FRUCTAN

ASSAY PROTOCOL

K-FRUC

11/22

(with recombinant inulinases and levanase)

100 Assays per Kit

AOAC Method 999.03
AACC Method 32-32.01
Codex Type III Method

INTRODUCTION:

Fructans are defined as any compound where one or more fructosyl- fructose linkage constitutes a majority of the linkages.[1] This refers to polymeric material as well as oligomers as small as the disaccharide, inulobiose. Material included in this definition may or may not contain D-glucosyl substituents. The terms oligomer and polymer are used by fructan researchers to distinguish between materials which can be specifically characterised and those which cannot.[1] Fructans are widely distributed in the plant kingdom. They are present in monocotyledons, dicotyledons and in green algae. Fructans differ in molecular structure and in molecular weight. They may be classified into three main types: the inulin group, the levan group and the branched group. The inulin group consists of material that has mostly or exclusively the (2→1) fructosyl-fructose linkage. Levan is material which contains mostly or exclusively the (2→6) fructosyl-fructose linkage. The branched group has both (2→1) and (2→6) fructosyl-fructose linkages in significant amounts (*e.g.* graminan from *Gramineae*).

Several procedures have been described for the measurement of fructan in plant material and food products, but it is generally accepted that they are best measured after hydrolysis to D-fructose and D-glucose. This introduces the challenge of independently removing, or measuring, sucrose, D-fructose and D-glucose. Pontis (1966)[2] hydrolysed sucrose with crystalline yeast invertase and destroyed the resulting D-glucose and D-fructose as well as existing monosaccharides by boiling with sodium hydroxide. However, yeast invertase also hydrolyses lower degree of polymerisation (DP) fructo-oligosaccharides (FOS). At a concentration of 10 mg/mL, 1-kestose is hydrolysed at approx. 20% the rate of sucrose and 1,1-kestotetraose is hydrolysed at approx. 10% the rate of sucrose.[3]

In a procedure for fructan determination introduced by McCleary and Blakeney (1999), sucrose in the sample is hydrolysed with a specific sucrase and the released D-glucose and D-fructose as well as all other reducing sugars in the sample are removed by borohydride reduction to the corresponding sugar alcohols (which are not measured with PAHBAH reagent). Inulin-type and branched-type fructans are then hydrolysed to D-glucose and D-fructose with highly purified *endo*- and *exo*-inulinases. Subsequently, ultra-pure *exo*- and *endo*-inulinases were produced recombinantly and introduced into the method. Use of these enzymes removed problems of partial hydrolysis of β-gluco- oligosaccharides by the trace levels of β-glucosidase and present in the non-recombinant enzymes. In the most recent development, a recombinant *endo*-levanase has been incorporated into the fructanase mixture, extending the use of the method to the measurement of levan-type fructans as are present in grasses such as timothy, cocksfoot, ryegrass and red fescue. In the absence of the *endo*-levanase, levan-type fructans are underestimated (Table 1, page 10).[4,5]

The method described in this booklet employs ultra-pure, recombinant enzymes and specifically measures fructans including inulin-type fructans from chicory, dahlia, jerusalem artichoke; highly branched fructans from onion and wheat stems and leaves; and levan-type fructans from pasture grasses such as timothy grass. Commercial, partially hydrolysed frucan products such as Raftilose P-95® are underestimated by this method (approx. 20%) to an extent related to the degree of hydrolysis of the fructan. The actual degree of underestimation is readily determined analytically if samples of the fructan/FOS ingredient

are available (see Appendix A). Agave fructan is measured quantitatively. Commercial *Agave* fructan preparations contain up to 20% of fructose, glucose and sucrose (which is not fructan and thus is not measured).

PRINCIPLE:

Sucrose is hydrolysed by a specific sucrase enzyme which has no action on lower degree of polymerisation (DP) FOS such as 1-kestose and 1,1-kestotetraose.[3] Starch and maltodextrins are hydrolysed to maltose and maltotriose by pullulanase and β-amylase, and these oligosaccharides are then hydrolysed to D-glucose by maltase (1 & 2). Since sucrase is more stable at 30°C, this is now the recommended temperature for this incubation step.

