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Cereal Fructosans: Part 1—Isolation and Characterization of Fructosans from Wheat Flour

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ABSTRACT

Wheat fructosans were isolated by two different methods—One, described earlier by Montgomery & Smith (1957), and one new, simpler method.

The preparations were characterized by chemical analysis and by gel filtration chromatography. Their carbohydrate composition was practically identical. The ash content was lower in fructosans prepared by Montgomery and Smith's method, since it involves an ion exchange treatment. The yield of total fructose was higher with our method (68% compared with 32%).

Acid hydrolysis was performed with wheat fructosans and, for comparison with inulin, to investigate the possibility of degradation of fructosans in the stomach. The results indicated very slow breakdown at physiological conditions. Wheat fructosans were slightly more acid resistant than inulin.

INTRODUCTION

Fructosans are present in all cereals at a concentration of 1–4% of the dry matter. They are composed of β-fructofuranosyl units in 2→1 (inulin) or 2→6 (levan) positions. There also seem to exist branched varieties with both kinds of link (Aspinall, 1970).

The fructosans are formed from sucrose by transfructosylation and

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therefore each molecule also contains an α-glucopyranosyl unit in the end position, indicating the sucrose molecule from which the synthesis started. Sucrose can also be regarded as the smallest possible fructosan.

The cereal fructosans are, in general, rather small in size, containing up to about 20 hexose units (MacLeod & Preece, 1954).

As part of a cereal project, supported by the Swedish Technical Development Board, a study on the fructosans has been started with the purpose of elucidating their nutritional and technological implications.

Little is known about the importance of fructosans in the baking of bread, although we have indications that the fructosans can partly replace sucrose, since they are hydrolyzed by yeast invertase to fructose which then is incompletely fermented (Tanaka & Sato, 1969; Zelazowska-Majer & Jacobzyk, 1979).

We do not (except for sucrose) know to what extent fructosans can be digested and absorbed. There is no β-fructosidase in the intestine, but it has been suggested that fructosans may be hydrolyzed in the stomach since they are extremely easy to hydrolyze by acids. Experimental evidence for this concept has never been given.

As a first step in an investigation of the cereal fructosans we have isolated the fructosans from wheat flour. Two methods have been used, one described by Montgomery & Smith (1957) as modified by Medcalf & Cheung (1971) based on precipitation with barium, and our own, simpler, method based on ethanol precipitation. The yield with these two methods, the purity of the preparations obtained and some of their properties have been studied.

MATERIAL AND METHODS

Carbohydrates

Inulin (used for comparison with the cereal fructosans) was obtained from Nutritional Biochemicals Co–ICN, Cleveland, Ohio. Other carbohydrates were commercial preparations of analytical grade purity.

Assay of total hexoses

These were assayed with anthron as described by Scott & Melvin (1953). Both free and bound hexoses react. Fructose gives 10% stronger reaction than glucose.

Assay of total fructose

The amount of fructose was assayed by means of the method of Dische & Borenfreund (1951). Both free and bound fructose react. The unspecific reaction by glucose is very weak. Pure glucose, maltose or starch give approximately 0·8% of the reaction of fructose and therefore fructose and fructosans can also be measured in the presence of a rather large excess of aldohexoses or carbohydrate composed of such.

Assay of reducing power

Reducing sugars were assayed with the dinitrosalicylate reagent (Hostettler *et al.*, 1951). Glucose and fructose give equally strong reactions.

Assay of free glucose

Free glucose was assayed with Tris-buffered glucose oxidase reagent (Dahlqvist, 1961).

Gel filtration chromatography

We used 90 cm long mantled columns packed with Biogel P2 200-400 mesh (wet). The diameter was 1·5 cm; the flow rate, 6 ml/h and the temperature was kept at 60 °C. The effluent was collected in 3 ml fractions which were analyzed for total hexoses and total fructose. The effluent volume, given in the figures is measured from the moment at which the sample was applied to the upper surface of the gel.

γ-amylase digestion

We used amyloglucosidase (1,4-α-D-glucan glucohydrolase EC 3.2.1.3 from *Aspergillus niger*, crystal suspension 10 mg/ml) obtained from Boeringer AG Mannheim GmbH, Germany.

