



Extraction and characterization of water-extractable and water-unextractable arabinoxylans from spelt bran: Study of the hydrolysis conditions for monosaccharides analysis

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ABSTRACT

Monosaccharide analyses were performed, statistically treated and adopted for spelt bran fractions; with regard to the arabinoxylan content. The AX content of the initial spelt bran reached 9.2% with an A/X ratio of 0.39. The initial spelt bran was rich in starch (41.2%) and protein (18.9%). WE- and WU-AXs from micronised spelt bran were extracted after improved enzymic destarching and deproteinisation treatments. WU-AXs were obtained by two successive extractions with 2% alkaline hydrogen peroxide at 60 °C during 4 h. 55% of the AX present in spelt bran was extracted by using the three extraction steps (WE- and WU-AXs), among AX, 13% were WE and 87% were WU. A/X ratios were different depending on the extraction process. WE-AXs were less rich in arabinose than WU-AXs. Each fraction contained two populations of AX. The first one consisted of low MW AX (7–8 kDa). The second population had a higher MW, 310–415 kDa for WU-AXs and 28 kDa for WE-AXs. The extracts had to be purified in order to improve the AX content. Results were compared to those obtained with wheat bran in the literature. This research was, to the best of our knowledge, the first study on AX extraction from spelt bran.

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1. Introduction

Spelt (*Triticum aestivum* ssp. *spelta*) is an ancient subspecies of common wheat (*Triticum aestivum* ssp. *aestivum*) whose main characteristic is that the hull is attached to the grain. Namely, both wheat and spelt have a hull, but in wheat it is free, and in spelt it is attached to the grain. In agronomic terms, spelt shows a high resistance to environmental factors (diseases...) and might perform better under less advantageous growing conditions (Campbell, 1997). Spelt is suitable for organic farming and contributes to agro-biodiversity, meeting consequently the objectives of the European Union on growing practices. Moreover, spelt has a higher protein content than wheat,

a higher lipid content, especially Δ^7 -avenasterol (Bonafaccia et al., 2000; Ruibal-Mendieta et al., 2004) and higher magnesium, phosphorus, iron, copper and zinc contents (Ruibal-Mendieta et al., 2005). It has been mainly used in specialty breads, organic food and products with characteristics that differ from regular wheat products (Ranhotra and Gelroth, 1995).

Bran is a by-product of the milling industry which accounts for 14–19% of the wheat grain. It comprises the outer layers, the aleurone layer and remnants of the starchy endosperm (Pomeranz, 1988). Contrary to spelt bran, wheat bran has been much studied. It is one rich source of dietary fibers including 46% of non-starch polysaccharides (NSPs) (Ralet et al., 1990), 15–25% of starch (Beaugrand et al., 2004), 3–10% of lignin (Bergmans et al., 1996; Ralet et al., 1990), 11–25% of proteins (Beaugrand, 2004; Brillouet and Mercier, 1981; Dupont and Selvendran, 1987), 4–6% of ash (Bataillon et al., 1998; Maes and Delcour, 2001), 6% lipids (Bataillon et al., 1998), pectins and some minor components (e.g. 5% uronic acids) (Brillouet and Mercier, 1981). In destarched bran, ferulic acid occurs at an average of 5000 µg/g, p-coumaric acid at 150 µg/g, and at sinapic acid 200 µg/g (Beaugrand et al., 2004). The main NSPs are arabinoxylans (AX), cellulose and (1→3), (1→4)-β-D-glucans which represent respectively 73%, 24% and 6% of the NSP of the bran (Brillouet and Mercier, 1981; Ralet et al., 1990; Selvendran et al., 1980). Small

Abbreviations: AE1, first alkali extract; AE1D, first alkali extract after dialysis; AE2, second alkali extract; AE2D, second alkali extract after dialysis; AX, arabinoxylan; A/X ratio, arabinose to xylose ratio; CRR, cellulose rich residue; dm, dry matter; GAX, glucuronoarabinoxylan; MW, molecular weight; NSP, non-starch polysaccharide; PWEM, purified water-extractable material; WE, water-extractable; WEM, water-extractable material; WU, water-unextractable; WUM, water-unextractable material.

