A Simple and Accurate Method for Determining Wheat Grain Fructan Content and Average Degree of Polymerization

Joran Verspreet,*,[†] Annick Pollet,[†] Sven Cuyvers,[†] Rudy Vergauwen,[‡] Wim Van den Ende,[‡] Jan A. Delcour,[†] and Christophe M. Courtin[†]

[†]Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), KULeuven, Kasteelpark Arenberg 20 - box 2463, 3001 Leuven, Belgium

[‡]Laboratory for Molecular Plant Physiology, KULeuven, Kasteelpark Arenberg 31 - box 2434, 3001 Leuven, Belgium

Ⓢ *Supporting Information*

ABSTRACT: An improved method for the measurement of fructans in wheat grains is presented. A mild acid treatment is used for fructan hydrolysis, followed by analysis of the released glucose and fructose with high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Not only the amount of fructose set free from fructans but also the released glucose can be quantified accurately, allowing determination of the average degree of polymerization of fructans (DP_{av}). Application of the mild acid treatment to different grain samples demonstrated that a correction should be made for the presence of sucrose and raffinose, but not for stachyose or higher raffinose oligosaccharides. The fructan content and DP_{av} of spelt flour, wheat flour, and whole wheat flour were 0.6%, 1.2%, and 1.8% of the total weight and 4, 5, and 6, respectively. Validation experiments demonstrate that the proposed quantification method is accurate and repeatable and that also the DP_{av} determination is precise.

KEYWORDS: *fructans, acid hydrolysis, wheat, degree of polymerization*

■ INTRODUCTION

There is a strong interest in the application of fructans in the food industry due to their potential to improve physicochemical properties of food products[1] and because of their potential health benefits.[2] Fructans are dietary fibers[3] and may have an additional positive health effect by the selective stimulation of beneficial gut bacteria. Indeed, fructans and, more specifically, inulin-type fructans are generally accepted as prebiotics since their fermentation induces specific changes in the composition and/or activity of the gastrointestinal microbiota that confer benefits upon host health.[2] Similar to other natural carbohydrates, fructans show intrinsic antioxidant properties as well.[4] Inulin-type fructans, like fructo-oligosaccharides (FOS) and inulin, are linear oligo- and polymers of fructose with at most one glucose unit per molecule and mostly or exclusively $\beta(2-1)$ linkages between the fructosyl units. Several other fructan-types, e.g. graminans, have been described and are classified based on the type of linkages between the fructose units.[5] Graminan-type fructans with both $\beta(2-1)$ and $\beta(2-6)$ fructosyl linkages can be found in the vegetative tissues of wheat.[6] The fine structure of fructans in wheat grains, however, is unknown. Only some smaller fructan oligosaccharides, like 6-kestose and neokestose, were identified in wheat flour.[7] The presence of these oligosaccharides demonstrates that not only $\beta(2-1)$ but also $\beta(2-6)$ fructosyl linkages occur in the fructans of wheat flour, which is consistent with the results of Montgomery and Smith.[8] Hence, it is clear that the structure of at least a part of the wheat grain fructans strongly differs from that of inulin-type fructans. This different, more complex structure may alter the effects on gastrointestinal health since structurally different fructans can result in different prebiotic effects.[9,10] Furthermore, the high consumption of wheat based food products makes wheat by far the most important fructan source in our diet[11] and stresses the possible nutritional importance of wheat grain fructans.

Several methods have been described to quantify fructans in food products. Quantification of fructans in wheat flour, however, is complicated because several mono-, di-, and oligosaccharides and starch present in wheat grains can interfere with the analysis. Moreover, fructans occur at relatively low concentrations (0.7−2.9% on dry basis) in wheat grains.[12] Most of the currently used methods are based on enzymatic hydrolysis of fructans into glucose and fructose followed by detection of the released sugars by spectrophotometry[13,14] or by high performance liquid chromatography.[15]

AOAC method 999.03 (Megazyme Fructan Assay Procedure, Megazyme, Bray, Ireland) and a modified version of this method (Megazyme Fructan HK Procedure) belong to the former and have found widespread use. These two methods do not provide information on the average degree of polymerization (DP_{av}) of fructans. The DP_{av} is, however, an important feature of fructans since it may influence their health-promoting effects[9,16] and physicochemical characteristics.[17] In addition, these two methods are laborious and may give unreliable results when the fructan content is lower than 1% on a dry weight basis.[18]