Incubation conditions were: temperature, 60 °C; 0·01 M Na–acetate buffer, pH 4·75; 5 mg of total carbohydrate and 50 μg of enzyme in a volume of 1·0 ml; incubation for up to 60 min. The enzyme hydrolyzes maltose and malto-oligosaccharides as well as starch and glycogen. The glucose liberated was measured with glucose oxidase (see above).

Isolation of fructosans from wheat flour

Method of Montgomery & Smith (1957) as modified by Medcalf & Cheung (1971)

Mix 260 g of wheat flour, 2·5 g of $CaCO_3$ and 750 ml of 80 % ethanol. Reflux with stirring for 60 min. Add 160 ml of distilled water (will give an ethanol concentration of 70 %) and stir overnight.

Centrifuge and extract the sediment with further 900 ml of 70 % ethanol for 60 min at room temperature. Combine the supernatants and concentrate them *in vacuo* at 40 °C to about 40 ml. Remove recipitated proteins repeatedly by centrifugation during the evaporation. Mix the concentrated solution with 95 % ethanol until slight turbidity occurs. Next, immediately add a solution of 10 g $Ba(OH)_2$ in 37·5 ml of hot water. A precipitate is formed at once. Add 95 % ethanol to a final volume of 1000 ml, mix and leave overnight.

On the next day add another 10 g $Ba(OH)_2$ dissolved in 37·5 ml hot water and mix. Centrifuge, and discharge the supernatant. Dissolve the sediment in 50 ml of cold water and neutralize with diluted H_2SO_4.

Then $BaSO_4$ will precipitate. Centrifuge, deionize the supernatant first with Dowex 50 (H^+) and then with Amerlite IRA 904 (OH). Lyophilize the eluate.

We were not able to obtain a dry powder with this method, but dissolved the syrup in 20 ml of water.

Own method

Mix 150 g of flour with 1000 ml of *boiling* 80 % ethanol. Reflux with stirring for 30 min. Let the insoluble particles sediment and then remove the supernatant by decantation.

Wash the sediment twice with water.

Combine the three supernatants and concentrate them in the evaporator at 40 °C to between 200 and 300 ml volume.

Recombine the concentrated supernatants with the sediment and dilute to 700 ml with water. Extract with stirring at room temperature for 2 h.

Centrifuge, and extract the sediment with further 300 ml of water for 1 h at room temperature. Centrifuge again, discard the sediment and combine the two supernatants. Measure the volume and add four volumes of 95 % ethanol. Leave at room temperature overnight.

Centrifuge, discard the sediment and concentrate the supernatant in the evaporator at 40 °C to about 100 ml.

Centrifuge again, discard the sediment and lyophilize the supernatant. The powder obtained contains the fructosans.

RESULTS

Recovery

The wheat flour used contained 12% moisture and 79% of total carbohydrate. Total fructose assay indicated 1·5% of fructose. This has to be corrected for the unspecific reaction of the starch, corresponding to about 0·6% of fructose, which leaves about 0·9% of fructosans in the flour.

When fructosans were prepared with the modified Montgomery and Smith method, a final preparation containing 738 mg of total fructose was obtained from 260 g of flour, indicating a yield of 32%.

With our method a fructosan preparation containing 914 mg of total fructose was obtained from 150 g of flour, indicating a yield of 68%.

Purity of the products

The composition of the fructosan preparations obtained by the two methods is shown in Table 1. The main difference is the higher ash content of the preparation obtained with our own method. In Montgomery and Smiths' method an ion exchange process is included to remove salts and if we apply that to our method it will give a product of higher purity. For most purposes, however, we do not regard the ash content as important.

We could never obtain a powder on lyophilization of the fructosans

TABLE 1
Comparison of Fructosan Preparations Obtained by the Two Different Methods

Contents calculated on dry matter basis	*Fructosans isolated by modified Montgomery and Smith method* (%)	*Fructosans isolated by our method* (%)
Total hexoses (anthron)	85	79
Fructose	60	54
Ash	<1	9·7

prepared by Montgomery and Smith's method, but had to handle it in water solution. With our method a powder was obtained on lyophilization, although this powder was hygroscopic. The higher ash content of our preparation may facilitate the preparation of a powder.

