

Measurement of Inulin and Oligofructan

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Fructans are defined as any compound in which one or more fructosyl-fructose linkages constitute a majority of the linkages (1). This refers to polymeric material as well as to oligomers as small as the disaccharide inulobiose. Fructans are widely distributed in the plant kingdom. They are present in monocotyledons, dicotyledons, and green algae. Fructans differ in molecular structure and in molecular weight. They may be classified into three main types: the inulin type, the levan (previously called phlein) type, and the graminan type (2). The inulin group consists of material that has mostly or exclusively the (2→1) fructosyl-fructose linkage. Levan is material that contains mostly or exclusively the (2→6) fructosyl-fructose linkage. The graminan (or branched) type has both (2→1) and (2→6) fructosyl-fructose linkages in significant amounts (e.g., graminan from Gramineae). The distribution of fructans in nature, and the production of fructooligosaccharides, such as neosugar, using fructosyltransferase, has been reviewed in a monograph (3). In the context of this article and the analytical procedure described, the term *fructan* relates only to inulin and graminan. The current analytical procedure has not been evaluated on levan.

In recent years, interest in the measurement of fructans such as inulin and oligofructose has been stimulated by applications made to regulatory authorities for acceptance of fructan as a dietary fiber component for food labeling purposes. Like soluble dietary fiber, fructans are not metabolized in the human upper digestive tract but are fermented in the large bowel. Since fructans are largely soluble in 80%

ethanol, they are not significantly measured by present dietary fiber methods. However, if fructans can be specifically measured, accurately and precisely, then these components can be added to total and soluble dietary fiber values. Of course, this would require that other dietary fiber analyses not include any of the high molecular weight fractions of fructan. The analysis of fructans is also of interest to food technologists, who wish to exploit the unique textural properties of inulin solutions (4), and of wheat, oat, and barley physiologists, who measure fructan content as a means of estimating the energy reserves of growing crops (5).

It is generally accepted that the most quantitative methods for measurement of fructans involve enzymatic hydrolysis of all fructan materials to fructose and glucose, followed by measurement of these sugars. A prime requirement is that sucrose, fructose, and glucose be independently removed, allowed for, or measured. Sucrose is rapidly hydrolyzed by enzymes used to hydrolyze fructan. A method for the removal of glucose, fructose, and sucrose was proposed by Pontis (6). Sucrose was hydrolyzed to glucose and fructose with a crystalline yeast invertase, and these sugars, as well as other monosaccharides in the extract, were destroyed by boiling with sodium hydroxide. The major limitation of this proposal is that yeast invertase enzymes also rapidly hydrolyze fructooligosaccharides that have a low degree of polymerization (DP) (Fig. 1). An alternative approach introduced by Quemener and coworkers (7) involves the use of capillary gas chromatography or high-performance liquid chromatography to analyze extracts of samples, either untreated or treated with amyloglucosidase or amyloglucosidase plus inulinase (fructanase). By measuring sucrose, fructose, and glucose in the various samples, and using appropriate calculations, it is possible to determine free glucose and fructose, sucrose, starch, and fructan.

The method described in this article measures fructan after removal of starch, sucrose, glucose, and fructose. Starch is hydrolyzed to glucose with a mixture of high-purity starch-degrading enzymes, and a highly purified sucrase enzyme is used to hydrolyze sucrose to glucose and fructose.

All of the reducing sugars in the sample are then removed by reduction to the sugar alcohols with sodium borohydride. Inulin and fructooligosaccharides are then hydrolyzed to glucose and fructose with a mixture of highly purified *exo*- and *endo*-inulinases. These reducing sugars are measured with the *p*-hydroxybenzoic acid hydroxide (PAHBAH) reducing-sugar method (8), previously shown to give an equal color yield with glucose and fructose (9).

MEASUREMENT OF ENZYME ACTIVITY

Units of Activity

Pure *exo*- and *endo*-inulinases, pullulanase, β -amylase, maltase, and sucrase were obtained from Megazyme International Ireland Ltd. (Bray, County Wicklow, Ireland). Pullulanase was purified from Promozym (*Bacillus acidopullulyticus*, Novo Nordisk, Bagsvaerd, Denmark) by ion-exchange chromatography and affinity chromatography on β -cyclodextrin-Sepharose 4B columns. β -Amylase from barley extracts was purified by chromatography on diethylaminoethyl-Sepharose followed by crystallization and recrystallization from ammonium sulfate (α -amylase contamination <1 in 10^6). Inulinases (both *endo*- and *exo*-) were purified from the commercial enzyme preparation Fructozyme (*A. niger*; Novo Nordisk), using a combination of anion and cation exchange resins, gel filtration, and hydrophobic chromatography (10).