Huynh et al.[12] developed a quantification method to analyze fructans in wheat grain samples. A mixture of exo- and endoinulinase is used to hydrolyze fructans while amyloglucosidase and α-galactosidase are used to remove interference from starch and raffinose oligosaccharides, respectively.[12,14] This method is generally an improved version of the high performance anion exchange chromatography (HPAEC) based methods of Quemener et al.[15] and AOAC method 997.08. The main advantage of this method is that the interference of raffinose oligosaccharides (like raffinose, stachyose, and verbascose) can be taken into account. The concentrations of glucose (G_f) and fructose (F_f) released from fructans are measured separately and, based on the assumption that every fructan molecule contains one glucose unit, the DP_{av} can be calculated. However, due to the high starch and low fructan concentrations in wheat flour, it is difficult to determine G_f in an accurate way with these methods.[19] Stöber et al.[19] circumvented the interference from glucose originating from starch by calculating G_f based on F_f. Nevertheless, this calculation requires an assumption on the DP_{av}, which is unknown in the case of wheat flour samples. In summary, the currently used methods are insufficient to determine the DP_{av} accurately. The aim of this study was to develop a quantification method that allows accurate determination of fructose as well as glucose released from fructans during their hydrolysis, even when high concentrations of starch or dextrins are present. In this way, determination of the DP_{av} of fructans in wheat flour samples becomes possible.

■ MATERIALS AND METHODS

Materials. All chemicals and reagents were purchased from Sigma-Aldrich (Bornem, Belgium) and were of analytical grade unless specified otherwise. 1-Kestose, 1,1,1-kestopentaose, α-galactosidase (E-AGLAN), and inulinase (E-FRMXPD) were from Megazyme (Bray, Ireland). A fructan control powder (25.5% fructan in α-cellulose) included in the Megazyme fructan assay kit was used as reference material. Native wheat starch (Meritena 200) was kindly provided by Tereos Syral (Aalst, Belgium). Flours used were a commercial whole wheat flour and spelt flour from Vanden Bempt (St-Joris Weert, Belgium) and a commercial wheat flour from Meneba (Rotterdam, The Netherlands).

Extraction of Wheat Grain Fructans. Approximately 150 mg of flour or fructan control was accurately weighed in a centrifuge tube, and 500 μL of rhamnose solution (8.00 mg/mL) was added as internal standard. Five different extraction conditions were tested in duplicate. Extractions were performed in a shaking incubator (200 rpm): (1) extraction with hot water (15 mL; 80 °C; 30 min), (2) extraction with hot water (15 mL; 80 °C; 30 min) followed by centrifugation (9000g; 10 min) and extraction of the pellet with hot water (15 mL; 80 °C; 30 min), (3) extraction with hot water (15 mL; 80 °C; 60 min), (4) extraction with ethanol (15 mL; 80% ethanol; 80 °C; 30 min) followed by centrifugation (9000g; 10 min) and extraction of the pellet with hot water (15 mL; 50 °C; 30 min), (5) extraction with ethanol (15 mL; 80% ethanol; 80 °C; 30 min), centrifugation (9000g; 10 min), and extraction of the pellet with water (15 mL; 50 °C, 30 min). This extract was centrifuged again and the resulting pellet extracted with hot water (15 mL; 50 °C, 30 min). The additional extraction steps with water in (4) and (5) were performed at lower temperature (50 °C instead of 80 °C) in order to minimize coextraction of starch and other glucose containing components. After extraction, samples were cooled to room temperature. Total extract volume was determined by weighing the tubes before and after the addition of the extraction medium. Samples were centrifuged (9000g; 10 min) and supernatants were combined if multiple extractions were performed. Only the supernatant was used for analysis. Fructan content was determined

using mild acid hydrolysis and HPAEC sugar quantification as described below.