For measuring the content of starch, dextrins, malto-oligosaccharides and maltose we used γ-amylase digestion as described in the 'Materials and Methods' section. This experiment indicated an α-glucan content of 0·5% in the preparation obtained with Montgomery and Smith's method and 1·6% in that obtained with our method. Controls with wheat flour indicated 72% α-glucans. No glucose was released from inulin with this method.

The free glucose content of the preparation obtained with Montgomery and Smith's method was 1·1% and in the preparation with our method, 0·2%. Wheat flour contained 0·05% free glucose.

Gel filtration

The results of gel filtration chromatography with the two kinds of preparation are seen in Fig. 1. They gave identical curves, with practically no monosaccarides, a distinct sucrose peak and a series of peaks of increasing molecular size. The fructosans from wheat have much lower weight than inulin, as is seen in the Figure.

Acid hydrolysis

For these experiments we used the fructosan preparation obtained with our own method and, for comparison, inulin. Heating was performed at either 37 °C or 100 °C in dilute hydrochloric acid (0·001–0·1 M). The reducing power was measured in samples taken at intervals (Fig. 2). In one experiment (fructosans heated at 100 °C in 0·1 M hydrochloric acid) we also measured the amount of glucose formed, using the glucose oxidase reagent, after neutralization of the acid.

Both inulin and the fructosan preparation can be hydrolyzed by dilute hydrochloric acid. When heated at 37 °C in 0·1 M hydrochloric acid, however, only a small fraction of the fructosans are hydrolyzed after 1 h. This does not support the concept that fructosans might be hydrolyzed by hydrochloric acid in the stomach. Inulin is more acid-labile than the fructosans, but inulin will not be hydrolyzed in the stomach, either (Fig. 2).