Suitably diluted and preequilibrated enzyme preparation (0.2 ml) was added to 0.2 ml of preequilibrated substrate (kestose for *exo*-inulinase; fructan for *endo*-inulinase) solution (10 mg/ml) in 100 mM sodium acetate buffer (pH 4.5), and the mixture was incubated at 40°C. Reaction was terminated at 0, 5, 10, and 15 min by the addition of 0.5 ml of Nelson/Somogyi solution D, and the color was developed by the standard procedure (11). In crude enzyme mixtures, *endo*-inulinase was specifically measured with azo-fructan by reference to an *endo*-fructanase/azo-fructan standard curve (12). One unit of inulinase activity is defined as the amount of enzyme required to release 1 μ mol of fructose reducing sugar equivalents from kestose

(*exo*-inulinase) or fructan (*endo*-inulinase) in 1 min at pH 4.5 and 40°C.

β -Amylase was assayed by the method described by McCleary and Codd (13) (using *p*-nitrophenyl maltopentaose), and pullulanase was assayed by the method described by McCleary (14), using tablets containing dyed and cross-linked pullulan. In both cases, activity was converted through a regression equation or a standard curve to units of activity on the native substrate. One unit of β -amylase is the amount

of enzyme required to release 1 μ mol of *p*-nitrophenol from *p*-nitrophenyl maltopentaose in 1 min under standard assay conditions (pH 6.2, 40°C). One unit of pullulanase is the amount of enzyme required to release 1 μ mol of glucose reducing sugar equivalents from pullulan (10 mg/ml) under standard assay conditions (pH 5.0, 40°C). Maltase was assayed on maltose (10 mg/ml) at pH 6.5 and 40°C, and sucrase was assayed on sucrose (10 mg/ml) at pH 6.5 and 40°C. In both cases, the

released glucose was measured with a glucose oxidase/peroxidase/4-amino antipyrine reagent.

One unit of sucrase is the amount of enzyme required to release 1 μ mol of glucose from sucrose (10 mg/ml) under standard assay conditions (pH 6.5, 40°C). One unit of maltase is the amount of enzyme required to release 2 μ mol of glucose from maltose (10 mg/ml) under standard assay conditions (pH 6.5, 40°C). Hydrolysis of 1 μ mol of maltose releases 2 μ mol of glucose.

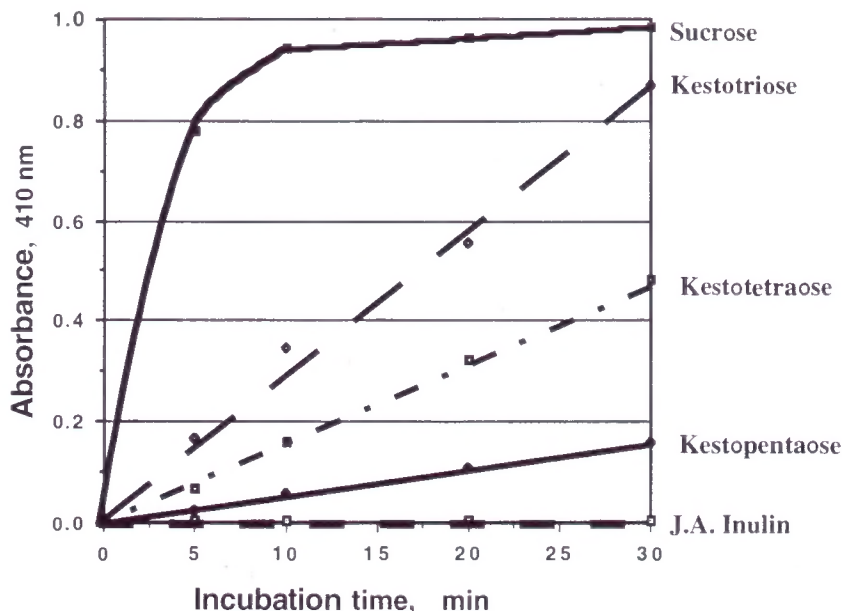


Fig. 1. Hydrolysis of sucrose and fructans by yeast invertase. Sugar compound (0.2 ml, 50 μ g) was incubated with invertase (2 U) in sodium acetate buffer (100 mM, pH 4.5) at 40°C. The reaction was terminated at various time intervals with *p*-hydroxybenzoic acid hydrazide working reagent, and color developed. Samples are Jerusalem artichoke inulin (JA inulin); 1-kestose; 1,1-kestotetraose; 1,1,1-kestopentaose; and sucrose.

