



Distribution and characterisation of fructan in wheat milling fractions

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ABSTRACT

Structure and health effects of inulin-type fructans have been extensively studied, while less is known about the properties of the graminan-type fructans in wheat. Arabinoxylan (AX) is another important indigestible component in cereal grains, which may have beneficial health effects. In this study, the fructan content in milling fractions of two wheat cultivars was determined and related to ash, dietary fibre and AX contents. The molecular weight distribution of the fructans was analysed with HPAEC-PAD and MALDI-TOF MS using ¹H NMR and enzymatic hydrolysis for identification of fructans. The fructan content (g/100 g) ranged from 1.5 ± 0.2 in flour to 3.6 ± 0.5 in shorts and 3.7 ± 0.3 in bran. A correlation was found between fructan content and dietary fibre content ($r = 0.93$, $P < 0.001$), but with a smaller variation in fructan content between inner and outer parts of the grain. About 50% of the dietary fibre consisted of AX in all fractions. The fructans were found to have a DP of up to 19 with a similar molecular weight distribution in the different fractions.

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1. Introduction

Fructans are carbohydrates consisting of fructosyl units with or without one glucosyl unit. The different types of fructans found in nature may be classified according to linkage and origin as suggested by Roberfroid (2005). Linear-type fructans contain predominantly one type of fructosyl-fructose linkage; β -(1 → 2) in inulin and β -(6 → 2) in levan (mostly from bacteria) and phlein (from plants). Graminan-type fructans contain both β -(1 → 2) and β -(6 → 2) linkages. Inulin from chicory root has been extensively studied regarding structure (De Leenheer and Hoebregs, 1994; Timmermans et al., 2001; van Loo et al., 1995) and health effects and has been found to increase mineral absorption, reduce cholesterol levels and have bifidogenic effects (Roberfroid, 2005). Interestingly, different molecular weights of inulin have been shown to affect the formation of colonic short-chain fatty acids (SCFAs) differently, i.e. the main end products of colonic fermentation, where some may have health-promoting effects (Nilsson and Nyman, 2005). Considerably less is known about the properties of

other types of fructans, such as the graminan-type fructans found in wheat.

The fructan content in wheat grains has been determined to be 0.9–1.8 g/100 g in five cultivars from five growth places (Fretzdorff and Welge, 2003). The content has been found to be higher in bran (2.0 g/100 g) and middlings (2.3 g/100 g) than in flour (1.6 g/100 g) and grain (1.5 g/100 g) (Knudsen, 1997). In the study of Fretzdorff and Welge (2003), fructans were estimated to have an average DP of 5–7 in most whole grain samples. Other studies have shown that 40–50% of fructans from wheat flour (Nilsson et al., 1986) and 60% from whole grains (Henry and Saini, 1989) have a DP of less than 6. Further, fructans in wheat flour have been reported to have a highest DP of 7–8 (van Loo et al., 1995) and ≥ 16 (Nilsson et al., 1986). The inconsistent results regarding DP in different studies may be caused not only by different samples but also by the use of different analytical methods. Today, more advanced and accurate methodologies are available. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) is a useful technique for separating fructans (Cataldi et al., 2000; Corradini et al., 2004). However, it is difficult to determine the DP of a non-linear series without available standards as several peaks may correspond to the same DP. These analyses can be complemented with matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS), which measures the molecular weight regardless of branching structure (Lopez et al., 2003; Losso and Nakai, 1997).

Fructan is an indigestible carbohydrate entering the colon, and therefore acts as a dietary fibre component, i.e. indigestible polysaccharides and lignin as defined by Trowell (1976). However,

Abbreviations: Ara, arabinose; Ara/Xyl, arabinose to xylose ratio; AX, arabinoxylan; DP, degree of polymerisation; Fru, fructose; Gal, galactose; Glc, glucose; HPAEC-PAD, high performance anion-exchange chromatography with pulsed amperometric detection; MALDI-TOF MS, matrix-assisted laser desorption time-of-flight mass spectrometry; NMR, nuclear magnetic resonance; SCFA, short-chain fatty acid; Xyl, xylose.

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current dietary fibre methods (Asp et al., 1983; Englyst and Cummings, 1984; Prosky et al., 1988; Theander et al., 1995) are based on precipitation in 80% ethanol and are thus unsuitable for fructan analysis. Inulin with DP ≤ 10 has been shown to be soluble in 80% ethanol, and inulin with DP 11–18 is soluble to some extent (Ku et al., 2003). Other methods for fructan analysis, based on enzymatic hydrolysis of the fructans followed by quantification of the released sugars, have therefore been developed (Hoebregs, 1997; McCleary et al., 2000; Steegmans et al., 2004).

Arabinoxylan (AX) is another important indigestible component in cereal grains. AX is present in the cell walls and has a linear backbone of (1 $\rightarrow$ 4)-linked β -D-xylopyranose residues, mainly substituted with α -L-arabinofuranose residues attached at O-2 and/or O-3 (Izydorczyk and Biliaderis, 1995). The AX content increases in outer layers of the grain (Nyman et al., 1984), with estimated contents of 5.5–7.8 g/100 g in whole grain and an average of 2.1 g/100 g in flour (Andersson and Åman, 2001). Physical properties of AX, such as viscosity, depend on the degree of substitution, distribution of substituents and degree of polymerisation. The extractability of AX also depends on interactions with other components of the cell wall, with lower extractability towards the outer layers of the grain (Andersson and Åman, 2001). Much attention has been paid to the influence of AX on bread-making (Andersson and Åman, 2001; Goesaert et al., 2005). More recently, some studies have also focused on potential prebiotic properties of arabinoxylan and arabinoxylan oligosaccharides (Grootaert et al., 2007).

