tag{1}$$

where M is the molecular weight of the solute (g mol^{-1}), 1860 is the factor used for milk freezing point, c is the concentration of the solution (g 100ml^{-1} of solution), and T is the depression of freezing point (°C).

Reducing carbohydrates were determined using the volumetric determination of sugars by copper reduction (Lane and Eynon titration) (Kirk & Sawyer, 1991).

Apparent specific volumes (ASV) and isentropic apparent molar compressibilities [$K_{\phi(s)}$] were determined using procedures as described by Birch, Parke, Siertsema and Westwell (1996). Isentropic apparent specific compressibilities were obtained using the equation:

$$K_{2(S)} = K_{\phi(s)}/M_2 \tag{2}$$

where $K_{2(S)}$ (cm^3 g^{-1} bar^{-1}) is the isentropic apparent specific compressibility, $K_{\phi(s)}$ (cm^3 mol^{-1} bar^{-1}) is the isentropic apparent molar compressibility, and M_2 (g mol^{-1}) is the molecular weight of the solute.

Spin-lattice relaxation times (T_1 values) of the protons were measured in these solutions using a Bruker PC 120 NMR Process Analyser, a low-resolution pulsed nuclear magnetic resonance spectrometer operating at a resonance frequency of 20 MHz and at a temperature of 37 ± 1°C. Three parameter fitting procedures were used for the calculation of relaxation times. The decay of magnetisation was checked and found to be monoexponential for all the solutions.

3. Results and discussion

Physicochemical measurements on inulin, Raftiline® ST, Raftilose® P, and Oligofructose are reported. Sucrose measurements are used as references for the data obtained by the different techniques.

The preliminary analysis of inulin, inulin oligomers, and sucrose consisted in the determination of molecular weight averages and reducing activity as listed in Table 1. The distribution variations among oligomers make these molecular weight averages somewhat questionable. Therefore, solution measurements are converted to specific parameters (i.e. per g).

Molecular weight averages for sucrose, oligofructose, and Raftilose® P were in line with reported data, while values obtained for Raftiline® ST and inulin showed lower molecular weights than literature data. Molecular weight calculations of inulin take into account the fact that the Sigma inulin composition does not include the fractions between DP2 and DP10 (Van Loo et al., 1995). Reducing activity was detected using 10 ml of fresh Fehling's solution against 5 g 100 ml^{-1} of carbohydrate solution. The results are given in function of the reducing activity calculated as dextrose equivalent (DE, that is, reducing power as g of dextrose in 100 g of dry matter). Inulin reducing activity was weaker than the limiting value of the method; the reducing powers for Raftiline® ST and oligofructose were quite the same; and Raftilose® P was the only sample which was high enough to be susceptible to deteriorative reactions such as the Maillard reaction (Table 1).

Apparent specific volume has proved very useful in the study of taste quality (Birch et al., 1996; Birch & Shamil, 1988; Kemp, Birch, Portmann & Mathlouthi, 1990). It appears to be an appropriate parameter for comparing the packing characteristics of carbohydrates with different molecular weights (differences being computed per unit weight of different types of molecule). Fig. 1 shows the ASVs of inulin, inulin oligomers, and sucrose. ASV gives a direct measurement of disturbance or displacement of water by solute.

ASVs at infinite dilution are reported in Table 2. These values are all within the range 0.60–0.64 cm^3 g^{-1} and show variation with degree of polymerisation (DP). The range of ASVs which broadly determines sweetness is 0.50–0.70 cm^3 g^{-1} and carbohydrates seem to be in the middle of this range. The central range of 0.60–0.64

Table 1
Molecular weight and reducing activity of inulin, Raftiline® ST, Raftilose® P, oligofructose, and sucrose

	Molecular weight from cryoscopy	Molecular weight from literature [a]	D.E.
Inulin	4620[b]	5000[c]	n.d.[d]
Raftiline® ST	1251	1800[c]	1.67
Raftilose® P	679	342–1638[c]	11.3
Oligofructose	579	504	1.35
Sucrose	340	342	0

[a] Literature values are expressed in DP.

[b] Inulin value considers the lack of DP2 to DP10, as demonstrated by Van Loo, Coussement, De Leenheer, Hoebregs and Smits (1995).

[c] Orafti report.

[d] not detected.