(1) Sucrose + H_2O $\xrightarrow{\text{(sucrase, pH 6.5, 30°C)}}$ D-glucose + D-fructose

(2) Starch + maltosaccharides $\xrightarrow{\text{(pullulanase, β-amylase, maltase, pH 6.5, 30°C)}}$ D-glucose

D-Glucose and D-fructose are reduced by sodium borohydride to the corresponding sugar alcohols, D-sorbitol and D-mannitol. In this reaction, the D-fructosyl residue at the reducing end of fructo- oligosaccharides in hydrolysed inulin preparations, is also reduced to the sugar alcohol. Native fructans and non-reducing FOS such as Neosugars® are not affected by this reaction (3).

(3) D-Glucose + D-fructose $\xrightarrow{\text{(sodium borohydride, 40°C)}}$ D-sorbitol + D-mannitol + reduced fructo-oligosaccharides

FOS, fructans and borohydride reduced FOS are specifically hydrolysed by *exo*- and *endo*-inulinase and *endo*-levanase to D-glucose and D-fructose (4).

(4) Fructan + H_2O $\xrightarrow{\text{(}exo\text{-, and }endo\text{-inulinases and }endo\text{-levanase, pH 4.5, 40°C)}}$ D-glucose + D-fructose

D-Fructose and D-glucose derived from fructan is measured using the PAHBAH reducing sugar method.[6] This method is simple to use and the colour response for D-fructose and D-glucose is the same (5).

(5) D-Glucose + D-fructose $\xrightarrow{\text{(PAHBAH reagent, 100°C, 6 min)}}$ PAHBAH colour complex

SPECIFICITY, SENSITIVITY, LINEARITY AND PRECISION:

This assay was the subject of a successful interlaboratory evaluation under the auspices of AOAC International and AACC International.[7] The assay is specific for all types of fructan, including; those containing mostly or exclusively (2→1) fructosyl-fructose linkages (inulin); those with both (2→1) and (2→6) fructosyl-fructose linkages in significant amounts (e.g. those from onion, *Gramineae* and *Agave*); and those containing mainly, or exclusively (2-6)

fructosyl-fructose linkages (levan). Borohydride reduced FOS are hydrolysed releasing D-mannitol from the "reducing end" terminus. Trace levels of fructan in cereal grain can be measured with no contribution to reducing sugar level by partial hydrolysis of mixed-linkage β-glucan. If the sample is likely to contain galactosyl-sucrose oligosaccharides (e.g. raffinose), these should be removed by hydrolysis with α-galactosidase (see page 7).

The smallest differentiating absorbance for the assay is 0.010 absorbance units. This corresponds to 12.8 µg/mL of D-glucose plus D-fructose in the sample extract being analysed. The detection limit is 25.6 µg of D-fructose and/or D-glucose per mL of sample extract, which is derived from an absorbance difference in the assay of 0.02.

The assay is linear over the range of 2.3 to 55 µg of D-fructose or D-glucose per assay. In duplicate determinations using one sample solution, an absorbance difference of 0.005 to 0.010 may occur, this corresponds to a D-fructose or D-glucose level of 0.023-0.046 µg per assay.

INTERFERENCE:

Interfering substances in the sample being analysed can be identified by including an inulin or levan internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding inulin or levan to the sample in the initial extraction steps.

SAFETY:

The reagents used in the determination of fructan are not hazardous materials in the sense of Hazardous Substances Regulation. The general safety measures that apply to chemical substances should be adhered to.

KIT CONTENTS:

Kits suitable for performing 100 assays are available from Megazyme. The kits contain the full assay method plus:

Bottle 1. **Sucrase plus β-amylase, pullulanase and maltase** as a freeze- dried powder.
Store below -10°C. See individual label for expiry date.

Bottle 2. (x2) **Fructanase.** Recombinant *exo*- and *endo*-inulinases and recombinant *endo*-levanase as a freeze-dried powder.
Store below -10°C. See individual label for expiry date.

Bottle 3. **Inulin Control Flour.** Inulin freeze-dried in the presence of α-cellulose.
Store at room temperature. See individual label for expiry date.

Bottle 4. **Levan Control Flour.** Timothy grass levan freeze-dried in the presence of α-cellulose.
Store at room temperature. See individual label for expiry date.

Bottle 5. **Sucrose Control Flour.** Sucrose freeze-dried in the presence of α-cellulose.
Store at room temperature. See individual label for expiry date.