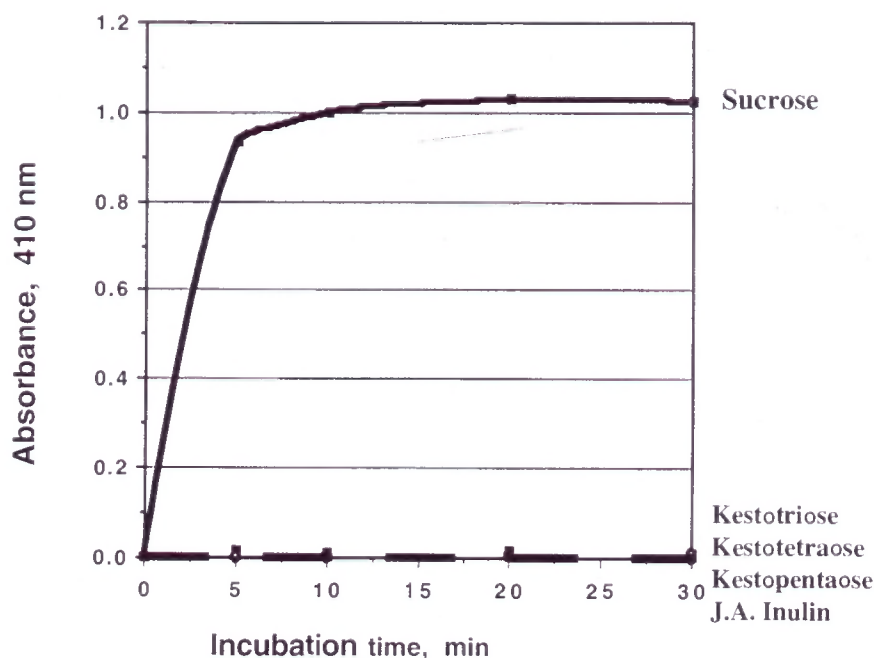


Fig. 2. Hydrolysis of sucrose and fructans by sucrase. Sugar compound (0.2 ml, 50 μ g) was incubated with sucrase (1 U) in sodium maleate buffer (100 mM, pH 6.5) at 40°C. The reaction was terminated at various time intervals with *p*-hydroxybenzoic acid hydrazide working reagent, and color developed. J. A. = Jerusalem artichoke.

Hydrolysis Reactions

Yeast Invertase or Sucrase. Hydrolysis of sucrose and fructooligosaccharides by yeast invertase or by sucrase was determined as follows. A sugar compound (0.2 ml, 50 μ g) was incubated at 40°C with either 1) invertase (2 U) in sodium acetate buffer (100 mM, pH 4.5) or 2) sucrase (1 U) in sodium maleate buffer (100 mM, pH 6.5). Reaction was terminated at various time intervals with PAHBAH working reagent (5) (5.0 ml), and the color was developed. Substrates employed were Jerusalem artichoke inulin, 1-kestose, 1,1-kestotetraose, 1,1,1-kestopentaose, and sucrose.

Hydrolysis by yeast invertase and sucrase of sucrose, fructooligosaccharides, and the high molecular weight fructan fraction of Jerusalem artichoke is shown in Figures 1 and 2. Figure 1 shows that, under conditions required to give complete hydrolysis of sucrose by invertase, 85% of kestose, 50% of kestotetraose, and almost 20% of kestopentaose is hydrolyzed. High molecular weight fructan is not hydrolyzed, consistent with the results of Pontis (6). Since fructans usually contain high levels of the tri-, tetra-, and pentasaccharides, invertase obviously cannot be used to selectively remove sucrose. In contrast, a sucrose-degrading enzyme (sucrase) identified by us, gives complete hydrolysis of sucrose to glucose plus fructose with no detectable hydrolysis of even kestose. The ratio of hydrolysis of sucrose to hydrolysis of kestose (under optimal assay conditions (i.e., pH 6.5 and 40°C, at a substrate concentration of 10 mg/ml) was 3,800:1.

***exo*-Inulinase.** Hydrolysis of fructans by *exo*-inulinase was determined by incubating fructan (5 g) in 100 ml of sodium acetate buffer (10 mM, pH 4.5) with 4,000 U of *exo*-inulinase at 40°C. Aliquots (20 ml) were removed at 0, 5, 20, and 60 min after addition of the enzyme and incubated at 100°C for 5 min to terminate the reaction. Aliquots (10 ml) were fractionated on a column (95 \times 5 cm) of Bio-Gel P-2 (<400 mesh) at 60°C and the eluate analyzed by the phenol/sulfuric acid method. Reaction samples (10 μ l) were subjected to thin-layer chromatography (TLC) (*n*-propanol-ethanol-water, 7:1:2). The TLC plates were developed once, and spots were visualized by spraying the plates with