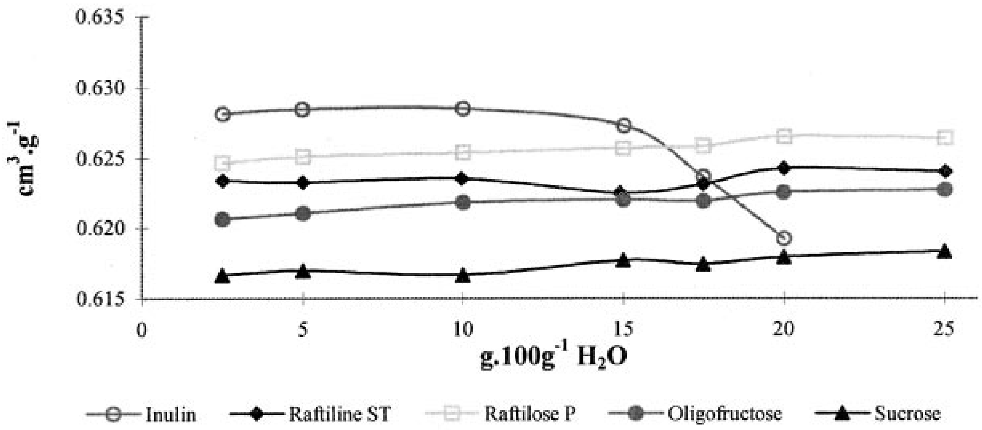

Fig 1. Changes in apparent specific volumes (cm^3 g^{-1}) of inulin, inulin oligomers, and sucrose with concentration (g 100 g^{-1} of H_2O).

cm^3 g^{-1} indicates pure sweetness for sugars, but also for glucose syrups with DP 1-8 (Birch & Karim, 1992) and for individual maltooligosaccharides (O'Donnell, 1984). ASVs increase with increasing molecular weights from sucrose to Raftilose® P while Raftiline® ST and inulin apparent specific volumes are both lower than expected. These data can be related to the chain lengths: molecules whose DP is higher than 9 start taking some secondary conformations with a more limited back-bone chain movement than oligomers with DP lower than 7–8 (Oka, Ota, Mino, Iwashita & Komura, 1992). Inulin supersaturated solutions, where concentration is above 15% (g 100 g^{-1} of water), lead to decreasing ASVs because of the formation of solute–solute interactions between inulin chains in addition to solute–water interactions. The general behaviour of this fructan is opposite to the behaviour of glucose syrups in which higher DP gives lower ASVs at the same concentration (Birch & Karim, 1992).

A lower ASV indicates better hydrostatic packing characteristic with water molecules, but it does not imply better compatibility with water structure. Isentropic apparent molar compressibility [$K_{\phi(s)}$] has been suggested as one of the best indicators of water compatibility (Galema & Høiland, 1991). Isentropic apparent specific compressibility [$K_{2(S)}$] appears to be an appropriate parameter for comparing the water compatibility of carbohydrates with different molecular weights. This parameter is not influenced by molecular weights of samples: using both the molecular weight values (from literature and cryoscopic analysis) for inulin, inulin oligomers and sucrose the $K_{2(S)}$ value does not change. Fig. 2 shows the changes of $K_{2(S)}$ with concentration.

Table 2
Partial specific volumes, isentropic partial molar compressibilities, and isentropic partial specific compressibilities of inulin, inulin oligomers, and sucrose at 20°C

	ASVs at infinite dilution (cm^3 g^{-1})	$K_{\phi(s)}$ at infinite dilution (cm^3 mol^{-1} bar^{-1})	$K_{2(S)}$ at infinite dilution (cm^3 g^{-1} bar^{-1})
Inulin	0.629	-20.6×10^{-4}	-4.3×10^{-7}
Raftiline® ST	0.623	-25.1×10^{-4}	-1.9×10^{-6}
Raftilose® P	0.625	-24.3×10^{-4}	-3.4×10^{-6}
Oligofructose	0.621	-24.4×10^{-4}	-4.0×10^{-6}
Sucrose	0.616	-21.3×10^{-4}	-6.0×10^{-6}

Results for solution properties are dependent on accurate weighings and these are therefore significant to four significant figures. This approach has been established to give reproducible and significant results (Galema & Høiland, 1991). $K_{2(S)}$ is really a measure of the compactness of the hydration layers around the solute molecules but it is a *specific* value, i.e. computed per g of solute. A low value of $K_{2(S)}$ therefore, indicates a very compact (i.e. incompressible) water layer, calculated per unit mass of solute. $K_{2(S)}$ values are therefore different from ASV$_s$ but vary is the same direction. The values reported in Table 2 fit within the range reported recently (Parke, Birch & Dijk, 1999) for sweet substances. $K_{2(S)}$ values for inulin are higher than the other solutes tested except at high concentration. This is attributed to conformational changes in the inulin polymer.

Isentropic apparent specific compressibility is a parameter that does not contain any intrinsic component since the solute molecules can be regarded as incompressible, at least over a moderate pressure range. Pure water has a positive molar compressibility (Galema & Høiland, 1991). $K_{2(S)}$ increases with increasing degree of polymerisation and concentration. The water around sucrose is less compressible than bulk water, while the hydration layer of high molecular weight inulin fractions like Raftiline® ST and inulin itself approaches the positive value of pure water. Compatibility of both inulin and inulin oligomers with water structure increases with increasing molecular weight of solute. The