Acid Hydrolysis of Fructans. Two aliquots (50 μL) of the extract were transferred to 2 mL Eppendorf tubes. The first aliquot was subjected to a mild acid treatment, carried out in 60 mM HCl (final concentration) in a total volume of 52.5 μL at 70 °C for 90 min. The reaction was stopped by adding 2 μL of Na_2CO_3 (1.0 M) followed by dilution of the sample with deionized water to 1 mL. Similarly, the nonhydrolyzed sample was diluted with water to 1 mL and both samples were filtered (0.22 μm) and analyzed with high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

Comparison of Enzymatic and Acid Hydrolysis of Fructans. Acid hydrolysis of grain fructans was compared with enzymatic degradation. Wheat flour, whole wheat flour, and spelt flour were extracted with ethanol (80%; 80 °C; 30 min) and centrifuged, and the pellet was extracted with water (50 °C; 30 min), as described before (extraction condition (4)). Because ethanol prevents proper enzyme action, it was evaporated (40 °C; 40 rpm) with a rotary evaporator (Buchi Labortechnik AG, Flawil, Switzerland) and the sample dissolved in hot water (15 mL, 80 °C). Aliquots for acid and enzymatic hydrolysis were taken from the same extract so that extraction conditions were identical. The method of Huynh et al.[12] was applied for the enzymatic degradation. Briefly, amyloglucosidase and α-galactosidase were added to a first aliquot in order to determine the free glucose, fructose, and sucrose concentration plus the concentration of glucose and fructose derived from extracted starch and raffinose oligosaccharides. The same enzymes were added to a second aliquot in combination with inulinase. Using HPAEC-PAD, the fructan concentration was calculated by the difference in glucose and fructose concentration in the two samples. Acid hydrolysis was performed as described above.

Determination of Interference from Raffinose Oligosaccharides and Other Carbohydrates. To determine the concentration of fructose derived from raffinose oligosaccharides present in wheat flour, aliquots of flour extracts (prepared as described in the previous paragraph) were subjected to enzymatic hydrolysis as in the method of Huynh et al.,[12] except that α-galactosidase was not included.

In addition, the interference of sucrose, raffinose, stachyose, verbascose, maltose, malto-oligosaccharides, β-glucan, and wheat starch during fructan determination via acid hydrolysis was determined by subjecting these compounds to mild acid hydrolysis followed by HPAEC-PAD analysis of the hydrolysis products.

High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection. Carbohydrates were analyzed by HPAEC-PAD performed on a Dionex ICS3000 chromatography system (Sunnyvale, CA, USA). Samples (25 μL) were injected on a CarboPac PA-100 guard column (50 × 4 mm) coupled to a CarboPac PA-100 anion exchange column (250 mm × 4 mm). Isocratic elution conditions (90 mM NaOH, 1 mL/min, 15 min) were used for the analysis of rhamnose, glucose, fructose, sucrose, and melibiose.

Calculations of the Fructan Concentration and DP_{av}. Calculation of the fructan concentration after acid hydrolysis is based on that described by Huynh et al.[12] An additional correction is made for raffinose. To this end, the melibiose concentration in the hydrolyzed sample is determined, since raffinose is split into fructose and melibiose under mild acid conditions. The concentrations of glucose and fructose released from fructan (G_f and F_f respectively) are then calculated as follows:

$$G_f\ (\%) = \frac{180.16 V_e (G_b - G_a - S_a)}{10000 m_s} \quad (1)$$

$$F_f\ (\%) = \frac{180.16 V_e (F_b - F_a - S_a - M_b)}{10000 m_s} \quad (2)$$

where 180.16 is the molecular weight of glucose or fructose, V_e is the volume of the extract (mL), m_s is the sample mass (mg), G_a and G_b are the glucose concentrations (μM) in the nonhydrolyzed and the hydrolyzed sample, respectively, F_a and F_b are the fructose

concentrations (μM) in the nonhydrolyzed and the hydrolyzed sample, respectively, S_a is the sucrose concentration (μM) in the nonhydrolyzed sample, and M_b is the melibiose concentration (μM) in the hydrolyzed sample. The fructan concentration and the DP_{av} are calculated with the following equations:[12]

$$\text{fructan (\%)} = k(G_f + F_f) \tag{3}$$

$$k = \frac{180 + 162(DP_{av} - 1)}{180 DP_{av}} \tag{4}$$

$$DP_{av} = \frac{F_f}{G_f} + 1 \tag{5}$$

Validation. After comparing extraction and hydrolyzing conditions, optimal conditions were chosen and the resulting quantification method was validated by conducting different recovery experiments. Samples were extracted with hot water (80 °C, 60 min), centrifuged, hydrolyzed by mild acid treatment, and analyzed with HPAEC-PAD as described before. Fructan concentrations and DP_{av} were calculated as in the previous section.