5% sulfuric acid in methanol followed by charring in an oven at 120°C (~5 min). Aliquots of the diluted samples were analyzed for reducing sugar level (with PAHBAH) and total carbohydrate (with the phenol/sulfuric acid method) (15), and percentage hydrolysis values were calculated.

endo-Inulinase. Hydrolysis of fructan by *endo*-inulinase was measured as follows. Fructan (1 g) in 50 ml of 100 mM sodium acetate buffer (pH 4.5) was incubated with 50 U of *endo*-inulinase (0.2 ml, 250 U/ml) at 40°C. Aliquots (5 ml) were taken at a range of time intervals up to 1,000 min, and *endo*-inulinase was inactivated by incubation at 100°C for 5 min. Aliquots (10 µl) were subjected to TLC (*n*-propanol-ethanol-water; 7:1:2). The TLC plates were developed once, and spots were visualized by spraying the plates with 5% sulfuric acid in methanol followed by charring in an oven at 120°C (~5 min). Aliquots of the diluted samples were analyzed for reducing sugar level (by the PAHBAH method) and total carbohydrate (by the phenol/sulfuric acid method), and percentage hydrolysis values were calculated.

FRUCTAN ASSAY PROCEDURE

Reagents

Sucrase/Amylase and Fructanase Preparations. One vial of sucrase/amylase preparation contains sucrase (50 U), β -amylase (barley, 500 U), pullulanase (*Bacillus acidopullulyticus*, 100 U), and maltase (yeast, 1,000 U) (fructan assay kit, Megazyme). This is dissolved in 22 ml of 10 mM sodium maleate buffer (pH 6.5). On dissolution, this solution should be stored frozen between uses.

Since starch and maltosaccharides are unstable in the highly alkaline conditions used in the PAHBAH reducing sugar method, it is essential to remove these from the sample extract by hydrolysis to glucose by the combined action of pullulanase, β -amylase, and maltase, followed by borohydride reduction. The level of each enzyme used is 10 times that required for complete hydrolysis. This combination of enzymes was chosen to allow starch hydrolysis to be performed concurrently with hydrolysis of sucrose by sucrase (i.e., at pH 6.5 and 40°C).

One vial of fructanase preparation from the kit, containing 8,000 U of *exo*-inulinase and 800 U of *endo*-inulinase, is dissolved in 22 ml of 100 mM sodium acetate buffer (pH 4.5). On dissolution, this solution should be stored frozen between uses.

PAHBAH Reducing Sugar Determination Reagent. To prepare solution 1, add 10 g of *p*-hydroxybenzoic acid hydrazide (cat. no. H-9882, Sigma Chemical Co., St. Louis, MO) to 60 ml of water in a 250-ml beaker on a magnetic stirrer. Stir the slurry and add 10 ml of concentrated hydrochloric acid. Adjust the vol-

ume to 200 ml with distilled water. Store at room temperature (stable for two years).

To prepare solution 2, add 24.9 g of trisodium citrate to 500 ml of distilled water and stir to dissolve. Add 2.2 g of calcium chloride dihydrate and dissolve by stirring. Then add 40.0 g of sodium hydroxide and dissolve with stirring. (The solution may be milky, but it will clarify on dilution.) Adjust the volume to 2 L. This solution is stable for at least two years at room temperature.

Immediately before use, make the PAHBAH working reagent by adding 20 ml of solution 1 to 180 ml of solution 2 and mixing thoroughly. The solution is stored on ice and is stable for about 4 hr.

Alkaline borohydride solution. Approximately 50 mg of sodium borohydride (Sigma, cat. no. S-9125) is added into polypropylene containers (10-ml volume with screw cap). Record the exact weight on the tubes (approximately 10 for convenience); seal the tubes and store them in a desiccator for future use. Immediately before use, dissolve the sodium borohydride (at 10 mg/ml) in 50 mM sodium hydroxide solution. This solution is stable for 4–5 hr at room temperature.

Test Samples, Standards, and Controls

Test Samples. Dry food samples (~50 g) are ground to pass a 0.5-mm sieve. All material is transferred into a wide-mouthed plastic jar and mixed well by shaking and inversion. Solid moist samples (such as chocolate) are warmed to room temperature and are then reduced in size with a cheese grater. A representative sample is taken for analysis. Soft, very moist food samples (such as low-fat spreads) are warmed to liquidize the sample. The material is stirred vigorously with a spatula, and a representative sample is taken. Liquid or semiliquid samples (such as juices or yogurt), are first adjusted to pH 6.5 before heating. Samples are directly diluted in sodium maleate buffer (100 mM, pH 6.5). All samples must be warmed to room temperature before weighing.