Bottle 6. **D-Fructose Standard Solution** (1.5 mg/mL) in 0.2% (w/v) benzoic acid.
Store at room temperature. See individual label for expiry date.

PREPARATION OF ENZYMES:

1. Dissolve the contents of bottle 1 (Sucrase and β-amylase etc) in 22 mL of Buffer 1 [sodium maleate (100 mM, pH 6.5 plus Bovine serum albumin (0.5 mg/mL)] **(Enzyme Solution A)**. Divide into aliquots of appropriate volume and store in polypropylene tubes.
Stable for ≤ 2 years below -10°C.

2. Dissolve the content of **one** of bottle 2 (Fructanase) in 11 mL of **Buffer 2** [sodium acetate (100 mM, pH 4.5]. This is **Enzyme Solution B**. Divide into aliquots of appropriate volume and store in polypropylene tubes. Stable for 4 months below -10°C. **Do not dissolve the remaining bottle 2 (Fructanase) until it is required.**

NOTE: The volume required to re-suspend bottle 2 (Fructanase) differs from that stated in the AOAC methods (999.03, 2016.14, 2018.07) but the concentration of the resulting **Enzyme solution B** remains the same. Therefore it can be used as normal in the remaining steps of these methods.

3, 4, 5 & 6 Use the contents of bottles 3, 4, 5 and 6 as supplied.
Stable for > 5 years at room temperature.

BUFFERS (not supplied):

Buffer 1: **Sodium maleate buffer** (100 mM, pH 6.5)
Dissolve maleic acid (11.6 g, Sigma cat. no. M0375) in 900 mL of distilled water and adjust the pH to 6.5 with sodium hydroxide solution (2 M). Adjust volume to 1 L.

To 100 mL of this buffer add 50 mg of Bovine serum albumin to give a concentration of 0.5 mg/mL. This buffer is used to dissolve the contents of bottle 1 (Sucrase preparation). Buffer should be stored below -10°C.

Buffer 2: **Sodium acetate buffer** (100 mM, pH 4.5)
Add glacial acetic acid (5.8 mL) to 900 mL of distilled water. Adjust to pH 4.5 using 1 M sodium hydroxide. Adjust the volume to 1 L. This is **Buffer 2.**

REAGENTS (not supplied):

1. **PAHBAH Reducing Sugar Assay Reagent**

   Solution A. Add 10 g of *p*-hydroxybenzoic acid hydrazide (Sigma cat. no. H9882-100G) (PAHBAH) to 60 mL of distilled water in a 250 mL beaker on a magnetic stirrer. Stir the slurry and add 10 mL of concentrated hydrochloric acid. Adjust the volume of the solution to 200 mL with distilled water.

   Solution B. Add 24.9 g of trisodium citrate dihydrate (MW = 294.1) to 500 mL of distilled water and stir to dissolve. Add 2.2 g of calcium chloride dihydrate (MW = 147.01) and dissolve. Add 40.0 g of sodium hydroxide and dissolve with stirring (the solution may be milky, but will clarify when diluted to 2 L). Adjust the volume to 2 L.

   PAHBAH Working Reagent. Immediately before use, add 20 mL of Solution A to 180 mL of Solution B and mix thoroughly. The mixed solution is stable for ~ 4 h on ice.

2. **Sodium hydroxide (50 mM)**

   Dissolve 2.0 g of sodium hydroxide in 900 mL of distilled water. Adjust the volume to 1 L.

3. **Alkaline borohydride (10 mg/mL sodium borohydride in 50 mM sodium hydroxide)**

   Accurately weigh approx. 50 mg of sodium borohydride (Sigma cat. no. 213462-100G) into polypropylene containers (10 mL volume with screw cap). Record the exact weight on the tubes (approx. 10 for convenience), seal the tubes and store them in a desiccator for future use. Stable for > 2 years at room temperature.

   Immediately before use, dissolve the sodium borohydride (at 10 mg/mL) in 50 mM sodium hydroxide. This solution is stable for 4-5 h at room temperature. This is **Reagent 3** (Alkaline borohydride solution).

4. **Acetic acid (200 mM)**

   Add 11.6 mL of glacial acetic acid to 600 mL of distilled water and adjust the volume to 1 L. This is **reagent 4** (200 mM acetic acid).

