



Structural changes of arabinoxylans in refrigerated dough

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ABSTRACT

Wheat flour mainly consists of the starchy endosperm of the kernel, and contains starch, proteins, lipids and non-starch polysaccharides. Arabinoxylans (AXs) are the main non-starch polysaccharides found in wheat flour. Studies showed that degradation of AXs in refrigerated dough has negative effect on quality. The objectives of this research were to structurally characterize the AXs from refrigerated dough samples and determine any correlations between the AX structural changes and variation in dough quality. We observed that the molecular weight of the AXs was changing during the extended storage, and we detected variation in arabinose to xylose ratio for water extractable and unextractable AXs. The ratio of unsubstituted xylose in water extractable AXs increased during storage. These results showed that changes in AX chemistry is correlated to the refrigerated dough quality.

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1. Introduction

Wheat flour, consisting mainly of the starchy endosperm of the kernel, contains starch (70–80%), proteins (8–18%), lipids (1.5–2.5%) and non-starch polysaccharides (2–3%) all expressed as percentage of dry matter (MacRitchie, 1989). The non-starch polysaccharides (NSP), originating from the cell wall of the aleurone and endosperm of the wheat kernel, are polysaccharides of pentose sugars and/or hexose sugars. The pentose polymers are termed pentosans, of which arabinoxylan (AX) is the most important; other polysaccharides include cellulose, β -glucan, arabinogalactan-peptide and minor constituents like glucomannan and xyloglucan. AXs are divided into water extractable AX (WE-AX) and water unextractable AX (WU-AX), which comprise 25% and 75% of the AXs present in wheat flour, respectively (Courtin & Delcour, 2002). AX content in wheat flour increases with the extraction rate of the flour due to the contamination with bran and aleurone fragments during the milling process. The wheat flour industry aims for maximal starch yield, so high-extraction-rate flour, which contains more AXs, is used. AX consists of a linear backbone of β -1,4 linked xyloses with some residues carrying either a single arabinose residue on C-3 or two arabinose residues on C-2 and C-3 (Gruppen, Kormelink, & Voragen, 1993). A few of the arabinose groups are esterified with ferulic acid (FA). FA (4-hydroxy-3-methoxycinnamic acid) is concentrated in the cell wall of the outer coverings of wheat where it is mainly esterified to the arabinose branches of AX (Geissman & Neukom, 1973). Therefore, it is a natural component of WE-AX and WU-AX. Free, soluble-bound and

insoluble-bound FA has been found in wheat flour and gluten (Autio, 2006). The molecular weight of AX is in the range between 22,000 and 5,000,000 Da (Dervilly, Saulnier, Roger, & Thibault, 2000).

Wheat flour contains several functionally significant enzymes, such as amylases, proteases, xylanases, lipoxxygenase, polyphenol oxidase and peroxidase. Although these enzymes are not active during the storage of the grain and flour, as water is added they become active and play a critical role in determining the functional characteristics of the flour (Rani, Prasada Rao, Leelavathi, & Haridas Rao, 2001). AXs are degraded by xylanolytic enzymes. Endo-(1,4)- β -D-xylanase (EC 3.2.1.8), α -L-arabinofuranosidase (EC 3.2.1.55), (1,4)- β -D-xylosidase (EC 3.2.1.37) and feruloyl esterases (EC 3.1.1.73) are all xylanolytic enzymes and generally highly specific in their catalytic reactions. These enzymes hydrolyze the AXs or their crosslinks with other macromolecules in dough, decreasing their water holding capacity (Courtin, Gys, & Delcour, 2006).

Today's refrigerated dough industry traces its origin to a small bakery that started business in Louisville, KY, in 1937. By definition, refrigerated dough is a flour-based, unbaked product that is stored between 4 and 7 °C. The first refrigerated dough product was a chemically-leavened biscuit with shelf life of about 3 weeks (Allenson, 1982). Today, the refrigerated dough market encompasses a wide range of products available in the United States as well as the international market including Western Europe and Canada. The products in these processing categories include dinner rolls, breakfast rolls, fruit rolls, pizza crusts, French croissants, and biscuits. These and other products have increased in popularity due to their ease of preparation, similarity to "homemade" products and because they remain fresh for an extended period of refrigerated storage. In spite of extensive applications, very limited

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peer-reviewed reports are available about refrigerated doughs. However, several patent applications verify the tremendous interest in the subject. The objectives of this research were to structurally characterize the AXs from refrigerated dough samples and determine any correlations between the AX structural changes and variation in dough quality.

2. Experimental

2.1. Materials

Flour samples were provided from ADM and Horizon milling companies. All the analyses were performed in triplicates and averaged values were used. The assay kit for total starch determination was purchased from Megazyme (Megazyme International Ireland Ltd., Bray, Ireland). All other chemicals were provided from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Standard analyses

An air oven method was used to determine the moisture content of the flours by drying the flour and weighing the residue (Approved Method 44-15A, AACC International 2004). Protein content (14% moisture basis, mb) of each of the flours was determined by the combustion method (Approved Method 46-30, AACC International 2004) using a LECO FP428 nitrogen analyzer (LECO Corporation, St. Joseph Michigan). The total starch assay kit was used to determine the total starch content (% dry weight basis, dwb) of each of the flours (Approved Method 76-13, AACC International 2004).