First, the fructan control was analyzed in triplicate at five concentrations (2.5, 6.5, 12, 20, and 26 mg/15 mL). Second, fructan recovery was examined by fortification of wheat flour with the fructan control at three different concentrations (1, 2, and 3% fructan of the flour weight) in 10 replications and was calculated for each individual sample with the following formula:

$$\text{recovery (\%)} = \frac{(m_1 C_1) - (m_2 C_2)}{(m_3 C_3)} \tag{6}$$

where m_1 and C_1 are the mass and the measured fructan concentration of the fortified sample, respectively, m_2 and C_2 are the mass and the fructan concentration of the unfortified flour sample, and m_3 and C_3 are the mass and the fructan concentration (25.5%) of the added fructan control. The relative standard deviation on the fructan concentration in the wheat flour, analyzed in 10 replications, was used as an indicator for the repeatability.

Finally, determination of the DP_{av} was validated by the application of the proposed method to fructan standards (1-kestose and 1,1,1-kestopentaose) with known DP. These fructan standards were added to starch at low concentrations (0.4−0.7% of total weight). Concurrently, sucrose (0.4%) and raffinose (0.25%) were added to the mixture in concentrations comparable to those found in wheat flour.[20,21]

Statistical Analysis. Statistical evaluation of the results was performed with SAS software 8.1 (SAS institute Inc., Cary, NC, USA) using a two-way ANOVA model ($P < 0.01$). A Tukey correction for multiple comparisons was applied. When analyses were performed in duplicate, the range was used to estimate the standard deviation, as described by Nelson.[22]

■ RESULTS AND DISCUSSION

Extraction of Wheat Grain Fructans. Hot water is normally used for fructan extraction in order to inactivate enzymes and because of the lower solubility of fructans in cold water. Besides, also hot ethanol solutions are used to extract fructans[23,24] in order to minimize the coextraction of starch.

The use of different extraction conditions had no statistical influence ($P > 0.01$) on the measured fructan concentrations in flour samples (Figure 1). However, since the average fructan concentrations of the flour samples always appeared to be slightly higher after the 60 min water extraction than after 30 min water extraction, the 60 min water extraction (80 °C) was preferred in further analyses to be on the safe side. For these extraction conditions, fructan concentrations were higher in whole wheat flour (1.76% of total weight) than in wheat flour (1.17%) and spelt flour (0.69%). The higher fructan concentrations in whole wheat flour is in line with the higher
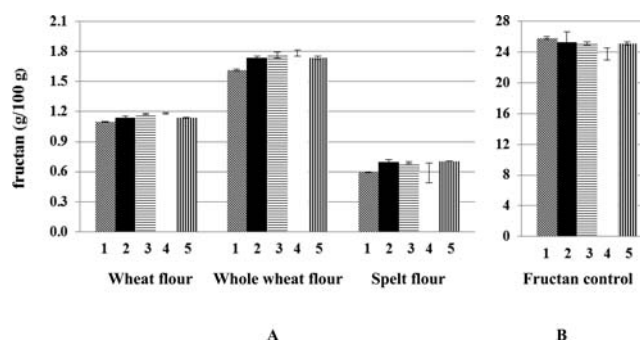

Figure 1. Fructan content (g/100 g total sample) of flour samples (panel A) and fructan control (25.5% fructan; panel B) determined at different extraction conditions: (1) extraction with water (80 °C, 30 min), (2) extraction with water (80 °C, 30 min), centrifugation, and extraction of the pellet with water (80 °C; 30 min), (3) extraction with water (80 °C, 60 min), (4) extraction with ethanol solution (80% ethanol; 80 °C; 30 min), centrifugation, and extraction of the pellet with water (50 °C; 30 min), (5) extraction with ethanol solution (80% ethanol; 80 °C; 30 min) and two centrifugation−water extraction steps (50 °C, 30 min). Error bars represent standard deviations on two replications.

fructan concentrations found in wheat bran than in wheat flour.[24,25] In contrast to the observations of Marques et al.,[26] higher fructan concentrations were measured in wheat flour than in spelt flour. However, Marques et al.[26] measured FOS concentrations instead of fructan concentrations and possibly did not include the higher DP fructan fraction. In addition, only one wheat variety and one spelt variety were analyzed as test samples in the present study while fructan contents can differ significantly between varieties.[12]

Haska et al.[24] demonstrated that extraction with ethanol (80%; 80 °C; 30 min) is insufficient for the extraction of fructans with a high DP and that an additional water extraction step is required. Our results indicate that, after this additional water extraction step, a second water extraction step is not necessary since the measured fructan concentrations were comparable for extraction conditions (4) and (5).