Standards and Controls. Fructose working standard solution (in quadruplicate), reagent blank (in duplicate), fructan control flour, and sucrose control powder are analyzed with each of set of samples. Fructose standard stock solution (0.2 ml, 1.5 mg/ml) is added to 0.9 ml of acetate buffer (100 mM, pH 4.5) and mixed thoroughly. Aliquots of this fructose standard working solution (0.2 ml, containing 54.5 mg of fructose) are dispensed in quadruplicate to the bottoms of four glass test tubes. An aliquot (0.1 ml) of acetate buffer (100 mM, pH 4.5) is added to each tube. Immediately before incubation in the boiling water bath, 5.0 ml of PAHBAH working reagent is added to each tube.

The fructan control flour is prepared by adding α -cellulose to a solution of high-DP

fructan, mixing thoroughly, and then lyophilizing and milling (to pass a 0.5-mm screen).

The reagent blank is prepared by adding an aliquot (0.3 ml) of acetate buffer to two test tubes, followed by PAHBAH working reagent (5.0 ml), and the assay is performed as for the samples. This is used to zero the spectrophotometer.

Samples (1.0 g) of the sucrose control powder (containing approximately 20% sucrose) mixture are analyzed by the same procedure as used for samples containing 0–12% fructan. The sample contains no fructan and is used to check the effectiveness of the sucrase and borohydride treatments. The calculated fructan content should be no more than 0.3%.

If sucrose is not completely hydrolyzed by the sucrase treatment, it will then be hydrolyzed by the fructanase mixture and give erroneously high fructan values. Indicative controls such as soluble starch and α -cellulose are occasionally used. The absorbance from α -cellulose should be negligible, i.e., <0.001, and for soluble starch, the absorbance should be very low, i.e., <0.003. This result for starch demonstrates the effectiveness of the borohydride reduction step.

Procedures

Extraction of Fructan. For samples containing 0–12% fructan, the sample (1.0 g) is accurately weighed into a dry Pyrex beaker (200-ml capacity). Hot distilled water (80 ml, ~80°C) is added, and the beaker and contents are stirred and heated on a hot-plate magnetic stirrer (at ~80°C) for approximately 15 min (i.e., until the sample is completely dispersed). In this step, it is important that the pH of the solution/slurry be above pH 5.5, otherwise the fructan may be partially depolymerized. The solution is cooled to room temperature and then quantitatively transferred to a 100-ml volumetric flask. The volume is adjusted to the mark with distilled water, and the contents are mixed thoroughly. An aliquot of the solution is filtered and analyzed immediately. The filtrate may be slightly turbid, depending on the nature of the sample extracted. If this filtrate is stored for several hours at low temperature before analysis, the fructan may tend to precipitate from solution. In such cases, the solution should be reheated to ~80°C and allowed to cool to room temperature before samples are removed for analysis.

For samples containing 12–50% fructan, a finely ground test sample (90–100 mg) is accurately weighed directly into a dry Pyrex beaker (100-ml capacity). Hot distilled water (40 ml, ~80°C) is added, and the beaker and contents are stirred and heated (~80°C) on a hot-plate magnetic stirrer for ~15 min (i.e., until the sample is completely dispersed). Again, it is important that the pH of the solution/slurry be above pH 5.5. The solution is cooled to

room temperature and then quantitatively transferred to a 50-ml volumetric flask, which is adjusted to volume with distilled water. The contents are mixed thoroughly. (For samples containing 50–100% fructan, the solution is transferred to a 100-ml volumetric flask and adjusted to the mark). An aliquot of this solution is filtered and analyzed immediately.

Removal of Sucrose, Starch, and

Reducing Sugars. Aliquots (0.2 ml) of the filtrate to be analyzed (containing fructan at approximately ~0.1–1.0 mg/ml), or of the control, are transferred to the bottoms of two glass test tubes. Sucrase/amylase solution (0.2 ml) is added to each tube, and the tubes are incubated at 40°C for 30 min. Alkaline borohydride solution (0.2 ml) is added to each tube. The tubes are vigorously stirred and then incubated at 40°C

for 30 min to effect the complete reduction of reducing sugars to sugar alcohols. An aliquot (0.5 ml) of acetic acid (100 mM) is added to each tube with vigorous stirring on a vortex mixer. If the borohydride is fresh, a vigorous effervescence should be observed. If an effervescence is not observed, the analysis should be repeated with fresh borohydride. (This treatment removes excess borohydride and adjusts the pH to ~4.5). This is termed Solution A. The optimal conditions for complete borohydride reduction and subsequent removal of borohydride and pH adjustment to about pH 4.5 were determined empirically, and the optimal conditions were chosen.