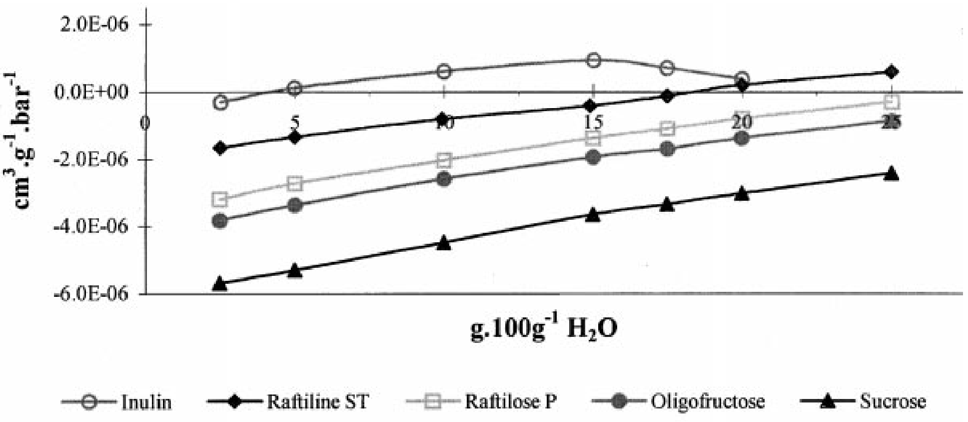

Fig. 2. Changes in isentropic apparent specific compressibilities (cm^3 g^{-1} bar^{-1}) of inulin, inulin oligomers, and sucrose with concentration (g 100 g^{-1} of H_2O).

apparent specific volumes, isentropic apparent specific compressibilities and isentropic apparent molar compressibilities at infinite dilution are reported in Table 2.

ASV values at infinite dilution decrease with decreasing molecular weight. Inulin and Raftiline® ST values are smaller than anticipated, presumably because of some secondary conformations of the back-bone chains. However, the differences are in fact small. $K_{\phi(s)}$ values at infinite dilution also lie in the same range, so it is not possible to find a clear correlation between the different samples and the mono-layer of water around them, while $K_{2(S)}$ values at infinite dilution clearly define the higher compatibility of inulin polymers towards water structure than oligomers. The well-organised polymers offer greater numbers of hydration sites. In fact, inulin and Raftiline® ST $K_{2(S)}$ values reach the positive water range at high concentrations.

^{1}H-NMR pulse relaxation studies provide another method of analysing the state of order of the inulin fraction of the solution protons. *Spin-lattice relaxation* is a process which allows nuclear spins to return to equilibrium following some disturbance of Proton Magnetic Resonance (PMR). As the nuclei approach equilibrium, the energy released is dissipated in the surroundings (the '*lattice*'). Shorter relaxation times mean greater degrees of order, probably due to greater hydrogen-bond energy, creating hydration sites and loss of motional freedom. Table 3 lists the differences between the spin-lattice relaxation times (T_1 values) of the samples.

Two clear trends can be identified: first, within any particular solution, an increase in the concentration of solute entails a decrease in T_1; second, between the samples, at any given concentration, increasing DP entails a decrease in T_1.

The first of these trends occurs simply because the ordered solute protons constitute an increasing proportion of the total protons. The second similar trend is due to the increasing degree of order as the average DP of the solute increases. However, a better explanation is that the larger molecules have less mobility, therefore, the relaxation times (T_1 values) are shorter.

The T values represent an average of all protons. In view of the concentrations of the various solutes, most protons are water protons. Therefore, the T, values mostly reflect average mobilities of water protons and they change with concentration as well as nature of solute.

Table 3
Changes in ^{1}H-NMR spin lattice relaxation times (s) of inulin, inulin oligomers, and sucrose with concentration (g 100 g^{-1} of H_2O)

	Spin-lattice relaxation times T_1 (s)					
	Concentration (g of solute in 100 g of H_2O)					
	2.5	5	10	15	17.5	20
Inulin	3.266	2.936	2.326	1.942	–	–
Raftiline® ST	3.576	3.141	2.771	2.469	2.311	2.153
Raftilose® P	3.486	3.263	2.992	2.617	2.449	2.347
Oligofructose	3.463	3.374	3.091	2.838	2.697	2.520
Sucrose	3.554	3.441	3.122	2.854	2.755	2.641

4. Conclusion

In conclusion, this work describes the behaviour of inulin and inulin oligomers in water. The apparent specific volumes (ASV) determined are typical of carbohydrates and fall within the pure sweetness taste range, in agreement with data of mono-, and di-saccharides, individual maltooligosaccharides, and glucose syrups. The isentropic apparent specific compressibilities [$K_{2(S)}$] and ^{1}H-NMR T_1 values are solution parameters which are useful for the characterisation and quantification of inulin fractions for rapid control purposes in the food industry. The solution behaviour exhibited by the different solutes is very dependent on the size and average

DP of the molecules. This ultimately affects the conformation that the molecules adopt in solution and hence their ability to pack efficiency in water.

Overall, the results, of the current work demonstrate that the solution properties of inulin oligomers are as expected for carbohydrate sweeteners. Both apparent isentropic compressibilities and apparent specific volumes indicate good hydration characteristics and a corresponding pure sweetness quality (middle of apparent specific volume sweetness range).

The Lane and Eynon titration shows a significant reducing activity for Raftilose® P and not for the other oligomers. For this reason, Raftilose® P is susceptible to the Maillard reaction.

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