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amounts of xyloglucan are also present in the pericarp (Dupont and Selvendran, 1987). Glucomannan (Mares and Stone, 1973) and arabinogalactan (Fincher et al., 1974) were reported in aleurone and endosperm cells. Wheat bran carbohydrates contain 7.1–9.9% arabinose, 1–1.8% galactose, 24.4–35.7% glucose, 10.8–16.6% xylose; the whole represents 50.6–59.8% of total dry weight (DW). Regarding free sugars, 7.2% was reported, from which 2.4% was sucrose and 1.3% was raffinose (Beaugrand et al., 2004).

Recent research has been focused on the major component of wheat bran: AX. AX are constituted by a main chain of β -linked (1 \rightarrow 4)-3-D-xylopyranose and are substituted mainly by side chains of α -L-arabinofuranose (Bacic et al., 1988). The substituent arabinoses are in position α -(O-2) and/or α -(O-3) of the xylose residues (Bacic and Stone, 1981). The substitution rate arabinose/xylose of the AX increases from the centre towards the outer layers of the wheat grain (Lempereur et al., 1997), the higher rate is observed in the pericarp where, on average, one xylose carries one arabinose (Brillouet and Mercier, 1981; Ring and Selvendran, 1980). For wheat bran, 31% of the xylose is unsubstituted, 24% is monosubstituted and 39% is disubstituted. The presence of 3,5-dimethyl-arabinose and 5-methyl-arabinose suggests that around 25% of arabinose residues are present under oligomeric side chains (Edwards et al., 2003). Uronic acids are associated with AX (such as glucuronic acid) (Brillouet and Joseleau, 1987).

Some AXs in wheat bran are physically and chemically interlinked and linked also to lignin and cellulose through diferulic acid bridges and hydrogen bonds (Iiyama et al., 1994). However, Mares and Stone (1973) found evidence that WE-AXs are not bound to the other cell wall polymers and that they are located at the surface of the cell wall. Concerning the WU-AXs, they are maintained in the cell wall structure by ester linkages (Delcour et al., 1999). Consequently, most of the AXs can only be extracted with alkaline media (Bergmans et al., 1996; Dupont and Selvendran, 1987; Shiiba et al., 1993). The procedure with peroxide hydrogen uses cheap chemicals with inherent low toxicity (Hollmann and Lindhauer, 2005) contrary to barium hydroxide used for example by Bergmans et al. (1996).

During the last decades, a renewed interest rose for spelt as human food due to its image as a “healthier, more natural, less over-bred” cereal than modern wheat (Schober et al., 2006). To the best of our knowledge, the only application of spelt bran is as a feeding ingredient and no study deals with other potential uses. Consequently, evaluation of spelt bran as a high value-added product is an important objective to support spelt development. From a technological point of view, AXs have a role in bread making performance and have functional properties interesting for use as food additives. They can be used as viscosity enhancers or thickeners because they have a high water-holding capacity and are able to stabilise protein foams (Courtin and Delcour, 2002). They can also be used as texturizers, more especially WE-AXs (Saulnier et al., 2007). Besides, they can be used for the production of oligosaccharides with physiological functions (Kabel et al., 2002a) and AX soluble fibers may have the potential to reduce glucose and lipid absorption (Saulnier et al., 2007). While a lot of works concern wheat AXs, few or none are carried out on spelt. The objectives of this study were to extract and characterize spelt bran AXs in order to open new ways for spelt bran applications as is already the case for wheat bran. In the present study, AXs from spelt bran were extracted with water and with alkali peroxide hydrogen. Different AX fractions were characterized and results obtained here were compared to those of the literature on wheat bran. Besides, different conditions of hydrolysis were tested on the spelt extraction fractions (Englyst and Cummings, 1984; Maes and Delcour, 2001; Southgate, 1995).