EQUIPMENT (RECOMMENDED):

1. Glass test tubes (round bottomed; 16 x 100 mm).
2. Pyrex screw cap culture tubes (25 x 150 mm) with PTFE lined phenolic caps (Fisher cat. no. 14-933D).
3. Micro-pipettors, *e.g*. Gilson Pipetman® (100 µL and 200 µL).
4. Positive displacement pipettor *e.g.* Eppendorf Multipette®
   - with 5.0 mL Combitip® (to dispense 0.2 mL aliquots of sucrase mixture and 0.1 mL aliquots of fructanase, and other solutions and buffers).
   - with 50 mL Combitip® (to dispense 5.0 mL aliquots of **PAHBAH Working Reagent**).
5. Brand® Dispensette® S Digital bottle-top dispenser (2.5-25 mL) (Sigma cat. no. BR4600351).
   - to dispense 10 mL of 200 mM sodium acetate buffer (pH 5.0).
6. Analytical balance.
7. Spectrophotometer set at 410 nm.
8. Vortex mixer (e.g. Vortex-Genie® 2 mixer).
9. Thermostated water baths (set at 30°C and 40°C).
10. Boiling water bath.
11. Microfuge (required speed 13,000 rpm).
12. Disposable 1.5 mL polypropylene microfuge tubes, e.g. Sarstedt cat. no. 72.690 (www.sarstedt.com).
13. Stop watch.

CONTROLS AND PRECAUTIONS:

1. The time of incubation at 100°C with PAHBAH reagent is critical and should be timed with a stop watch.
2. With each set of determinations, reagent blanks and D-fructose controls should be included and analysed concurrently.
   a) The reagent blank consists of 0.3 mL of 100 mM sodium acetate buffer (**Buffer 2**) plus 5.0 mL of PAHBAH Working Reagent.
   b) D-fructose controls are prepared by adding 0.2 mL of D-Fructose Standard Solution (1.5 mg/mL) to 0.9 mL of 100 mM sodium acetate (pH 4.5) (**Buffer 2**) with thorough mixing. Dispense 0.2 mL aliquots of this solution (containing 54.5 µg of D-fructose), in quadruplicate, into glass test tubes (16 x 100 mm). Add 0.1 mL of **Buffer 2** to each tube plus 5.0 mL **PAHBAH Working Reagent** (immediately before incubation in the boiling water bath).
3. With each set of determinations an **inulin/cellulose control powder** and/or a **levan/cellulose control powder** is included. The fructan content of these powders is given on the vial label.

4. A **sucrose/cellulose control powder** should be analysed with each new lot of reagents. If the sucrase treatment step is completely effective, the determined fructan value should be approx. 0.2% (w/w). If the sucrase is not effective, the determined value will reflect the sucrose content of the control sucrose/ cellulose powder [approx. 10% (w/w); see vial label].
5. D-Fructose **controls** (quadruplicate) and **reagent blank** solutions (duplicate) are run with each batch of samples and are incubated in the boiling water bath at the same time as the samples.
6. The effectiveness of borohydride reduction can be checked using D-fructose standard solution (0.2 mL, 1.5 mg/mL) and proceeding from **Step B.1** (page 8) of the assay procedure. Treatment with fructanase enzyme (**Step C.2**) is replaced with addition of acetate buffer (0.1 mL, 0.1 M, pH 4.5). The solution should be colourless following incubation with PAHBAH Working Reagent.
7. If the sample being analysed contains **galactosyl-sucrose oligosaccharides**, these can be removed by incubation with *A. niger* α-galactosidase (Megazyme cat. no. **E-AGLANP**). Refer to Frequently Asked Questions (FAQs) under **K-FRUC** on the Megazyme website.

ASSAY PROCEDURE:

Dry samples are milled to pass a 0.5 mm screen. Solid fatty samples (e.g. chocolate) are cut into fine shavings with a sharp knife; soft food products (e.g. spreads) are analysed without further preparation. All samples should be at room temperature before they are weighed.

A. Fructan Extraction

Samples containing 0-10% (w/w) fructan

Accurately weigh approx. 400 mg of the sample into a dry pyrex screw cap culture tube (25 x 150 mm) and add 25 mL of distilled water. Loosely cap the tube. Place the tube into a boiling water bath and heat for a total of 10 min. After 5 min, tighten the tube cap and vigorously mix the contents by inversion and shaking the tube. Return the tube to the boiling water bath. After a further 5 min, remove the tube from the boiling water bath and again mix the contents by inversion and shaking.