Determination of Interference from Raffinose Oligo-saccharides and Other Carbohydrates. Several carbohydrates present in wheat flour contain glucose and/or fructose and may therefore interfere with fructan quantification after acid hydrolysis. Wheat starch, maltose, malto-oligosaccharides, and β-glucan did not release glucose or fructose during mild acid treatment, while sucrose did (results not shown). Accordingly, a correction for the last sugar should be made. The concentration of sucrose, which is split into glucose and fructose, can be measured in the nonhydrolyzed sample. A recovery experiment also demonstrated that glucose and fructose were not degraded under the conditions of the mild acid treatment (results not shown).

Since raffinose oligosaccharides contain fructose, they can interfere with the quantification of fructans. After mild acid treatment of a wheat flour extract, only melibiose, the hydrolysis product of raffinose, could be detected by HPAEC-PAD. Manninotriose and verbascotetraose, the hydrolysis products of stachyose and verbascose, respectively, were absent (results not shown). To confirm these results quantitatively and to exclude the presence of raffinose-oligosaccharides with a DP higher than that of verbascose, fructan concentrations of flour samples was determined in four different ways. First, the fructan concentration was determined

Table 1. Fructan Concentrations (g/100 g total sample) in Wheat Samples Determined via Either Enzymatic or Acid Hydrolysis[a]

	fructan concn (g/100 g)					
	enzymatic hydrolysis			acid hydrolysis		
	with α-galactosidase	without α-galactosidase	Δ_1	with raffinose corrn	without raffinose corrn	Δ_2
whole wheat flour	1.84 (0.01)	1.99 (0.01)	0.15 (0.02)	1.77 (0.03)	1.91 (0.03)	0.14 (0.01)
spelt flour	0.61 (0.01)	0.71 (0.01)	0.09 (0.01)	0.63 (0.05)	0.71 (0.05)	0.07 (0.01)
wheat flour	1.06 (0.03)	1.04 (0.04)	−0.02 (0.07)	1.17 (0.02)	1.19 (0.02)	0.02 (0.01)

[a]Enzymatic hydrolysis was performed either with or without α-galactosidase. The difference in the calculated fructan concentrations is denoted by Δ_1. The fructan concentration was also calculated after acid hydrolysis either with or without correction for raffinose, and the difference is denoted by Δ_2. Values within parentheses are standard deviations on two replications.

via the enzymatic method of Huynh et al.[12] In a second approach, the same method was applied but α-galactosidase was not used in the hydrolysis step. When α-galactosidase is not included, fructose is released from raffinose and from other raffinose oligosaccharides by the action of inulinase.[14,27] Consequently, the calculated fructan concentration is higher when α-galactosidase is not included in the enzymatic method. Table 1 shows the difference between fructan concentrations from the two analyses (denoted as Δ_1) and gives information about the total amount of raffinose oligosaccharides in flour. In a third approach, samples were subjected to mild acid conditions as described earlier. Finally, these results were used to calculate fructan concentrations a fourth time but without making a correction for the presence of raffinose. Since mild acid treatment releases fructose from raffinose, the fructan concentration increased when the correction was omitted. This increase (Δ_2 in Table 1) is a measure of the raffinose concentration. Because Δ_1 and Δ_2 do not differ significantly ($P > 0.01$), raffinose seems to be the only important raffinose-oligosaccharide in wheat grains. In conclusion, a correction should be made for the presence of sucrose and raffinose when acid hydrolysis is used for fructan quantification since they release fructose and/or glucose during mild acid conditions. No correction has to be made for raffinose oligosaccharides with a DP higher than raffinose since these oligosaccharides either were not present or were present only in negligible quantities in the analyzed wheat grain samples. This is in agreement with previous observations that stachyose could not be detected in wheat flour[12] or could be detected only at very low concentrations (0.06 mg/g flour),[21] while verbascose could not be detected at all.[12,28]