2. Material and methods

2.1. Materials

The bran came from the spelt cv. Ressay, harvested in August 2007. The spikelets were hulled and the grains milled at “Le Moulin de Hollange” in November 2007. The milled fractions: white flour, bran and hulls were stored at 4 °C. Bran was screened at 1 mm to remove parts of the hulls, and was then micronised by jet milling (Alpine 100 AFG, Augsburg, Germany) with 6–7 bar of pressure and turbine at 2000 rpm. The granulometry was measured in triplicate with a Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK).

All reagents were of analytical grade. All enzymes were supplied by Novozymes (Bagsvaerd, Denmark).

2.2. Methods

2.2.1. Monosaccharide composition

Monosaccharide analysis was based on the method of Englyst and Cummings (1984). A sample (in triplicate) was accurately weighed and the acid was added. The hydrolyses were performed with two acids: H₂SO₄ or TFA, at three concentrations: 0.5 M; 1 M and 2 M, during 3 h at 100 °C and the trial was completed with TFA 2 M at 110 °C during 2 h. The solution was then neutralized with concentrated NaOH and the alkalinity of the solution was checked. Sugars (0.4 mL of supernatant) were reduced to their corresponding alditols with 2 mL of DMSO containing 2 mL NaBH₄. Reduction was performed for 90 min at 40 °C. The excess of NaBH₄ was eliminated by adding 6 mL glacial acetic acid. Acetylation was then performed with acetic anhydride (4 mL, 10 min at room temperature) in the presence of 1-methyl-imidazole (0.4 mL) as a catalyst. Acetylation was stopped with 10 mL deionized water and the acetylated alditols were partitioned between dichloromethane (4 mL) and water. After the phase separation, 1 mL of the lower one was transferred in a septum-cap vial. 2-Deoxy-D-glucose was used as internal standard and standards of D-arabinose, D(+)-xylose, D(+)-mannose, D(+)-glucose, D(+)-galactose (purity >99.5%, Sigma Chemical Co., St-Louis MO, USA) were used. The analyses were performed with a Hewlett–Packard Agilent 6890 series gas chromatograph equipped with a high-performance capillary column, HP1-methylsiloxane (30 m \times 0.32 mm, 0.25 μ m film thickness) (Scientific Glass Engineering, Melbourne, Australia). 0.2 μ L of derivatized sugars in dichloromethane was injected. Helium was the carrier gas with a flow of 1.6 mL/min. The injection temperature was 290 °C, and the temperature program was 1 min at 120 °C, followed by a linear increase in 4 min to 220 °C and then in 35 min–290 °C which was maintained for 4 min. Compounds were detected using a flame ionization detector at 320 °C.

2.2.2. Preparation of destarched deproteinised bran

Spelt bran in 0.05 M pH 6.5 phosphate buffer (1:7, w/v) was heated under continuous stirring to 75 °C and α -amylase Termamyl 120 L (Novozymes) was added (10 μ L/g bran). The suspension was then heated to 90 °C and maintained 1 h. It was then cooled to 50 °C and Fungamyl 800 L (Novozymes) was added (0.4 μ L/g bran). The suspension was kept 30 min at 50 °C. Three proteases were successively added: Neutrase (50 μ L/g bran), Alcalase (50 μ L/g bran) and Flavourzyme 1000 L (2 μ L/g bran) (all three from Novozymes). The suspension was heated to 55 °C and left 4 h under continuous stirring. The solution was heated at 100 °C for 15 min. The mixture was vacuum filtered through a 20 μ m nylon filter. The residue, i.e. destarched and deproteinised spelt bran or water-unextractable material (WUM), was washed several times with distilled water and dried at 50 °C in an oven for 24 h. The filtrate and the residue

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