Hydrolysis and Measurement of Fructan. Aliquots (0.2 ml) of Solution A are accurately and carefully transferred (in duplicate) to the bottoms of glass test tubes. Fructanase solution (0.1 ml) is added to each test tube; the tubes are stirred on a vortex stirrer and incubated at 40°C for 20 min to effect the complete hydrolysis of fructan to fructose and glucose. The contents of all tubes (including the fructose standard working solution, the reagent blank, and the extracts of both the fructan control sample and the sucrose control sample) are treated with PAHBAH working reagent (5.0 ml) and incubated in a boiling-water bath for exactly 6 min. All of the tubes are placed in the water bath and are removed from the bath at the same

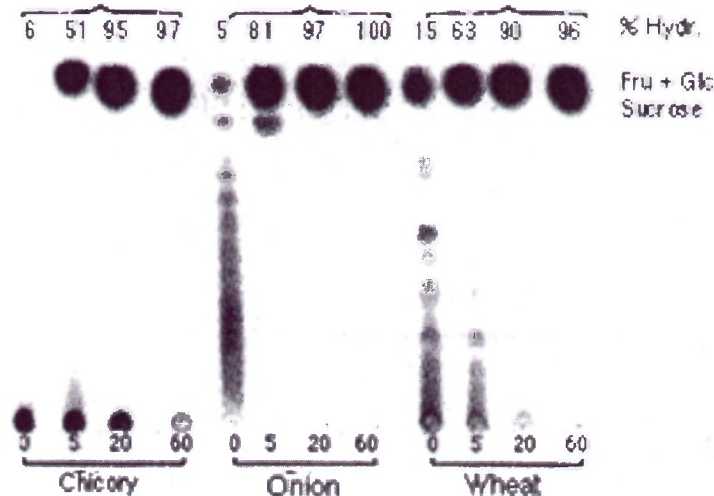


Fig. 3. Thin-layer chromatography (TLC) of the sugars produced on hydrolysis of chicory (high degree-of-polymerization fraction), onion, and wheat fructans by *exo*-inulinase (conditions as described in the text). TLC. plates were developed once with *n*-propanol-ethanol-water (7:1:2).

time. On removal from the bath, the rack of tubes is immediately placed in cold water (18–20°C) for about 5 min. The absorbance of each solution is measured at 410 nm against the reagent blank. Absorbance values should be measured as soon as possible after cooling the tubes. The PAHBAH color complex changes with time.

The total fructan content (% on as-is basis) in test samples is calculated as follows:

Total fructan, % =

$$A \times F \times 5 \times V \times \frac{1.1}{0.2} \times \frac{100}{W} \times \frac{1}{1,000} \times \frac{162}{180}$$

where:

A = PAHBAH absorbance of reaction solutions (0.2 ml) read against the reagent blank

F = factor to convert absorbance values to micrograms of fructose (= 54.5 µg fructose/absorbance value for 54.5 µg fructose)

5 = factor to convert from 0.2 ml, as assayed, to 1.0 ml

V = volume (ml) of extractant used (i.e., 100 or 50 ml)

1.1/0.2 = factor showing that 0.2 ml was taken from 1.1 ml of enzyme digest for analysis

W = weight (mg) of sample extracted

100/W = factor to express fructan as a percentage of flour weight

1/1,000 = factor to convert from micrograms to milligrams

162/180 = factor to convert from free fructose, as determined, to anhydrofructose and anhydroglucose (as occurs in fructan).

MEASUREMENT OF INULIN AND FRUCTOOLIGOSACCHARIDES

Action of Inulin-Degrading Enzymes

In the development of the current procedure for the assay of inulin and fructooligosaccharides, two inulin-degrading