Comparison of Acid and Enzymatic Hydrolysis. HPAEC-PAD profiles of hydrolyzed flour extracts demonstrated that both mild acid and inulinase treatment degrade wheat grain fructans completely (results not shown). To confirm this observation, fructose and glucose concentrations after acid or enzymatic hydrolysis of flour samples were compared. As expected and observed in Table 1 (enzymatic hydrolysis with α-galactosidase vs acid hydrolysis with raffinose correction), the calculated fructan concentrations did not differ significantly. It is generally accepted that inulinases are able to degrade fructans with a linear structure.[29] Our results show that they also degrade wheat grain fructans, although these fructans probably have a more complicated, branched structure.[8] However, due to the fact that glucose is released from starch and maltose during the enzymatic procedure but not during mild acid hydrolysis, much less glucose is set free from nonfructan compounds during mild acid hydrolysis and the concentration of glucose derived from fructans can be determined more accurately. This is a prerequisite to calculate

the DP_{av} of fructans since small changes in the measured G_f have major effects on the calculated DP_{av}. In addition, a one-step extraction with water is sufficient when acid hydrolysis is used and extraction with ethanol to avoid coextraction of starch or dextrins is redundant.

Outline of the Optimized Procedure and Validation. Based on the previous results, an optimized fructan quantification procedure is presented. Hot water is used to extract fructans (80 °C, 60 min) and, after centrifugation, a mild acid treatment for fructan hydrolysis. Glucose and fructose are measured with HPAEC-PAD in the hydrolyzed sample. As other sugars than fructans, like sucrose and raffinose, also release fructose and/or glucose during mild acid treatment, a correction is made for their presence in wheat grains. Sucrose concentrations are measured directly in the nonhydrolyzed extract together with free glucose and fructose. Raffinose is split into melibiose and fructose during mild acid treatment. By measurement of melibiose in the hydrolyzed sample, the interference from raffinose is taken into account. Fructan concentrations and DP_{av} are calculated as described in Materials and Methods.

Regression analysis revealed a linear correlation between the recovered and the actual fructan concentrations when a fructan control sample was analyzed with the proposed method (Figure S1 in the Supporting Information).

Acceptable fructan recoveries (99.3−102.4%) were also found when wheat flour was spiked with the fructan control (Table 2), demonstrating the accuracy of the method. The

Table 2. Average Recovery (and Standard Deviation) of Fructans Spiked in Wheat Flour, Determined via Acid Hydrolysis in 10 Replications

	recovery (%)
wheat flour + 3% fructan	99.3 (2.6)
wheat flour + 2% fructan	102.4 (3.1)
wheat flour + 1% fructan	100.9 (2.9)

relative standard deviation was small (2.94%, 10 replications) when the fructan concentration of the wheat flour was analyzed and indicates that the method has a good repeatability as well.

Small amounts of 1-kestose or 1,1,1-kestopentaose were added to a mixture of starch, sucrose, and raffinose to verify if the proposed method can be used to determine the DP_{av}. Calculated and actual DP_{av} were comparable (Table 3).

Since only few pure fructan samples with a high and known DP are available, it is more difficult to assess the suitability of the method for DP determination of high DP fructans. Nevertheless, a DP_{av} of 30.96 was found for inulin from

Table 3. DP$_{av}$ (and Standard Deviation) Determined via Acid Hydrolysis and Expected DP$_{av}$[a]

	DP$_{av}$	
	calcd	expected
spelt flour	4.08 (0.21)	
wheat flour	4.87 (0.37)	
whole wheat flour	5.62 (0.17)	
inulin	30.96 (3.39)	≥25
1-kestose	2.98 (0.03)	3
1,1,1-kestopentaose	4.86 (0.13)	5

[a]Flour samples and inulin were analyzed in duplicate. 1-Kestose and 1,1,1-kestopentaose were added in low concentrations (0.4−0.7% of total weight) to starch together with sucrose (0.4%) and raffinose (0.25%) and analyzed in 3 replications.

chicory (expected DP$_{av}$ ≥ 25), and therefore the authors presume that the method is suitable for these samples as well.

Application of mild acid treatment to water extracts of flour samples demonstrated a DP$_{av}$ of 4, 5, and 6 for the spelt flour, wheat flour, and whole wheat flour, respectively. A slightly higher DP$_{av}$ was observed for the whole wheat flour than for the wheat flour. This may be a consequence of the almost complete disappearance of fructans in the outer pericarp during kernel maturation. Indeed, Schnyder et al. (1993) observed that the fructans in the outer pericarp of immature wheat kernels had a higher molecular weight than those from the inner pericarp, testa, and endosperm.[30] Since the fructans from the outer pericarp disappeared almost completely, a low DP$_{av}$ for both whole wheat flour and wheat flour can be expected. The described method is suitable to investigate this further by a detailed analysis of the DP$_{av}$ of wheat flours and wheat bran.