The purpose of this study was to quantify and characterise fructan in milling fractions of wheat and to relate it to ash, dietary fibre and AX contents. The molecular weight distribution of wheat fructans was analysed with HPAEC-PAD and MALDI-TOF MS while ^1H NMR and enzymatic hydrolysis were used for identification of fructans.

2. Experimental

2.1. Materials

Whole grains and five milling fractions (flour of about 79% extraction rate, the middling flour with the highest ash content included in the flour, germ, shorts and bran) of the wheat cultivars Harnesk (conventionally and organically grown) and Kosack (conventionally grown) were obtained from Lantmännen Reppe (Lidköping, Sweden). The organically grown Harnesk was processed at a different mill and without separation of a germ fraction. Prior to analysis, samples were milled on a Cyclotec 1093 mill (Foss Tecator, Höganäs, Sweden) to pass through a 0.5 mm screen. If not further specified, Harnesk refers to conventionally grown Harnesk.

2.2. Analyses

2.2.1. Fructan

Fructan content was analysed in duplicate with the enzymatic/spectrophotometric AOAC method 999.03 (McCleary et al., 2000) using the enzyme assay kit K-FRUC (Megazyme, Bray, Ireland). Standard blanks were used and, since wheat contains the raffinose family oligosaccharides, these carbohydrates were corrected for by hydrolysis with *Aspergillus niger* α -galactosidase (Megazyme, Bray, Ireland). This correction was performed in a separate step before the degradation of starch, maltosaccharides and sucrose. For comparison, samples were analysed without incubation with α -galactosidase.

2.2.2. Dietary fibre

Dietary fibre content was analysed in duplicate as described by Theander et al. (1995). In this method, starch is degraded by α -amylase and amyloglucosidase followed by precipitation with 80%

(v/v) ethanol and hydrolysis with H_2SO_4 . The released neutral sugars are quantitated by gas–liquid chromatography, uronic acids are determined by colorimetry and Klason lignin is determined gravimetrically as ash-free acid-insoluble residue. To verify that no fructans were precipitated with the dietary fibre, an 80% ethanol precipitate of Harnesk bran was analysed for fructan content with the Megazyme assay kit. Further, Raftilose®P95 (ORAFIT, Tienen, Belgium) was analysed from the H_2SO_4 hydrolysis step at 125 °C to verify the degradation of fructose at these conditions. Dietary fibre content was calculated as the sum of neutral sugar residues, uronic acid residues and Klason lignin. Arabinoxylan and arabinogalactan contents were calculated from arabinose, xylose and galactose residues as suggested by Delcour et al. (1999), assuming arabinose to be included in arabinogalactans with an Ara/Gal ratio of 0.7.

2.2.3. Ash

Ash content was determined by burning the sample at 600 °C for 3 h in a muffle furnace.

2.3. Fructan extraction parameters

To find suitable extraction parameters for the fructans, different experimental conditions were tested. Triplicate samples of shorts were extracted at two ethanol concentrations (50 and 80% v/v), two times (30 and 60 min) and at two temperatures (21 and 80 °C). The sample was stirred every 10 min during the extraction. In samples containing 50% ethanol, the concentration was increased to 80% before all samples were cooled at -20 °C for 30 min (to precipitate proteins) and centrifuged for 10 min (1000 $\times$ g). The pellet was washed twice with cold 80% ethanol and dried with nitrogen gas. The supernatants were combined and the ethanol evaporated. Fructan contents of fractionated (pellets and supernatants) and unfractionated samples were analysed with the Megazyme assay kit. Extracts prepared with 80% ethanol were analysed with HPAEC-PAD to determine the molecular weight distribution. Additional tests were done to check whether ethanol insolubility or precipitation during the cooling step excluded the largest fructans. For this purpose, samples were extracted (80%, 80 °C, 30 min) and cooled to 21 °C or -20 °C. The washed pellets were extracted in water (50 °C, 30 min) and analysed with HPAEC-PAD. D-Lactose monohydrate (BioChemika, Fluka) was used as internal standard and added before the extraction. Before the HPAEC-PAD analysis, acetate buffer (5 ml, pH 5.0) was added and maltodextrins were degraded by incubating with amyloglucosidase (100 μl , 140 U/ml) (Megazyme) at 60 °C for 4 h. Samples for HPAEC-PAD analysis were prepared in duplicate.

2.4. Identification and molecular weight distribution of fructans

Duplicate samples from all fractions of Harnesk and shorts from Kosack were extracted in 80% (v/v) ethanol at 80 °C for 30 min and then centrifuged at 1000 $\times$ g for 10 min. The pellet was extracted twice with water (50–55 °C, 30 min), the water extracts and acetate buffer were added to the evaporated ethanol extract and maltodextrins were degraded (see above). For identification of fructans and the raffinose family oligosaccharides, aliquots were also incubated with inulinase and α -galactosidase (40 °C, 30 min), respectively. Standard solutions of glucose (1), fructose (2), sucrose (3), raffinose (4), stachyose (5), verbascose (6), inulin-type fructooligosaccharides with DP 3–5 (1-kestose – Glc(Fru) $_2$, 1,1-kestotetraose – Glc(Fru) $_3$ and 1,1,1-kestopentaose – Glc(Fru) $_4$) (7) and Raftilose®P95 with DP 2–8 (8) were prepared in water. Standards were obtained from Merck (Darmstadt, Germany) (1–4), Sigma (St. Louis, Missouri, USA) (5), Megazyme (Bray, Ireland) (6–7) and ORAFIT (Tienen, Belgium) (8).

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