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Molecular characterization of water extractable arabinoxylans isolated from wheat fine bran and their effect on dough viscosity

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ABSTRACT

Water extractable arabinoxylans (WE-AXs) from different tissues of wheat exhibit large natural variations in their structure and are the basis of the functional properties of wheat in bread dough. The objective of this work was to characterize the molecular features of fine bran WE-AXs from two wheat genotypes (*Triticum aestivum* and *T. durum*) grown in field trials in Northwest Mexico, and explore their effect on the viscosity of flour-fine bran doughs. The monosaccharide composition and spatial arrangement of WE-AXs were influenced by wheat genotype and determined their molecular characteristics. *Durum* fine bran WE-AXs showed a low degree of substitution. Fine bran WE-AXs showed also a wider range of molecular sizes than the flour. The thermal stability of the WE-AXs were in the following order: *durum* fine bran < *aestivum* fine bran, and < flour. The lower arabinose/xylose substitution of WE-AXs was in accordance to high intrinsic viscosity, and produced a higher extensional viscosity of flour-fine bran doughs. These findings provide an additional knowledge about other important factors that affect the rheological properties of the dough, such as WE-AXs structure, which appears to play a more important role than expected.

1. Introduction

Fine bran was recently shown to be an ingredient in bread flour formulations (Pavlovich-Abril, Rouzaud-Sández, Romero-Baranzini, Vidal-Quintanar, & Salazar-García, 2015), and the bread-making quality is significantly related to dietary fiber, starch, and protein contents in the wheat flour-fine bran blends. Once the effect of dietary fiber on dough and bread properties, a study polysaccharides to the structural level is suggested, specifically arabinoxylans; since, the substitution pattern of arabinose on the xylose backbone determines their physicochemical properties (Cyran & Saulnier, 2012).

The structure of AXs varies within different tissues. The AXs of aleurone and starchy endosperm tissues have similar structures with a backbone of β-(1 → 4)-linked D-xylopyranosyl residues (xylose), some of which are either mono-substituted at the C(O)-3 position, or di-substituted at the C(O)-2 and C(O)-3 positions with some L-arabinofuranosyl residues (arabinose) (Toole et al., 2011). The AXs may have some of the arabinose residues ester-linked on C(O)-5 to ferulic acid (3-methoxy, 4 hydroxycinnamic acid) (Saeed, Pasha, Anjum, & Sultan, 2011). In contrast, the wheat bran AXs are more complex with some uronic acids, mostly glucuronic acid, on the C(O)-2 position of xylose (Izydorczyk & Biliaderis, 1995).

The AXs are characterized as water extractable (WE-AXs) or water un-extractable. The difference in their extractability accounts for various physicochemical properties. The AXs are an important component of dough because they bind water and contribute to the viscosity. The WE-AXs play a more important structural role in dough and bread than the water un-extractable AXs (Gudmundsson & Eliasson, 1991). High content of AXs disturb the protein network formation during dough development and could affect the dough properties. This applies particularly for wheat dough where the extensibility and gluten yield are widely affected by the presence of AXs (Wang, Oudgenoeg, van Vliet, & Hamer, 2003).

Knowledge of the structural characteristics of WE-AXs fine bran, which is a mixture of different tissues of wheat (Posner & Hibbs,

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1989), might be useful to explain the effects of fine bran on dough properties and bread. The objective of this research was to characterize the structure and molecular properties of the WE-AXs, extracted from fine bran and relate those to dough viscosity of flour-fine bran blends.

2. Materials and methods

2.1. Materials

Fine bran from two different wheat genotypes (*Triticum aestivum* and *Triticum durum*), referred to as hard wheat fine bran (HFB) and *durum* wheat fine bran (DFB), respectively, and white flour (WF) were obtained previously (Pavlovich-Abril et al., 2015). Termamyl (EC 3.21.1. from *Bacillus licheniformis*), β-glucosidase (EC number 3.2.1.21 from *Aspargilus niger*), and protease (EC number 3.4.21.14 from *Bacillus lichenformis*) were purchased from Megazyme International Ireland (Bray Co. Wicklow, Ireland). Table 1 shows the composition of white flour-fine bran blends.

2.2. Isolation of WE-AX

The WE-AXs were isolated as described by Hromádková, Paulsen, Polovka, Kost'alova, & Ebringerová (2013), with some modification: 100 g of fine bran (and WF) were treated with 1 L of ethanol (95%) at 25 °C for 12 h while stirring to remove lipids and filtered using a fine strainer (0.2 mm). The defatted sample was suspended in 1 L of distilled water and heated at 95 °C for 30 min with Termamyl (*Bacillus licheniformis*, 1 mL of XII-A type, 611 U/mg) while stirring, and cooled. After the pH was adjusted with 3 mol/L HCl, the sample was incubated with an amyloglucosidase (*Aspergillus niger*, 0.3 mL, 3200 U/mL) for 3 h at 50 °C while stirring. The starch free extract was adjusted to pH 7.5 with 2 mol/L NaOH, followed by hydrolysis of protein with protease (*Bacillus licheniformis*, 0.8 U/g) for 18 h at 37 °C and inactivation of the enzyme by heating at 100 °C for 10 min. The extract was centrifuged (Heraeus Primo, Primo R Centrifuge; Thermo Fisher Scientific, USA) (10 000 rpm for 22 min at 20 °C) to obtain a supernatant consisting of WE-AXs. After centrifugation, the AXs were precipitated by adding ethanol (100% vol) until the concentration of alcohol in the solution reached 65% vol. Additionally, the sample underwent three washing steps with ethanol solutions (65%, 80%, and100%) and one with pure acetone to dry.