2.3. Preparation of refrigerated dough samples and extraction of arabinoxylans (AX) from dough samples

In order to avoid confounding factors arising from the presence of other ingredients, a lean dough formula was used. The dough was prepared by using 100 g of flour (14% moisture basis), 1.8 g of salt, and a certain amount of water, containing 0.06% w/v of sodium azide (Mallinckrodt baker Inc. Paris, KY) to prevent microbial spoilage, to reach the desired moisture content previously determined according to the specific farinograph absorption test (see Table 1). Dough was mixed in a 100 g pin mixer (National Manufacturing, Lincoln, NE) for the previously determined optimum mixing time (3 min 45 s), sheeted, molded, and stored in plastic containers for 0 (analysis was done immediately after mixing), 1, 2, 3, 6, 10, 16 and 34 days at 6 °C.

The freeze dried dough samples were ground in a mortar and pestle to fine consistent grind and the flour samples were used directly. Deionized water was chilled to a temperature of 4 °C and added to 5.0 g of sample in a 50 mL centrifuge tube. The tubes were then vortexed to disperse the sample and shaken at 4 °C for 30 min at 200 rpm. The tubes were then centrifuged at 7850 rpm for 15 min at 4 °C. The supernatant was removed and frozen immediately at –80 °C. The supernatant was then dispersed in 20 mL of

4 °C deionized water and shaken and centrifuged as before. The supernatant was added to the previously collected supernatant and frozen at –80 °C. The precipitate and the supernatant were then freeze dried. After freeze drying 40 mL of boiling water was added to the supernatant to dissolve the material and boiled for 30 min. The samples were then freeze dried again and dissolved in 40 mL room temperature water. Next the dissolved samples were centrifuged at 7850 rpm for 15 min at 4 °C. The samples are treated with alpha-amylase to hydrolyze any starch residue and dialyzed against water at 4 °C for 3 days. The samples were filtered through qualitative filter paper and, finally the extracted AX was freeze dried.

2.4. Dough syringing determination

Dough syringing was measured as the liquid released by the dough after centrifugation at 13570 rpm. The dough was divided in pieces of approximately 10 g and after centrifugation of accurately weighed dough pieces, the liquid inside of the centrifuge tube was removed with a glass pipette. The syrup released was calculated as the difference in weight between the tubes before and after syrup removal and was expressed as a percentage of the initial dough weight.

2.5. Determination of apparent endoxylanase activity levels

An Endo-β-Xylanase assay kit with AZCL-AX tablets was obtained from Megazyme International (Megazyme International Ireland Ltd., Wicklow, Ireland). For the assay procedure, 4 g of flour was weighed into a 50 mL centrifuge tube and 20 mL of 25 mM sodium acetate buffer pH 4.7 was added. The slurry was extracted at 6 °C for 1 h. The tubes were centrifuged at 5000 rpm for 10 min at 6 °C. From each sample 1.0 mL was taken from the supernatant and placed in 4 test tubes. The test tubes were preincubated for 10 min at 40 °C. AXCL-AX tablets were added to two of the four tubes and incubated for 17 h at 40 °C. The reaction was stopped with 10 mL of 2.0% trizma base solution and the samples were filtered through #1 Whatman filter paper. Finally absorbance was read at 590 nm and the enzyme activity was calculated against a standard curve.

2.6. Microextension studies

Microextension test was used to investigate changes in the dough extensibility and resistance. A Texture Analyzer (TA-XT2 from Texture Technologies Corp., Scarsdale, NY) was used to perform extension tests on small scale (0.8 g) dough strips (Kieffer, Wieser, Henderson, & Graveland, 1998). The maximum resistance (R_{max}) and the extensibility (E) of refrigerated doughs were determined from the test.

2.7. Determination of dough consistency

Consistency was determined by using a farinograph (Gys, Courtin, & Delcour, 2003) and calculated as a percentage of the decrease in initial dough consistency.

Table 1
Proximate analysis of the flours used in this study.

Sample	Moisture (%)	Protein (%)	Total Starch (%)	Total AX ^a (%)	A/X (in total-AX)	Farinograph Absorption (%)	Xylanase activity (XU) ^b
QL	12.6	13.3	74.6	3.29	0.79	64.2	0.051858
PB	13.5	12.0	75.3	3.26	0.74	63.8	0.008496

^a Total AX% = (Ara + xyl)*0.88

^b One xylanase unit (XU) is determined as described in (Courtin et al., 2006). One xylanase unit (XU) corresponds to an increase in E_{590} of 1.0 per gram of sample and per hour under the conditions of the assay.

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