Journal of Food Composition and Analysis 34 (2014) 12–17

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original research article

Determination of water-extractable polysaccharides in triticale bran

Rania Agil [a], Farah Hosseinian [a,b,*]

[a] Food Science & Nutrition Program, Department of Chemistry, Carleton University, Ottawa, ON, Canada K1S 5B6
[b] Institute of Biochemistry, Carleton University, Ottawa, ON, Canada K1S 5B6

ARTICLE INFO

Article history:
Received 29 July 2012
Received in revised form 4 February 2014
Accepted 6 February 2014

Keywords:
Triticosecale
Triticale bran
Food composition
Food analysis
Water-extractable polysaccharides
Arabinoxylans
Molecular weight distribution
Ethanol fractionation
Simple sugars
Scanning electron microscopy

ABSTRACT

The content, composition, and molecular weight distribution (MWD) profile of water-extractable polysaccharides (WEP) in triticale (× *Triticosecale* Whitm.) bran were determined. Results were compared against wheat (*Triticum* sp.) and rye (*Secale* sp.) bran, as well as triticale straw and flakes. The effects of the following conditions on the extractability of sugars were compared and respective fractions obtained: (1) boiling water extraction, WEP-I; (2) successive enzyme treatment and dialysis, WEP-II; (3) successive ethanol fractionation, WEP-III. Although the highest yield of WEP was generally found in fraction II, fraction III had the least level of simple sugars as unwanted co-extractives. The extractability of xylose (Xyl) and arabinose (Ara) in all bran samples significantly increased ($P < 0.05$) by up to 23.3% and 3.2% respectively, after precipitation with 80% ethanol. Amongst the cereal brans, wheat bran possessed a significantly higher ($P < 0.05$) molar % of Ara (19.5%) and Xyl (29.6%), followed by triticale bran (14.5% Ara, 17.2% Xyl), and lowest in rye bran (7.2% Ara, 13.4% Xyl). Triticale bran (TB) had higher molecular weight (MW) polysaccharides and the widest MWD range (4400–401,000 Da) of WEP, suggesting greater bioactive potential and high grain hardiness; prospects to be further investigated in future studies.

1. Introduction

Triticale (× *Triticosecale* Whitm.) is a hybrid of wheat (*Triticum* sp.) and rye (*Secale* sp.), containing high levels of nutritionally beneficial compounds (Rakha et al., 2011). Modern triticales AC Ultima) are mostly hexaploid which originated from *Triticum turgidum* subsp. *durum* × rye (*Secale cereale*) cross. Triticale is rich in phenolics and dietary fibres consisting of both soluble and insoluble fibres (Hosseinian and Mazza, 2009; Oatway, 2010). Triticale possesses a combined advantage of the high yield potential of wheat, and the disease and environmental tolerance of rye. Animal studies have shown the digestibility of triticale to be generally higher than barley and relatively equal to rye (Chrenková et al., 2012; Salabi et al., 2010). The poor bread making quality of rye and the digestibility problems of barley in cattle increase the need for exploring other avenues of grain sources such as triticale. Although triticale is an excellent candidate for animal feed due to high protein, amino acid, polysaccharide and B vitamin content, it has yet to be well-recognized for human food applications (Hansen, 2010). The insufficient demand for its significant utilization in food products can be attributed to the limited research data available on the characterization of triticale composites, particularly in the bran.

Consumption of whole grains has been associated with a variety of health benefits such as a reduced risk of obesity, type II diabetes, cardiovascular disease and cancer. Although the exact mechanisms have yet to be discovered, it is understood that the physiological functions of their protective bioactive compounds reside in the bran fraction of cereal grains (Okarter and Liu, 2010). Cereal bran such as wheat bran is a by-product of milling and usually accounts for 14–19% of the grains' weight (Maes and Delcour, 2002). On average, WEP such as pentosans/arabinoxylans are part of soluble dietary fibre, making up to 30% of the total sugar content of cereal bran (Cyran and Saulnier, 2005). Whole wheat and rye grain are common ingredients of the human diet, serving as essential sources of dietary fibre. The dietary fibre content in triticale (13–16%) is less than that of rye (15–21%) but generally higher than wheat (11–14%) (Cyran and Saulnier, 2005; Rakha et al., 2011). This can be attributed to the higher amount of cell wall polysaccharides being an essential part of the dietary fibre composite.

