Food Chemistry **18** (1985) 131–138

Release of Inulin by Enzymatic Liquefaction of Chicory Roots

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(Received: 6 March, 1985)

ABSTRACT

During liquefaction of chicory roots with commercial pectolytic and cellulolytic enzymes the release of inulin has been followed with high-performance liquid chromatography. For the release of inulin pH 4·6–4·0 is optimal: almost all inulin is present in the liquid phase (supernatant). One commercial enzyme preparation showed inulinase activity.

INTRODUCTION

In The Netherlands about 10^5 tons of chicory roots (*Cichorium intybus* L.) are produced annually. After forcing, the heads are separated from the roots and sold as a fine vegetable. The remaining roots constitute a waste, which is mainly used as cattle feed. This waste might be a source of bitter compounds and inulin, a linear $\beta-2\rightarrow1$ linked fructose polymer terminated by a sucrose unit residue (Fig. 1). Inulin can be applied in the food industry after hydrolysis to fructose and glucose units (Zittan, 1981).

The purpose of this study was to liquefy this waste with commercial

Food Chemistry 0308-8146/85/$03·30

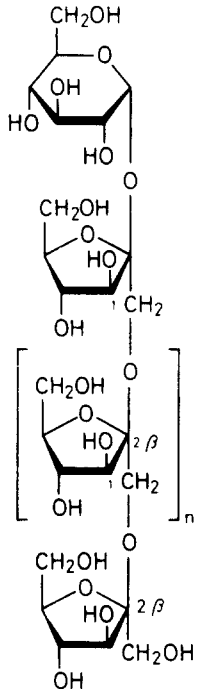

Fig. 1. Structure of inulin.

enzyme preparations to release inulin and, in addition, the bitter compounds (to be published). So far analytical methods of determining inulin are based on estimation of fructose and glucose after acid hydrolysis (Wight & van Niekerk, 1983). We wanted a method that would estimate inulin as such, for which purpose we developed one with high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Materials

Unforced and forced chicory roots were obtained from two different growers in 1983 and stored at 0 °C for one week before use.

Chemicals used were from Merck, unless stated otherwise.

Liquefaction of chicory roots

Unblanched chicory roots were cut into small pieces (about 4 mm^3) under liquid nitrogen and kept frozen at −25 °C until use. Portions of frozen roots (100 g) were suspended in 100 ml citrate buffer (22 mM).

For every 100 g of roots 0·1 g Rapidase C600 (pectolytic and cellulolytic enzymes, Gist-Brocades, The Netherlands) was added as a dry preparation under stirring of the suspension. Also liquefaction with some combinations of Rapidase C80 (pectinases) and Maxazym CL2000 (cellulases), both from Gist-Brocades, was carried out.

During liquefaction the material was stirred continuously and the temperature kept constant at 40 °C. After several periods of time (0, 1, 2, 5, 8 and 24 h) samples were removed with a pipette which had a wide orifice (9 mm ID) and centrifuged for 30 min at 3000 *g*. The supernatant was analyzed for dry matter, inulin, and after addition of methanol (up to 80 % v/v) for fructose, glucose and sucrose. The residue was also used for the estimation of dry matter.

The control was the same mixture but without the addition of commercial enzymes, and using a citrate buffer concentration of 48 mM instead of 22 mM to maintain the pH at 4.

Determination of inulin and sugars

A Waters Ass. (Milford, MA, USA) liquid chromatograph equipped with a 6000A pump, U6K injector and a R401 refractive index detector was used. The column for the separation of inulin was an Aminex HPX-42A from Bio-Rad (Richmond, CA, USA) connected in line with a guard column (80 × 4 mm) filled with 35 % AG50-WX4 (minus 400 mesh, H^+-form) and 65 % AG3-X4A (200–400 mesh, put in OH^--form) both from Bio-Rad (Brons & Olieman, 1983). The solvent was doubly distilled water, degassed by filtration through a Millipore-filter of pore size 0·45 μm for aqueous solutions. The column, solvent and detector were thermostatted at 85, 90 and 40·0 °C, respectively. The flow-rate was 0·4 ml/min. The samples were filtered through a Millipore-filter (0·45 μm) prior to injection. Inulin was purchased from BDH Ltd (England).

Fructose, glucose and sucrose were determined using a LiChrosorb-10-NH column from Chrompack (Vlissingen, The Netherlands) with ethylacetate–acetone–water (300:550:150 v/v) as eluent (Müller & Siepe, 1980). The temperatures of column and detector were both 30·0 °C. The flow-rate was 1·3 ml/min.