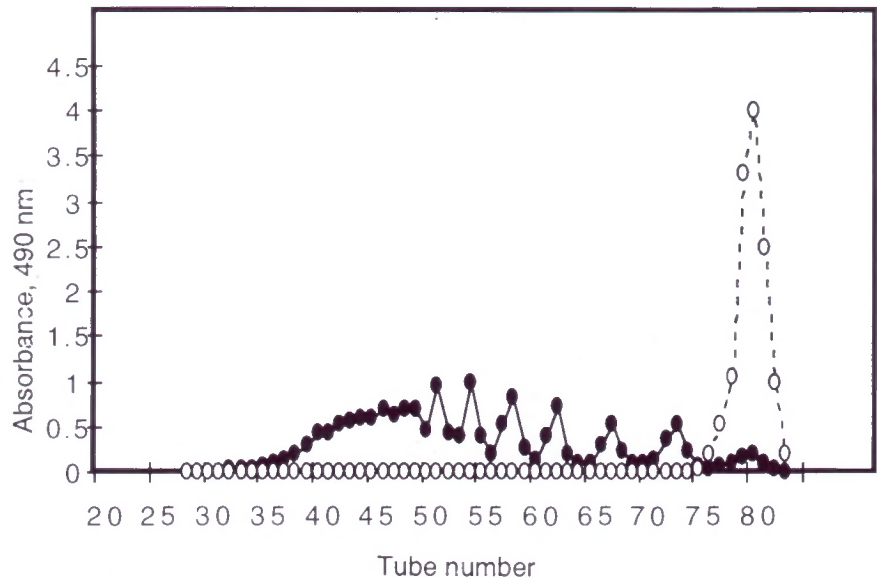


Fig. 4. Bio-Gel P-2 chromatography of the sugars produced on hydrolysis of onion fructan by *exo*-inulinase (incubation conditions as described in the text). Column eluates were analyzed by the phenol-sulfuric acid procedure. Incubated for 0 min (●) and 60 min (○).

enzymes were purified, *exo*-inulinase and *endo*-inulinase. The action of *exo*-inulinase on fructans is shown in Figures 3 and 4. Thin-layer chromatographic patterns of the product released on hydrolysis of onion, wheat, and high-DP chicory fructans by *exo*-inulinase are shown in Figure 3.

The high molecular weight fraction from inulin was a gift from Raffinerie Tirlemontoise S.A. Tienen, Belgium. The onion fructan (moderately branched fructooligosaccharides) and wheat stem fructan (highly branched fructooligosaccharides) were extracted from the appropriate source with hot water, decolorized with activated charcoal, and freeze-dried. Other fructan-containing materials used in the development of the current procedure were obtained commercially.

The only reaction products were monosaccharides (fructose and glucose). Wheat and chicory fructan were hydrolyzed less rapidly than onion fructan. The lower rate of hydrolysis of the chicory fructan is thought to be due to the high DP of this substrate and thus the lower concentration of nonreducing end groups available for reaction. The slower rate of hydrolysis of the wheat fructan is probably due to the highly branched nature of this mixture of oligosaccharides. The Bio-Gel P-2 chromatographic patterns of the onion fructooligosaccharide mixture before and after incubation with *exo*-inulinase (Fig. 4) demonstrate that, with the level of enzyme used, hydrolysis to fructose and glucose is complete.

Incubation of chicory fructan with *endo*-

fructanase produced an accumulation of oligosaccharides of DP 4, 5, and 6 (Fig. 5). Very extended incubations with this enzyme resulted in near-complete hydrolysis of these oligosaccharides to fructose, glucose, and sucrose. The reason for such extensive hydrolysis is not clear. Most *endo*-polysaccharases release little monosaccharide, even on extended incubation with the substrate (16).

Sodium dodecyl sulfate-gel electrophoresis and isoelectric focusing patterns of *exo*- and *endo*-inulinase enzymes are shown in Figures 6 and 7. These patterns indicate that there were good separations of *exo*- and *endo*-inulinases by the chromatographic procedures used. Each enzyme appeared as a single major band on SDS-gel electrophoresis but as multiple bands with a narrow pI range on isoelectric focusing. Some properties of the *exo*- and *endo*-inulinases purified and used in these studies are shown in Table I.

The effect of concentration of *exo*-inulinase, and the presence or absence of *endo*-inulinase, on the determined fructan content of three materials is shown in Table II. For quantitative measurement of fructan in all of these materials, an enzyme level of at least 18 U per assay is required. At low levels of *exo*-inulinase, the addition of *endo*-inulinase resulted in more effective hydrolysis (and thus measurement) of high-DP chicory fructan. This increase was minimal and much lower than expected.

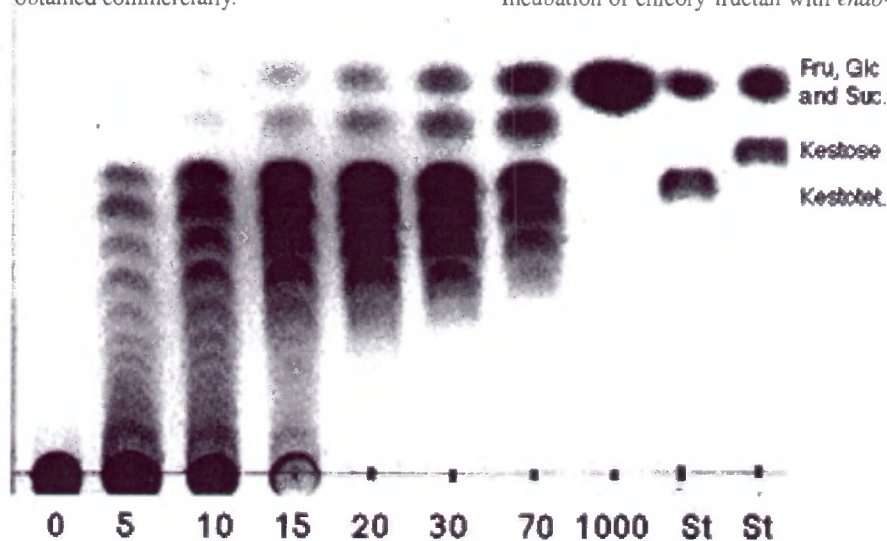


Fig. 5. Thin-layer chromatography (TLC) of the sugars produced on hydrolysis of high degree-of-polymerization chicory inulin by *endo*-inulinase (conditions as described in the text). TLC plates were developed once with *n*-propanol-ethanol-water (7:1:2).