2.3. Chemical and physicochemical methods

2.3.1. Gas chromatography analysis

The neutral sugar content in WE-AXs extracts (10–20 mg) were determined by hydrolysis of the polysaccharides with 1 mol/L sulfuric acid (100 °C, 2 h) and the released monosaccharides were converted to alditol acetates, according to the method described by Rouau & Surget (1994). Alditol acetates were quantified on a DB 225 column (Agilent Technologies, Santa Clara CA, USA) in a Clarus 580 Perkin Elmer gas chromatograph (Perkin Elmer Inc, Waltham, MA, USA) equipped with a flame ionization detector and helium was used as the carrier gas. The column was held at 180 °C for 2 min, ramped from 180 to 220 °C at 5 K/min, and held at 220 °C for 10 min. Glucose originating as glucomannan was calculated by multiplying mannose by 7.3, according to Mansberger et al. (2014). Arabinose originating as arabinogalactan was calculated by multiplying galactose by 0.7, as reported by Mansberger et al. (2014). The AX content was the sum of the remaining arabinose and xylose. The nitrogen content (%N) was obtained by the Dumas method using a Leco-FP 528 nitrogen analyzer (46–30, AACC, 2000), and protein was calculated by N × 6.25. Starch was determined using the KI/I_2 test (NMX-F-374-1983, 2013).

2.3.2. High-performance size-exclusion chromatography (HPSEC)

The molecular weight distribution of WE-AXs extracts was determined by HPSEC using an Ultrahydrogel 1000 column (7.8 × 300 mm) eluted isocratically (Carvajal-Millan et al., 2005). The mobile phase was 0.2 mol/L $LiNO_3$ at flow rate 0.6 mL/min. The calibration was performed using pullulan standards P10-P800 (Shodex Standard P-28, Macherey-Nagel, Germany), with detection between 800 and 50 kDa. 20 μL of sample (20 mg/mL AXs) was injected and a Waters 600 differential refractometer was used for detection. The molecular weights of WE-AXs samples were calculated from their intrinsic viscosity (η) using the Mark-Houwink equation, as reported by Carvajal-Millan et al. (2005).

2.3.3. Intrinsic viscosity determinations

Viscosity measurements were made by determination of the flow times of WE-AXs solutions in water (from 0.5 to 1.0 mg/mL in WE-AXs) using an AVS 400 capillary viscometer (Schott Geräte, Hofheim, Germany), equipped with an Oswald capillary tube at 25 ± 0.1°C and immersed in a temperature controlled water bath. The [η] was estimated from relative viscosity measurements (η_{rel}) of solutions by extrapolation of Kraemer and Mead and Fouss curves to "zero" concentration, as reported by Carvajal-Millan et al. (2005). The viscosimetric mass [Mν] was calculated from the Mark-Houwink relationship, $M\nu = ([\eta]/k)1/\alpha$, where $k = 0.00347$ and $\alpha = 0.98$ (Carvajal-Millan et al., 2005).

2.3.4. Differential scanning calorimetry (DSC)

A DSC instrument (DSC 8000, Perkin-Elmer Norwalk, CT, USA) was used to determine the thermal decomposition of WE-AXs. Samples (5 mg) were placed in stainless steel pans and equilibrated at 100 °C for 3 min, and then heated at 5 K/min to 300 °C, against an empty reference pan. Indium was used as a standard for temperature and enthalpy calibration, and Perkin Elmer Pyris Version 3.50 software was used to control the experimental conditions and analysis. Approximated endothermic enthalpy (ΔH)

Table 1
Chemical composition[a] of the flour-fine bran blends (70–30%, w/w).

Sample[b]	Chemical components[c]					
	TF	IF	Protein	Starch	AX[d]	B-G
HFB	10.45 ± 0.7[a]	6.84 ± 0.3[a]	13.13 ± 0.1[a]	61.78 ± 0.2[b]	6.12[a]	0.35 ± 0.1[a]
DFB	9.87 ± 0.3[a]	5.88 ± 0.6[a]	13.02 ± 0.7[a]	63.17 ± 2.2[b]	5.04[a]	0.25 ± 0.1[a]
WF	5.57 ± 0.3[b]	3.60 ± 0.1[b]	12.87 ± 0.1[a]	70.28 ± 0.0[a]	5.10[a]	0.26 ± 0.0[a]

Columns with different letters differ significantly ($p < 0.05$).
[a] Chemical Analysis are described in Pavlovich-Abril et al., 2015.
[b] HFB- hard wheat fine bran; DFB- *durum* wheat fine bran; WF- hard wheat white flour.
[c] TF-total fibre; IS-insoluble fibre; AX-Arabinoxylans; B-G-Beta-glucans.
[d] AX was calculated by material balance based on gas chromatography results (white flour-fine bran, 70-30%, w/w).