RESULTS AND DISCUSSION

Liquefaction of chicory roots

Several commercial enzyme preparations containing pectinases and cellulases were used to test their ability to liquefy chicory roots. Combinations of pectinases and cellulases, supplied simultaneously, caused an almost complete liquefaction, as already found by Pilnik *et al.* (1975) and Voragen *et al.* (1980), with other plant material than chicory roots. When we used pectinase or cellulase alone liquefaction was not complete.

With the preparation Rapidase C600, which contains both types of enzymes, good results were obtained at pH 4·6 to 4·0 (Table 1); almost all dry matter from the roots is solubilized and present in the supernatant.

Release of inulin

The release of inulin during enzymatic liquefaction of forced chicory roots at 40 °C and pH 4 (0·1 M Na-acetate buffer was used instead of

TABLE 1

pH and Mass Balance of Total Dry Matter During Enzymatic Liquefaction and Control as Distributed Between Supernatant (S) and Residue (R). (Data are calculated for 100 g suspension (50 g roots and 50 g citrate buffer; 22 mM and 48 mM, respectively).)

Time (h)	*Part of suspension*	*Liquefaction*		*Control*	
		pH	*Dry matter (g)*	*pH*	*Dry matter (g)*
	Suspension		12·80		12·80
0	S	4·6	7·09	4·1	6·64
	R		5·71		6·16
1	S	4·4	6·46	4·1	3·71
	R		6·34		9·09
2	S	4·3	8·60	4·1	4·71
	R		4·20		8·10
3	S	4·1	10·21	4·1	5·37
	R		2·59		7·43
8	S	4·1	10·23	4·1	6·52
	R		2·57		6·28
24	S	4·0	11·03	4·0	6·73
	R		1·77		6·07

citrate buffer) was followed as a function of time and of enzyme preparation.

When Rapidase C80 (pectinases) was used, alone or in combination with Maxazym CL 2000 (cellulases), an increase in fructose was observed indicating that inulin was being hydrolysed (Table 2). The isolation of

TABLE 2

Sugar Composition of Supernatant after 24 h of Enzymatic Liquefaction of Chicory Roots at 40 °C with Several Enzyme Preparations; pH 4, Obtained with 0·1 M Na-acetate Buffer. (Data are expressed as % of total dry matter.)

Enzyme preparation	*Enzyme concentration*[a]	*Glucose*	*Fructose*	*Sucrose*	*Sugars with DP > 3*
Rapidase C80	0·25	13·0	43·4	1·1	0·2
Rapidase C80 + Maxazym CL2000	0·20 + 0·20	16·2	36·4	0·8	0·6
Maxazym CL2000	0·20	2·4	2·1	12·4	3·8
Rapidase C600	0·20	5·0	5·0	12·0	5·2

[a] Expressed as percentage of fresh weight of chicory roots.

inulin is easier than the isolation of fructose (Kierstan, 1978), consequently the use of Rapidase C80 must be disapproved. So the application of Rapidase C600 was preferred to avoid hydrolysis of inulin.

A pH of 4·6 to 4·1 proved to be optimum for the release of inulin for unforced as well as forced roots. Almost all inulin of the root was solubilized in the supernatant in contrast to the control, where an average of 63 % of total inulin is present in the supernatant (Fig. 2). When the pH

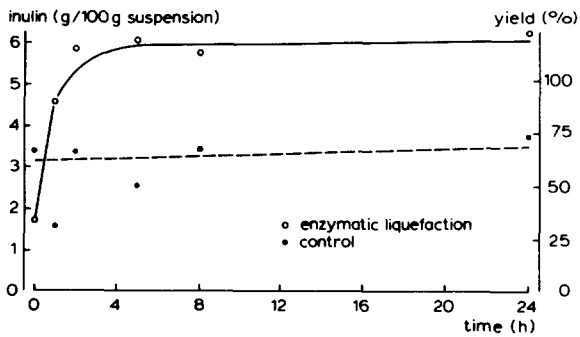

Fig. 2. Release of inulin in supernatant during enzymatic liquefaction of chicory roots calculated as g/100 g suspension (50 g roots + 50 g buffer). For conditions used see text.

of the control was not kept at 4 (buffer concentration too low), a lower recovery of inulin was obtained.

This low recovery of inulin coincides with an increase of fructose. With the low buffer concentration the pH of the control remained at *ca.* 5. It is possible that inulinase from the chicory root has hydrolyzed the inulin. Inulin could be separated by the Aminex HPX-42A column (Fig. 3(a)) (coefficient of variation = 15·4%, 13 replicates in various runs; the recovery was 90% as established by spiking; the minimum amount that could be detected was 5 μg).