Samples containing 10-40% (w/w) fructan

Accurately weigh approx. 100 mg of the sample into a dry pyrex screw cap culture tube (25 x 150 mm) and add 25 mL of distilled water. Loosely cap the tube. Place the tube into a boiling water bath and heat for a total of 10 min. After 5 min, tighten the tube cap and vigorously mix the contents by inversion and shaking. Return the tube to the boiling water bath. After a further 5 min, remove the tube from the boiling water bath and again mix the contents by inversion and shaking.

In each case, allow the tube contents to cool to room temperature and then transfer ~ 2.0 mL into 2.0 mL microfuge tubes and centrifuge at 13,000 rpm for 5 min.

NOTE: For samples containing 40-100% (w/w) fructan, add 1 mL of centrifuged extract to 2 mL of water and proceed with the assay.

B. Removal of Sucrose, Starch and Reducing Sugars:

1. Accurately dispense a 0.2 mL aliquot of the solution to be analysed (containing approx. 0.1-1.0 mg/mL of fructan) into the bottom of a glass test tube (16 x 100 mm).
2. Add 0.2 mL of diluted Sucrase/Amylase solution (**Enzyme Solution A**) and incubate the tube at **30°C** for 30 min.

NOTE: If samples contain very high levels of sucrose and maltodextrins (e.g. baby milk formulations), increase this incubation time to 60 min to ensure complete hydrolysis of these oligosaccharides.

3. Add 0.2 mL of **Reagent 3** (alkaline borohydride solution) to the tube, stir vigorously and cover the tubes with Parafilm®. Incubate the tubes at **40°C** for 30 min to effect complete reduction of reducing-sugars to sugar alcohols.
4. Add 0.5 mL of **Reagent 4** (200 mM acetic acid) to the tube with vigorous stirring on a vortex mixer. **A vigorous effervescence should be observed** (this treatment removes excess borohydride and adjusts the pH to approx. 4.5). This is termed **Solution S.**

C. Hydrolysis and Measurement of Fructan:

1. Accurately and carefully transfer 0.2 mL aliquots of **Solution S** into the bottom of 3 glass test-tubes (16 x 100 mm).
2. Add 0.1 mL of fructanase solution (**Enzyme Solution B**) to 2 of these tubes (**samples**) and 0.1 mL **Buffer 2** (100 mM sodium acetate) to the third (**sample blank**).
3. Incubate the tubes at **40°C** for 30 min to effect complete hydrolysis of fructan to D-fructose and D-glucose. Seal the tubes with Parafilm® during incubation.
4. Add 5.0 mL of **PAHBAH Working Reagent** to all tubes [**samples, sample blanks, the D-fructose standard** (see Controls and Precautions 2.b), **reagent blank** (Controls and Precautions 2.a) and the extract of the **inulin/cellulose** control and/or the **levan/ cellulose control**] and incubate in a boiling water bath for exactly 6 min.
5. Remove the tubes from the boiling water bath and immediately place them in cold water (18-20°C) for approx. 5 min.
6. Measure the absorbance of all solutions at 410 nm against the reagent blank.

   Measure the absorbance values as soon as possible after cooling the tubes. **The PAHBAH colour complex fades with time.**

CALCULATIONS:

Fructan (% w/w as is):

$$= \Delta A \times F \times 5 \times 25 \times \frac{1.1}{0.2} \times \frac{100}{W} \times \frac{1}{1000} \times \frac{162}{180} \times D$$

$$= \Delta A \times \frac{F}{W} \times D \times 61.9$$

where:

ΔA = sample absorbance - sample blank absorbance (both read against the reagent blank)

F = factor to convert absorbance values to µg of D-fructose = (54.5 µg D-fructose)/(absorbance for 54.5 µg D-fructose)

5 = factor to convert from 0.2 mL as assayed to 1.0 mL

25 = volume (mL) of extractant used

$\frac{1.1}{0.2}$ = 0.2 mL was taken from 1.1 mL of enzyme digest for analysis

W = weight (mg) of sample extracted (i.e. 100 or 200 mg)

$\frac{100}{W}$ = factor to express fructan as a percentage of sample weight

$\frac{1}{1000}$ = factor to convert from µg to mg

$\frac{162}{180}$ = factor to convert from free D-fructose, as determined, to anhydrofructose (and anhydroglucose), as occurs in

D = further dilution of the sample extract

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears in the Megazyme web site (www.megazyme.com).