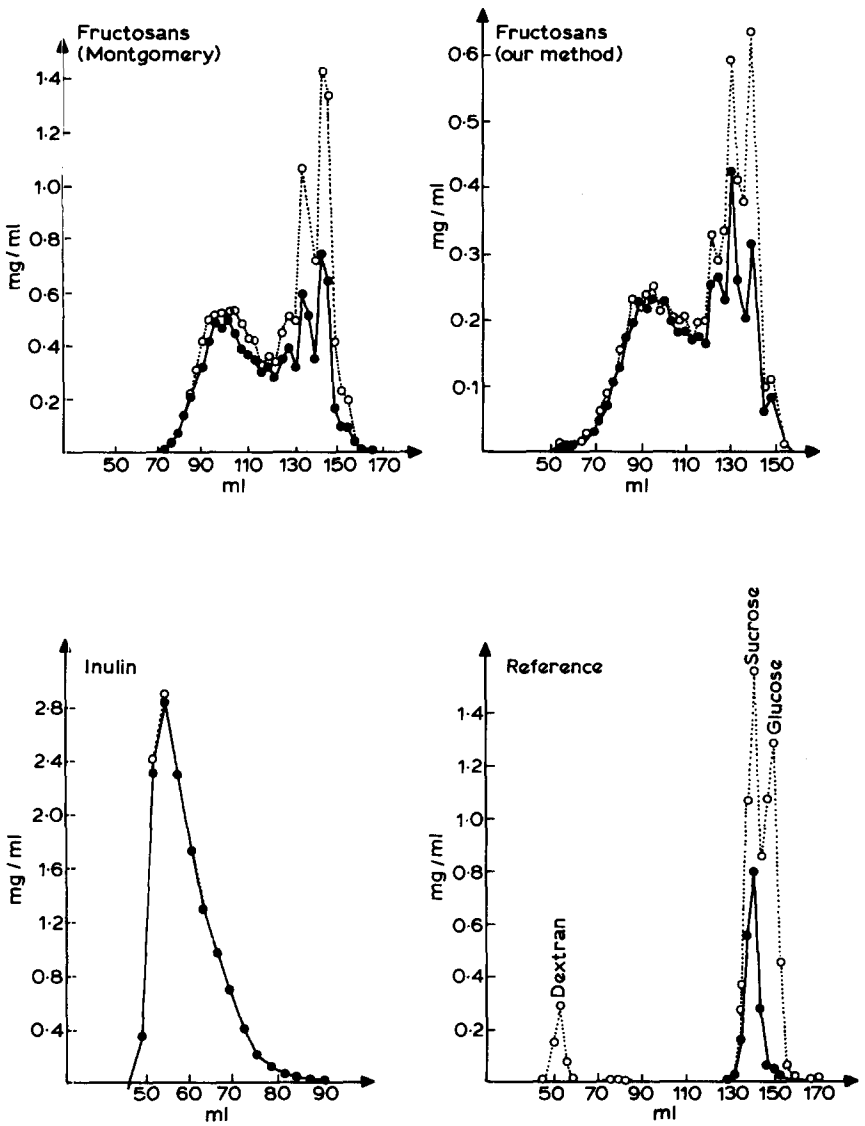

Fig. 1. Gel filtration chromatography of the two fructosan preparations studied, inulin and a reference. (○----○ total hexoses; ●——● total fructose).

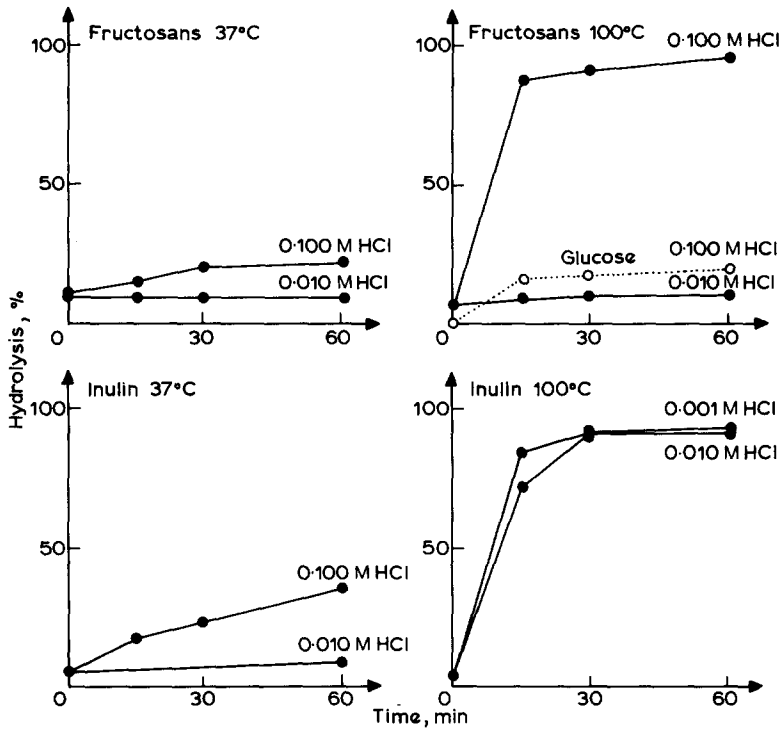

Fig. 2. Acid hydrolysis of fructosans and inulin, measured as increase in power (●——●). In one case glucose was also measured with glucose–oxidase (○ - - - - ○). At complete hydrolysis glucose amounts to 20% of the reducing sugars.

The fructosan preparation yielded about 20% of glucose on complete acid hydrolysis.

DISCUSSION

With the preparation method for fructosans we have described, it is important to keep the flour dry until the first extraction and perform this with hot 80% ethanol. Although the ethanol is afterwards removed in the evaporator it serves to inactivate the β-amylase present in the flour. In experiments where the flour was first suspended in water at room temperature, the final 'fructosan' preparation contained up to 50% maltose.

The fructosans obtained with our own method seemed to be identical

with those obtained with Montgomery and Smith's method, except for a 10% salt content. Only our own preparation could be lycophilized to a powder (possibly because of the salts). If such is wanted, these salts can be removed by ion exchange.

The recovery was higher with our method.

The wheat fructosans were of relatively small molecular size, as described earlier in the literature (MacLeod & Preece, 1954). There was very little free fructose, but significant amounts of sucrose.

Although the wheat fructosans, like inulin, are easily hydrolyzed by acids, 1 hour's incubation with 0·1M HCl at 37°C only gave a very low degree of hydrolysis. This makes it unlikely that the hydrochloric acid in the stomach will be able to hydrolyze the fructosans. The digestion of fructosans *in vitro* and *in vivo* will be subject to further study.

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