Recent studies have shown that water soluble pentosans (e.g. arabinoxylans), the major cell wall polysaccharides, may have

* Corresponding author at: Food Science & Nutrition Program, Department of Chemistry, Carleton University, Ottawa, ON, Canada K1S 5B6.
Tel.: +1 613 520 2600x2048; fax: +1 613 520 3749.
E-mail address: farah_hosseinian@carleton.ca (F. Hosseinian).

http://dx.doi.org/10.1016/j.jfca.2014.02.004
0889-1575/

potential health benefits, thus acting as quality markers of cereal grains (Agil and Hosseinian, 2012; Cyran et al., 2003). The objectives of this study were to: (i) characterize and quantify the WEP composition in triticale bran, (ii) measure the effect of ethanol fractionation on the yield potential of WEP, and (iii) measure the molecular weight distribution (MWD) of these WEP components in triticale bran and all results were compared with that of triticale straw and flakes, as well as wheat and rye bran. In this study, the term water-extractable polysaccharide mainly refers to that of pentosans or arabinoxylans.

2. Materials and methods

2.1. Sample preparation

Triticale bran, straw, and flakes (AC Ultima, spring triticale) were kindly provided by Agriculture and Agri-Food Canada (Lethbridge, AB). The bran is the hard outer layer of the cereal grain, while flakes are rolled and pressed wholegrain and straw are the dry stalks of the cereal plant that remain after the grain and chaff have been removed. Wheat and rye bran samples were purchased from a commercial source (Bulk Barn) in Kelowna, BC, Canada. All samples were ground to approximately 1.5 mm particle size using a Thomas Wiley Mill (Philadelphia, PA, USA); this was within the recommended range of a 1.0–2.0 mm particle size (Agil et al., 2011). The samples were dried in an oven at 105 °C overnight, cooled in desiccators, and weighed. All results are reported relative to the oven-dried weight (ODW) of the sample and calculated according to the following equations (Sluiter et al., 2008). Samples were stored in sealed plastic bags and kept in a freezer (–20 °C) prior to further analyses.

Calculations:

$$\%\,\text{Moisture (w/w)} = 100 - \left[\frac{\text{weight}_{\text{dry sample plus dry pan}} - \text{weight}_{\text{dry pan}}}{\text{weight}_{\text{sample as received}}} \times 100\right]$$

$$\%\,\text{Total solids (w/w)} = \frac{\text{weight}_{\text{dry sample plus dry pan}} - \text{weight}_{\text{dry pan}}}{\text{weight}_{\text{sample as received}}} \times 100$$

$$\text{ODW} = \frac{\text{weight}_{\text{air dry sample}} \times \%\,\text{total solids}}{100}$$

2.2. Scanning electron microscopy

Scanning electron microscopy (SEM) was employed to confirm the particle size of bran after grinding. Triticale bran was used as the representative sample whereby the sample was mounted on aluminium stubs, coated with a layer of gold (30 nm), and examined with a scanning electron microscope (SEM) (JSM-840A; JEOL Ltd., Tokyo, Japan) at an accelerating voltage of 10 kV (Gangadharappa et al., 2008).

2.3. Isolation of WEP

The WEP were sequentially extracted in triplicate from each grain sample, according to the procedures of Agil and Hosseinian (2012) and Lazaridou et al. (2008b). To remove fats, the fine powder (100 g) was extracted twice using hexane, at a 5:1 (v/w) solvent/solid ratio, for 1 h at room temperature (Liyana-Pathirana and Shahidi, 2006). The mixtures were filtered through a Whatman No. 4 (15 cm) filter paper, and the defatted residue was dried and kept at −20 °C until further analyses.