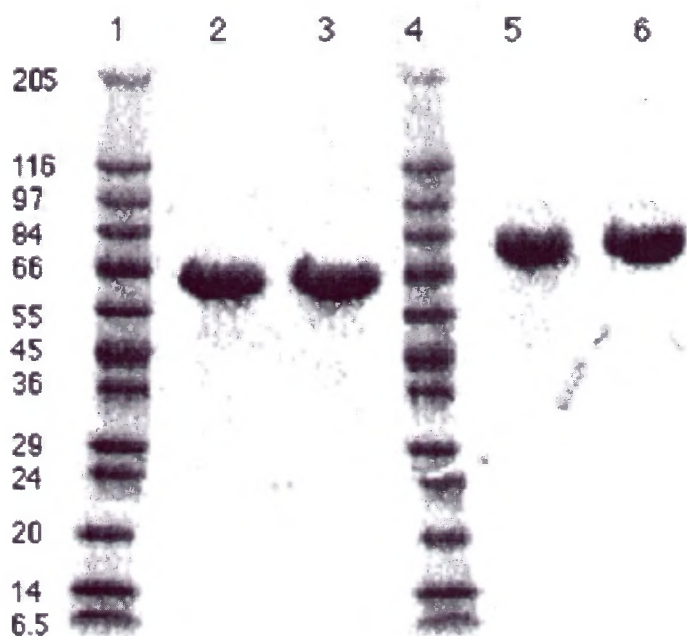


Fig. 6. Sodium dodecyl sulfate gel electrophoresis of purified *exo*- and *endo*-inulinases. Electrophoresis was in precast gradient gels (4–15% Tris-HCl). Lanes 1 and 4, molecular weight standards (wide molecular weight range); lanes 2 and 3, *endo*-inulinase; lanes 5 and 6, *exo*-inulinase.

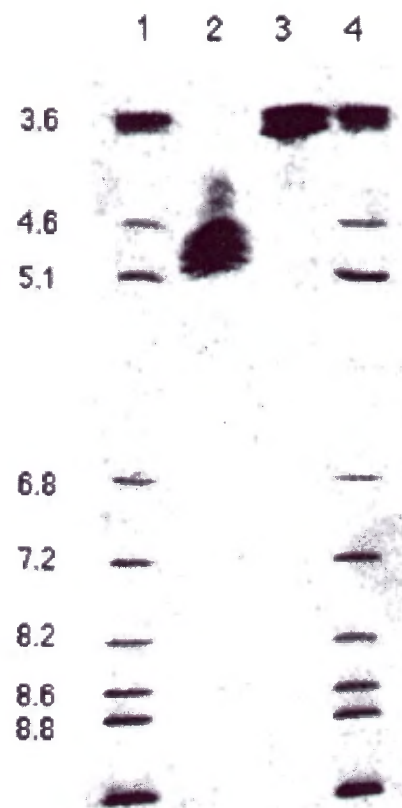


Fig. 7. Isoelectric focusing of purified *exo*- and *endo*-inulinases. Lanes 1 and 4, pI standards; lane 2, purified *exo*-inulinase; lane 3, purified *endo*-inulinase.

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18. AOAC INTERNATIONAL. Methods



The Authors

Barry McCleary is cofounder and technical director of Megazyme International Ireland Ltd. He received his Ph.D. degree in 1975 from the University of Sydney and his D.Sc. Agr. degree in 1989. Before founding Megazyme, he was principal research scientist with the New South Wales Department of Agriculture, N. S. W., Australia.



Tony Blakeney is head of Grain Processing and Nutrition at BRI Australia Ltd. in North Ryde, Australia. Before joining BRI in 1997, he worked with the Australian Rice Breeding Programme for over 25 years, selecting all the current varieties for quality. Blakeney is cochair of the AACC Rice Milling and Quality Technical Committee, is a past chairman of the Royal Australian Chemical Institute Cereal Chemistry Division, and was awarded the the F. B. Guthrie Medal in 1991 by that Institute. He has had a long-term interest in carbohydrate chemistry and analysis and is a current member of the AACC Approved Methods Committee.

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