Table 1. Fructan content of various samples determined with *exo*- plus *endo*-inulinases with and without added *endo*-levanase.

	Fructan content, % w/w (as is basis)	
Sample	***exo*- and *endo*-Inulinanases**	***exo*- and *endo*-Inulinanases Plus *endo*-Levanase**
Timothy grass (sample A)	**4.9**	**13.8**
Timothy grass (sample B)	**3.2**	**6.2**
Rye grass	**8.9**	**9.9**
Oaten hay	**10.7**	**10.9**
Barley MAX (grain)	**12.8**	**12.8**
Pure levan from timothy grass	**59.2**	**91.2**
Pure inulin from chicory	**95.0**	**92.3**

APPENDIX:

A. Determination of the Extent of Underestimation of Fructan Content as a Consequence of Borohydride Reduction of Hydrolysed Inulin:

1. Accurately weigh approx. 40 mg of FOS or pure fructan into a dry pyrex screw cap culture tube (25 x 150 mm) and add 40 mL of distilled water. Loosely cap the tube. Heat the tube in a boiling water bath for 10 min to dissolve the fructan and stir the tube contents on a vortex mixer after 5 and 10 min.
2. Allow the solution to cool to room temperature and then mix the tube contents thoroughly.
3. Accurately dispense a 0.2 mL aliquot of the solution into the bottom of a glass test tube (16 x 100 mm) and add 0.2 mL of 100 mM sodium acetate buffer, pH 4.5 (**Buffer 2**) and mix thoroughly.
4. Proceed with the standard Fructan procedure from step B.3 through to C.6 (inclusive, page 8) and measure the absorbance against the tube that had no fructanase incubation. This gives the absorbance for the borohydride reduced fructan sample (**BRF**).
5. In a parallel experiment, transfer 0.2 mL of solution from step 3 into the bottom of a glass test tube (16 x 120 mm) and add 0.9 mL of 100 mM sodium acetate buffer, pH 4.5 (Buffer 2) to the tube with vigorous stirring.
6. Accurately and carefully transfer 0.2 mL aliquots of this solution into the bottom of three glass test-tubes (16 x 100 mm) and proceed with fructan hydrolysis according to the standard Fructan procedure; step C.2 to C.6 (inclusive, page 8). This gives the absorbance for the non-borohydride reduced fructan sample (**NBRF**).

Calculate the percentage recovery of fructan in the standard procedure following borohydride reduction as:

Percentage recovery = absorbance BRF / absorbance NBRF x 100

REFERENCES:

1. Lewis, D. H. (1993). Nomenclature and diagrammatic representation of oligomeric fructans - paper for discussion. *New Phytologist*, **124**, 583-595.

2. Pontis, H. G. (1966). Observations on the *de novo* synthesis of fructosans *in vivo*. *Arch Biochem Biochem.,* **116**, 416-423.

3. McCleary, B. V. & Blakeney, A. B. (1999). Measurement of inulin and *oligofructan. Cereal Foods World*, **44**, 398-406.

4. Longland, A. C., Dhanoa, M. S. & Harris, P. A. (2011). Comparison of a colorimetric and a high-performance liquid chromatography method for the determination of fructan in pasture grasses for horses. *J. Sci. Food Agric*, **92**, 1878-1885.

5. McCleary, B. V., Charmier, L., McKie,V. A. & Rogowski, A. (2019). Determination of Fructan (Inulin, FOS, Levan, and Branched Fructan) in Animal Food (Animal Feed, Pet Food, and Ingredients): Single-Laboratory Validation, First Action 2018.07. *J. AOAC International*, 102(3), 883-892.

6. Lever, M. (1973). Colorimetric and fluorimetric carbohydrate determination with *p*-hydroxybenzoic acid hydrazide. *Biochem. Med*. **7(2)**, 274-281.

7. McCleary, B. V, Murphy, A. & Mugford, D. C. (1997). Determination of Oligofructans and Fructan Polysaccharides in Foodstuffs by an Enzymatic/ Spectrophotometric Method: Collaborative Study. *J. AOAC International*, **83**, 356-364.

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