The retention time of inulin in the chromatograms of the supernatant was corroborated with commercial inulin. For the separation of the heterogeneous oligosaccharides of low degree of polymerisation (DP) this column was not suitable; a bad resolution and double peaks of one sugar are seen (Fig. 3(b)). Also the retention times of, for example, maltose and sucrose (both DP 2) are different. Probably there is a different interaction between column and sugars with a five-membered or six-membered ring, or partial hydrolysis of the relatively weak β-fructoside bond is taking place in the column during elution.

Also the temperature of the solvent has to be higher than the temperature of the column (Brobst & Scobell, 1982) to avoid air bubbles,

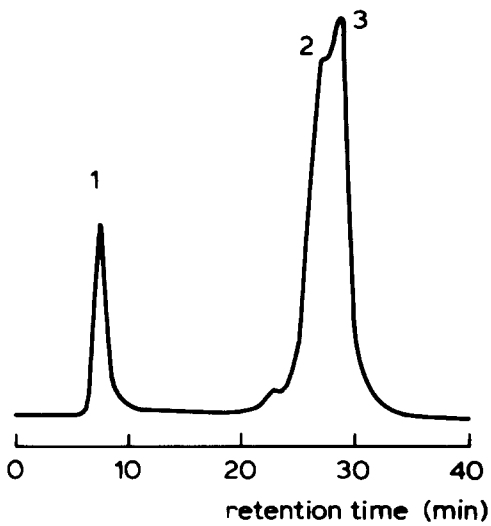

Fig. 3(a). Chromatogram of the supernatant of liquefied chicory roots after 24 h (RI × 4, sample size 20 μl). Peak 1 = inulin, peak 2 = sucrose, peak 3 = fructose.

which could disorder the RI-detector, even though the solvent was degassed.

Although inulin in the obtained supernatant after enzymatic liquefaction of chicory roots could be determined by HPLC, a good preparation of the samples is necessary to guarantee a long column life. The separation of heterogeneous oligosaccharides with this Aminex HPX-42A column has to be optimized as well.

ACKNOWLEDGEMENT

We thank Dr N. Gorin and Dr W. Klop from the Sprenger Institute for valuable discussions and G. Grevers for the HPLC analysis of sugars.

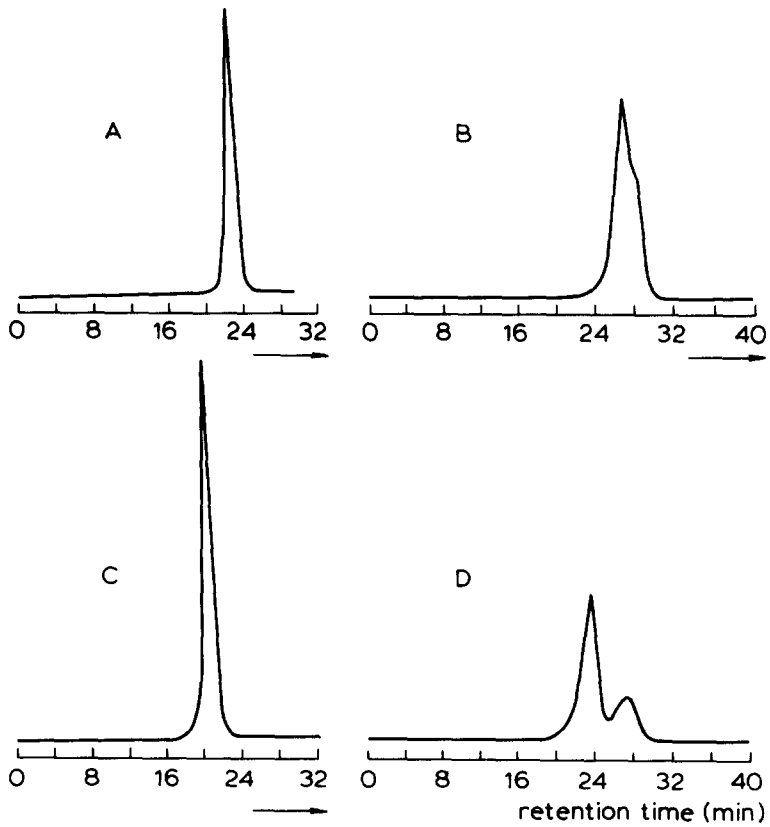

Fig. 3(b). Chromatograms of standard solutions in water (1 % w/v) of maltose (A), sucrose (B), maltotriose (C) and raffinose (D) on an Aminex HPX-42A column (sample size 20 μl, RI × 4).

This study was made possible with financial support from the 4 million Guilder Fund for Biotechnology of the Department of Agriculture and Fisheries.

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