ASSOCIATED CONTENT

ⓢ Supporting Information

One figure showing the correlation between the recovered and the actual fructan concentrations when a fructan control (25.5% fructan) was analyzed in triplicate at five concentrations (2.5, 6.5, 12, 20, and 26 mg/15 mL) with the proposed method. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel: + 32 16 32 14 82. Fax: + 32 16 32 19 97. E-mail: Joran. Verspreet@biw.kuleuven.be.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

HPAEC-PAD, high performance anion exchange chromatography with pulsed amperometric detection; DP$_{av}$, average degree of polymerization; FOS, fructo-oligosaccharides; G_f, concentration of glucose released from fructans; F_f, concentration of fructose released from fructans

REFERENCES

(1) Crittenden, R. G.; Playne, M. J. Production, properties and applications of food-grade oligosaccharides. *Trends Food Sci. Technol.* **1996**, 7, 353−361.

(2) Roberfroid, M.; Gibson, G. R.; Hoyles, L.; McCartney, A. L.; Rastall, R.; Rowland, I.; Wolvers, D.; Watzl, B.; Szajewska, H.; Stahl, B.; Guarner, F.; Respondek, F.; Whelan, K.; Coxam, V.; Davicco, M.-J.; Léotoing, L.; Wittrant, Y.; Delzenne, N. M.; Cani, P. D.; Neyrinck, A. M.; Meheust, A. Prebiotic effects: metabolic and health benefits. *Br. J. Nutr.* **2010**, 104 (Suppl. S2), S1−S63.

(3) McCleary, B. V. Development of an integrated total dietary fiber method consistent with the Codex Alimentarius definition. *Cereal Food World* **2010**, 55, 24−28.

(4) Van den Ende, W.; Peshev, D.; De Gara, L. Disease prevention by natural antioxidants and prebiotics acting as ROS scavengers in the gastrointestinal tract. *Trends Food Sci. Technol.* **2011**, 22, 689−697.

(5) Van Laere, A.; Van den Ende, W. Inulin metabolism in dicots: chicory as a model system. *Plant Cell Environ.* **2002**, 25, 803−813.

(6) Bancal, P.; Carpita, N. C.; Gaudillère, J. P. Differences in fructan accumulated in induced and field-grown wheat plants: an elongation-trimming pathway for their synthesis. *New Phytol.* **1992**, 120, 313−321.

(7) Nilsson, U.; Dahlqvist, A.; Nilsson, B. Cereal fructosans: Part 2 Characterization and structure of wheat fructosans. *Food Chem.* **1986**, 22, 95−106.

(8) Montgomery, R.; Smith, F. The carbohydrates of the gramineae. VII. The constitution of a water-soluble hemicellulose of the endosperm of wheat (*Triticum vulgare*). *J. Am. Chem. Soc.* **1955**, 77, 3325−3328.

(9) Ito, H.; Takemura, N.; Sonoyama, K.; Kawagishi, H.; Topping, D. L.; Conlon, M. A.; Morita, T. Degree of polymerization of inulin-type fructans differentially affects number of lactic acid bacteria, intestinal immune functions, and immunoglobulin A secretion in the rat cecum. *J. Agric. Food Chem.* **2011**, 59, 5771−5778.

(10) Kilian, S.; Kritzinger, S.; Rycroft, C.; Gibson, G.; du Preez, J. The effects of the novel bifidogenic trisaccharide, neokestose, on the human colonic microbiota. *World J. Microbiol. Biotechnol.* **2002**, 18, 637−644.

(11) Moshfegh, A. J.; Friday, J. E.; Goldman, J. P.; Ahuja, J. K. C. Presence of inulin and oligofructose in the diets of Americans. *J. Nutr.* **1999**, 129, 1407−1411.

(12) Huynh, B.-L.; Palmer, L.; Mather, D. E.; Wallwork, H.; Graham, R. D.; Welch, R. M.; Stangoulis, J. C. R. Genotypic variation in wheat grain fructan content revealed by a simplified HPLC method. *J. Cereal Sci.* **2008**, 48, 369−378.

(13) McCleary, B. V.; Murphy, A.; Mugford, D. C. Measurement of total fructan in foods by enzymatic/spectrophotometric method: Collaborative study. *J. AOAC Int.* **2000**, 83, 356−364.