Defatted ground sample (10 g) was extracted with distilled water (1:100, w/v), stirred for 4 h at 65 °C, cooled, and centrifuged at 2706 × *g* for 20 min using a Sorvall RC 5B Plus centrifuge (Mandel Scientific Company, Guelph, ON, Canada). The supernatant was kept, the extraction repeated, and supernatants from the first and second extraction combined. This extract portion was designated WEP-I, representing the primary fraction of extract that has not been enzyme or ethanol treated.

To eliminate starch molecules and proteins from the sample, 1 mL of α-amylase solution (pH 6.9, 0.5–1.0 U/g) and 1 mL protease (pH 7.5, 2.4 U/g) from heat resistant *Bacillus licheniformis* as well as 1 mL of amyloglucosidase (pH 4.5, 300 U/ml) from *Aspergillus niger* (Sigma–Aldrich Canada Ltd., Oakville, ON) were added to the supernatant solution and stirred overnight at 40 °C. To inactivate the enzymes, the solution was heated at 95 °C for 5 min, then cooled and centrifuged at 2706 × *g* for 20 min at room temperature. The supernatant solution was dialysed against deionized water (4 °C for 72 h) using membrane tubing (3500 molecular weight cut off, Spectrum Laboratories, Inc., CA, USA). This extract portion was designated WEP-II, representing enzyme treated extract.

This latter fraction of extract, WEP-II, was further fractionated by incremental increases of ethanol concentration including 20, 40, 60 and 80% (Maes and Delcour, 2002). The mixture was stored at 4 °C overnight (16 h), centrifuged at 16,915 × *g* for 30 min and the precipitate was collected. This resulted in four fractions WE-20, WE-40, WE-60 and WE-80, respectively. The last two digits refer to the ethanol concentration at which the precipitate was collected. Since the highest proportions of water-extractable polysaccharides were obtained by precipitation with 80% ethanol, this fraction was designated WEP-III representing enzyme and ethanol treated extract. All extract solutions (WEP-I, -II, and -III) were freeze-dried and stored in a −20 °C freezer until further analysis.

2.4. Molecular weight distribution

2.4.1. High performance liquid chromatography (HPLC)

The molecular weights of polymeric carbohydrates was estimated using high performance size-exclusion chromatography on an Agilent 1100 HPLC system with an RI detector, autosampler, and Agilent ChemStation Plus software (Agilent Technologies Inc., Palo Alto, CA). The column comprised of triple Ultrahydrogel (2000, 250, and 120, 7.8 mm × 300 mm, Waters, Milford, MA, USA) equipped with a guard column (TSK PWH, TosoHaas GmbH, Stuttgart, Germany). Samples of WEP-III fractions, were dissolved in water, filtered through a 0.2 μm PVDF filter (BD, Franklin Lakes, NJ, USA) and injected (20 μL). Separation ensued for 90 min at 0.4 mL/min, with an isocratic condition using 0.1 M $NaNO_3$, as a mobile phase at 25 °C. MWD profiles of WEP-III fractions were determined using dextran standards (1 mg/mL) as molecular weight markers measured in Daltons (Da). The varying molecular weights of dextran standards used are as follows: 4400, 9900, 21,400, 43,500, 124,000, 196,000, 277,000 and 401,000 Da. Processing and analysis of MWD data were performed in triplicate using GPC software (Agilent Technologies Inc., Palo Alto, CA, USA).

2.4.2. Fourier transform infrared (FTIR) spectroscopy

To confirm and compare results obtained by HPLC for molecular weight determination, FTIR was also employed in accordance with the method of Hamed and Allam (2006), using a Nicolet™ 380 FTIR (Thermo Electron Corp., Madison, WI, USA) equipped with OMNIC Spectra™ software. Samples in dried form (1 mg) were placed on a Diamond and ZnSe crystal plate as recommended by Thermo Electron Corp. Spectra were recorded in duplicates between 400