(14) Andersen, R.; Sorensen, A. An enzymatic method for the determination of fructans in foods and food products - Comparison of the results by high performance anion exchange chromatography with pulsed amperemetric detection. *Eur. Food Res. Technol.* **1999**, 210, 148−152.

(15) Quemener, B.; Thibault, J. F.; Coussement, P. Determination of inulin and oligofructose in food products and integration in the AOAC method for measurement of total dietary fiber. *LWT—Food Sci.Technol.* **1994**, 27, 125−132.

(16) Van de Wiele, T.; Boon, N.; Possemiers, S.; Jacobs, H.; Verstraete, W. Inulin-type fructans of longer degree of polymerization exert more pronounced *in vitro* prebiotic effects. *J. Appl. Microbiol.* **2007**, 102, 452−460.

(17) López-Molina, D.; Navarro-Martínez, M. D.; Rojas-Melgarejo, F.; Hiner, A. N. P.; Chazarra, S.; Rodríguez-López, J. N. Molecular properties and prebiotic effect of inulin obtained from artichoke (*Cynara scolymus* L.). *Phytochemistry* **2005**, 66, 1476−1484.

(18) Muir, J. G.; Shepherd, S. J.; Rosella, O.; Rose, R.; Barrett, J. S.; Gibson, P. R. Fructan and free fructose content of common Australian vegetables and fruit. *J. Agric. Food Chem.* **2007**, 55, 6619−6627.

2106

dx.doi.org/10.1021/jf204774n | *J. Agric. Food Chem.* 2012, 60, 2102−2107

(19) Stöber, P.; Bénet, S.; Hischenhuber, C. Simplified enzymatic high-performance anion exchange chromatographic determination of total fructans in food and pet food - Limitations and measurement uncertainty. *J. Agric. Food Chem.* **2004**, *52*, 2137−2146.

(20) Macarthur, L. A.; D'appolonia, B. L. Comparison of oat and wheat carbohydrates. I. Sugars. *Cereal Chem.* **1979**, *56*, 455−457.

(21) Henry, R. J.; Saini, H. S. Characterization of cereal sugars and oligosaccaharides. *Cereal Chem.* **1989**, *66*, 362−365.

(22) Nelson, L. S. Use of the range to estimate variability. *J. Qual. Technol.* **1975**, *7*, 46−48.

(23) Jenkins, C. L. D.; Snow, A. J.; Simpson, R. J.; Higgins, T. J.; Jacques, N. A.; Pritchard, J.; Gibson, J.; Larkin, P. J. Fructan formation in transgenic white clover expressing a fructosyltransferase from Streptococcus salivarius. *Funct. Plant Biol.* **2002**, *29*, 1287−1298.

(24) Haskå, L.; Nyman, M.; Andersson, R. Distribution and characterisation of fructan in wheat milling fractions. *J. Cereal Sci.* **2008**, *48*, 768−774.

(25) Knudsen, K. E. B. Carbohydrate and lignin contents of plant materials used in animal feeding. *Anim. Feed Sci. Technol.* **1997**, *67*, 319−338.

(26) Marques, C.; D'Auria, L.; Cani, P. D.; Baccelli, C.; Rozenberg, R.; Ruibal-Mendieta, N. L.; Petitjean, G.; Delacroix, D. L.; Quetin-Leclercq, J.; Habib-Jiwan, J.-L.; Meurens, M.; Delzenne, N. M. Comparison of glycemic index of spelt and wheat bread in human volunteers. *Food Chem.* **2007**, *100*, 1265−1271.

(27) Gill, P. K.; Manhas, R. K.; Singh, P. Purification and properties of a heat-stable exoinulinase isoform from Aspergillus fumigatus. *Bioresour. Technol.* **2006**, *97*, 894−902.

(28) Kuo, T. M.; VanMiddlesworth, J. F.; Wolf, W. J. Content of raffinose oligosaccharides and sucrose in various plant seeds. *J. Agric. Food Chem.* **1988**, *36*, 32−36.

(29) Vijayaraghavan, K.; Yamini, D.; Ambika, V.; Sowdamini, N. S. Trends in inulinase production - a review. *Crit. Rev. Biotechnol.* **2009**, *29*, 67−77.

(30) Schnyder, H.; Gillenberg, C.; Hinz, J. Fructan contents and dry matter deposition in different tissues of the wheat grain during development. *Plant Cell Environ.* **1993**, *16*, 179−187.